GENETIC VARIATION AND COGNITIVE ABILITY

DANIELLE POSTHUMA

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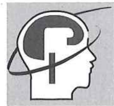

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VRIJE UNIVERSITEIT

GENETIC VARIATION AND COGNITIVE ABILITY

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Vrije Universiteit Amsterdam,
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Daniëlle Posthuma

geboren te Amsterdam

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copromotor : dr. J.C.N. de Geus

.. 'We stress this point as exemplifying the need for mathematical treatment of problems in population genetics. Intuitive verbal discussions are helpful in giving a feel for a problem, but may give rather distorted conclusions (and occasionally are downright misleading).'

\- JS Gale. *Population Genetics – 1980, p.10*

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prof.dr. NG Martin
prof.dr. MC Neale
prof.dr. RS Kahn
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Chapter 1

General introduction

Despite the wealth of studies indicating substantial genetic influences on general and specific cognitive abilities in children and adolescents (Bouchard and McGue, 1981; Boomsma, 1993; Cherny and Cardon, 1994; Devlin, Daniels and Roeder, 1997; Rijsdijk *et al.*, 1995, 1997; Boomsma and van Baal, 1998: Rietveld *et al.*, 2001; Bartels *et al.*, 2002), relatively few studies have investigated the heritability of cognitive ability during early and middle adulthood (Kallman, 1951; McGue, Bouchard and Iacono, 1993; Pedersen, 1992; McClearn *et al.*, 1997; also reviewed in Pedersen and Lichtenstein, 1997). Heritability for general cognitive ability increases from infancy (20%), to childhood (40%) to adolescence (50%), to early, middle and late adulthood (60%). Plomin *et al.*, (1994) showed that the observed stability in cognitive ability across the life span (i.e. smart kids will be smart adults) is largely mediated by stable genetic factors. This genetic stability suggests the existence of stable individual differences in underlying neurophysiological or biological mechanisms. We still have little knowledge of the nature of these mechanisms underlying cognitive ability.

The first goal of the present study is to investigate the heritability of cognitive ability in early and middle adulthood. A second goal is to gain more insight in the anatomical, electrophysiological, and behavioural substrates of cognitive ability. It will be investigated whether anatomical, electrophysiological, and behavioural indices of cognitive ability, derived mostly from non-genetic designs, show interindividual variability. Next, it will be investigated to what extent this interindividual variability can be ascribed to genetic or environmental variability between individuals. Finally, it will be investigated whether there is a correlation between the genes that influence individual differences in these biological indices and the genes that influence individual differences in cognitive ability. A significant genetic correlation may facilitate the future detection of genes for cognitive ability. Biological, neurophysiological, electrophysiological and behavioural indices of the pathways that connect genes and cognitive ability are called *endophenotypes* of cognitive ability. An extended twin design, i.e. including twins and additional siblings, will be used to quantify the relative contributions of genes and environmental influences to the (co) variance in cognitive ability and its endophenotypes.

Outline of this thesis

This introductory chapter will give a brief overview of the anatomical, electrophysiological and behavioural indices of cognitive ability used throughout this thesis, and of the extended twin families on which observations on cognitive ability and its endophenotypes were obtained. Recruitment procedures, structure of the families in terms of age, sex, zygosity, and family composition, and a description of tasks and measures will be given. In the ensuing chapter, the biometrical model underlying quantitative genetic analyses will be discussed and a solid biometrical basis for the extended twin design will be provided. In Chapter three the extended twin design is evaluated in terms of the statistical power to detect genetic and environmental influences.

In Chapters four and five the extent to which heritability estimates derived from twin studies can be generalized to the non-twin population is investigated, by comparing twins with their non-twin siblings. In Chapter four this is done for cognitive ability, in Chapter five this is done for brain volume.

Chapters 6 to 10 examine various endophenotypes for cognition. The ground plan for each endophenotype is to first test the contribution of genetic factors to individual differences in the endophenotype, followed by an examination of how the endophenotype correlates to the various aspects of psychometric IQ and the genetic or environmental source of this correlation. Chapter 6 reports on the genetic analysis of brain volumes and specifically investigates the heritability of cerebellar volume. In Chapter 7 the relation between brain volumes and intelligence is investigated in a multivariate genetic design. In Chapters 8, 9, and 10 a multivariate genetic design is employed to investigate the extent to which the relation between intelligence and alpha peak frequency (Chapter 9), perceptual speed (Chapter 9), latency of selective response activation (Chapter 10), and frontal inhibition (Chapter 10), is mediated through common genetic factors or through common environmental factors.

In Chapter 11 a description of the practical application of existing linkage and association methods to an extended twin design is given including some extensions of these methods (e.g. non-additive genetic influences).

In the general discussion (Chapter 12), the empirical results from the chapters 3 to 10 are discussed and integrated with existing literature. A short note on future linkage and association based gene-finding using the database on which this thesis is based, concludes this thesis.

Anatomical indices of cognitive ability

In the course of this thesis I was fortunate to become part of the collaboration of our department (Biological Psychology at the VU) with the structural Magnetic Resonance Imaging (MRI) laboratory of the Utrecht Medical centre. Brain volumes are an obvious source of individual differences in cognitive abilities. Since the second half of the 19th century positive relations between head size and intelligence have been observed, and these observations have gained tremendous weight by confirmation through the much more reliable assessments of 'head size' by structural MRI (Willerman *et al.*, 1991; Egan at al. 1994; Andreasen *et al.*, 1993; Raz *et al.*, 1993; Storfer, 1999; Wickett *et al.*, 2000; Pennington *et al.*, 2000). Recent progress in the scoring and segmentation of MRI scans make it possible to separately assess (regional) white and grey matter volumes. White matter volume may easily be construed as a possible source of individual processing speed. Existing evidence now explicitly suggests a link between (frontal) grey matter volume and the "g" factor of intelligence (Thompson *et al.*, 2001). Very little, however, is known about the

genetic architecture of cerebral and cerebellar brain volumes. In collaboration with drs Baaré, Hulshoff Pol and Kahn (Posthuma *et al.*, 2000; Baaré *et al.*, 2001; Hulshoff Pol *et al.*, 2002) this thesis has tried to remedy this situation somewhat.

Electrophysiological and behavioural indices of cognitive ability

Electrophysiological and behavioural indices of cognitive ability may be derived from two specific domains of brain functioning: working memory capacity (Kyllonen and Christal, 1990; Necka, 1992; Daneman and Merikle, 1996; Engle *et al.*, 1999) and processing speed (Vernon, 1983, 1985, 1991; Jensen, 1982; Reed and Jensen, 1992; Vernon, 1993; Vernon and Weese, 1993; Bowling and Mackenzie, 1996; Knorr and Neubauer, 1996; Rijsdijk, Vernon and Boomsma, 1998; Fry and Hale, 2000).

Recent advances in the cognitive neurosciences have provided powerful methods and designs for assessment within these domains, allowing the measurement of various stages of information processing, such as stimulus detection, stimulus perception, response selection, response initiation, response execution, memory consolidation, and memory retrieval.

These methods include behavioral test-batteries, neurophysiological techniques like electroencephalography (EEG) and event related potentials (ERP) (Gevins *et al.*, 1999), and neuroimaging methods like positron emission tomography (PET) (Raichle *et al.*, 1979: Colsher, 1980) or functional Magnetic Resonance Imaging (fMRI) (Ogawa and Lee, 1990; Howseman and Bowtel, 1999; Logothetis *et al.*, 2001). Currently, however, PET and fMRI are not feasible on the scale required by genetic epidemiology.

Working Memory

All reasoning involves the use of working memory in which people temporarily store and manipulate information. In fact, it has been suggested that reasoning ability is nothing more than working memory capacity (Kyllonen and Christal, 1990).

Working memory, according to the model of Baddeley and Hitch (Baddeley, 1986; Baddeley and Hitch, 1974) denotes a modular system composed of a *central executive* and two *multimodal storage systems*: the phonological loop and the visuo-spatial sketch pad (Baddeley, 1986, 1992, 2000). The recently added fourth component to Baddeley's modular model of working memory is the episodic buffer. This component is thought to be responsible for the integration of information from both the phonological loop and the visuospatial sketchpad, as well as from long-term memory traces (Baddeley, 2000, Prabhakaran *et al*, 2000). A recent study by Prabhakaran *et al* (2000) located this episodic buffer in the right frontal region (BA 9, 10, and 46).

The central executive, which has often been localized in the frontal lobes (e.g. Fuster, 1997; Smith and Jonides, 1999), controls the two slave systems responsible for the encoding and temporary storage of either visual material (the visuospatial

sketchpad), or verbal material (the phonological loop). It also allocates attentional resources during the simultaneous execution of two tasks, coordinates the capacity to switch retrieval strategies, controls the capacity to hold and manipulate information stored in long term memory, and controls the selective attention to relevant stimuli while inhibiting irrelevant stimuli (Baddeley, 1996). This latter inhibitory function is particularly interesting as a decline in this function has been suggested to be the central cause of cognitive decline (Dempster, 1991, 1992; Kramer *et al.*, 1994; West, 1996). Executive functioning and inhibitory control have been associated with cortical activity in the prefrontal cortex, specifically with Brodman's areas 45, 46 (Smith and Jonides, 1998, O'Reilly, Braver Barch and Cohen, 1999) and 8 (Rowe *et al.*, 2000), but their precise mechanisms are unknown.

Inhibitory functioning can be assessed with any task that involves a target and a (salient) distractor, such as the Eriksen Flanker Task (Eriksen and Eriksen, 1974). In this task subjects are required to respond to a central target stimulus that is flanked by stimuli that are either congruent or incongruent with the target stimulus. If the central target is congruent with the flankers, a fast response is feasible. If, however, the central target is incongruent with the flankers, the response that is automatically activated by the flankers needs to be inhibited, which slows response times and increases error probability (Eriksen and Eriksen, 1974; Coles *et al.*, 1985). Results from brain imaging studies have suggested that the anterior cingulate cortex (ACC) is a critical neurobiological substrate of frontal inhibition (Awh and Gehring, 1999) and is the executive area for attention (Posner *et al.*, 1988). The ACC is located on the medial surface of the frontal lobes and is thought to funnel command signals originating from lateral prefrontal areas to motor output systems (Turken and Swick, 1999). It is involved in evaluation processes such as error and response-conflict monitoring, and indicates when attentional control needs to be more strongly engaged (MacDonald *et al.*, 2000).

In this thesis the heritability of frontal inhibitory functioning, assed with the Eriksen Flanker Task, across two age cohorts will be investigated as well as its association with cognitive ability.

Processing Speed

The most salient feature of working memory is that it has a limited capacity that has to be distributed over the competing functions of storage and processing. If working memory storage increases due to activity of the visuospatial sketch-pad or phonological loop, reasoning and comprehension are impaired (see for example Fuster, 1997; Miyake and Shah, 1999). If reasoning demands are increased, storage capacity rapidly diminishes. Anything that speeds up either storage or processing will increase working memory capacity in terms of the number of items that can be processed in a fixed time interval. A reasonable idea, therefore, is that individuals with fast neural processing speed will also have the highest working memory capacity.

This is the main idea of the limited capacity theory of working memory (Vernon, 1987; Jensen, 1998), and ties in neatly to the most researched hypothesis for a neural basis of differences in cognitive ability: the neural speed hypothesis – the idea that individual differences in neural speed or more general processing speed determine individual differences in cognition (Vernon, 1983, 1985, 1991; Jensen, 1982; Reed and Jensen, 1992; Vernon, 1993; Vernon and Weese, 1993; Bowling and Mackenzie, 1996; Knorr and Neubauer, 1996; Rijsdijk, Vernon and Boomsma, 1998; Fry and Hale, 2000).

Speed of processing is the speed with which subjects can perform basic cognitive operations, including stimulus detection and perception, response selection, response initiation, response execution, memory consolidation, and memory retrieval. Differences in this speed are thought to reflect structural aspects of neural wiring, like myelin-sheathing, number of ion-channels, or the efficiency of neurotransmission. Thus, it can be expected that individual differences in speed clearly depend on genetic influences on the myriad of proteins influencing both axonal conduction and synaptic transmission. At the same time processing speed also critically depends on the actual neural networks wiring, which is, by its very nature, experiential. The extended twin approach in the current study allows the estimation of the relative contributions of genetic and environmental influences to processing speed.

In this thesis the heritability of several measures of processing speed and their relation with cognitive ability will be investigated. These measures include a general index of the speed of brain oscillations and indices of several stages of information processing: perceptual speed, speed of premotor response selection, speed of motor response selection, and speed of response initiation.

Experimental tasks and measures

The main tasks and measures reported on in this thesis are listed in Table 1.1 (for a full listing of all collected measures see Appendix I). The experimental protocol for the full set of tasks was divided in two sessions; an EEG session and an IQ/Reaction Time/Inspection Time session. Sessions lasted approximately 2 hours and 10 minutes each. In between sessions subjects had a 15 minutes break. The order of the sessions was randomised across subjects.

Psychometric IQ

Psychometric IQ was assessed with the Dutch version of the WAIS-IIIR (WAIS-III, 1997) of which eleven subtests were used to assess psychometric IQ. Block design, Letter-number sequencing, Information, Matrix reasoning, Similarities, Picture completion, Arithmetic, Vocabulary, Digit symbol-coding, Digit-symbol pairing and Digit symbol-free recall.

Verbal IQ, performance IQ and the four standard WAIS-IIIR dimensions were derived from these subtests. Performance IQ was derived from picture completion,

block design, matrix reasoning; Verbal IQ was based on information, similarities, vocabulary, arithmetic. Verbal Comprehension was based on information, similarities, and vocabulary; Working Memory was based on arithmetic and letter-number sequencing, Perceptual Organization was based on block design, matrix reasoning, and picture completion), and Processing Speed was based on digit-symbol substitution.

Table 1.1
Overview of tasks and measures reported on in this thesis.

DOMAIN	TASK	MEASURE
PSYCHOMETRIC INTELLIGENCE	Wechsler Adult Intelligence Scale-revised (WAISIIIR)	Block design, Letter-number sequencing, Information, Matrix reasoning, Similarities, Picture completion, Arithmetic, Vocabulary, Digit symbol-coding, Digit-symbol pairing, Digit symbol-free recall.
BRAIN VOLUME	-	MRI: Intracranial space, gray matter volume, white matter volume, cerebellar volume, lateral ventricular volume, third ventricular volume
	-	Head circumference
WORKING MEMORY / PROCESSING SPEED	Resting EEG	Alpha peak frequency
	Inspection time task	Inspection time
	Eriksen Flanker Task – EEG recording	LRP-onset, peak latency, decision time, performance; effects of stimulus-response incongruency on these
OTHER		Demographics (age, education) Body height

Brain volume

Magnetic Resonance Imaging (MRI) was carried out by dr. Wim Baaré at the Academic Hospital in Utrecht in the laboratory of dr. Kahn. Collaboration with this laboratory allowed the inclusion of the analyses of several brain structures in this thesis. The sample that participated in Baaré's study (Baaré, 2001) consisted of 258 subjects from 112 extended twin families. MRI acquisition details have been described in dr. Baaré's thesis (Baaré, 2001). One hundred thirty five subjects from 60 families also participated in the present study.

Head circumference was measured with a measuring tape in the sample (N=688) that participated in the IQ and EEG measurements.

EEG recording

Brain oscillatory speed (i.e. peak alpha frequency) was extracted from the electroencephalographic recordings. During the EEG measurements the subjects were seated in a comfortable reclining chair in a dimly lit, sound-attenuated electrically shielded room. EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems, (Enschede, The Netherlands). Signals were continuously represented online on a Nec multisync 17" computer screen using POLY 5.0 software (POLY, 1999) and stored for offline processing. Standard 10-20 positions were F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2 (Jasper, 1958). Additionally F1 and F2 were placed halfway between F3 and Fz, and between Fz and F4, respectively. Software-linked earlobes (A1 and A2) served as reference. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ impedances of the EOG electrodes below 10 kΩ. The EEG was amplified (0.05 - 30 Hz), digitized at 250 Hz and stored for offline processing.

Subjects were instructed to close their eyes, relax and minimize movement during the three minutes of EEG recording of the eyes closed (EC) task. During the three minutes recording of the eyes open (EO) task subjects were instructed to fixate on the dot presented at the center of the computer screen, and to avoid blinking.

A power density spectrum was calculated by using a Fast Fourier transform applied to 4 sec. epochs of the three minute-recordings of each condition. This yielded 44 epochs (epoch 45 was not used for computational reasons) and a 0.25 Hz resolution in the power spectra. The power density spectra on the occipital leads were used to derive the individual alpha peak frequencies (IAF).

The peak frequency in the EC condition was determined as the highest peak in a window of 7-14 Hz in the power spectrum, irrespective of the shape of the spectrum. Visual inspection was conducted for peak frequencies occurring at the boundaries of the search window. Final localization of the correct IAF was based on an automated comparison between the peak frequency as determined in the EC condition and the frequency at which alpha power was depressed most by opening of the eyes (i.e. finding the peak frequency in the spectrum obtained by subtracting the EO spectrum from the EC spectrum). If these two methods of peak detection yielded an identical peak frequency, this was taken as the IAF.

If the two methods yielded different peak frequencies, the spectra were visually inspected in order to determine the real alpha peak frequency. For example, in cases where the EC spectra showed two peaks of approximately the same magnitude, that peak was taken at which alpha depression was highest.

Inspection time task

Perceptual speed was assessed with an inspection time task. The stimulus was presented on a monitor at a viewing distance of approx. 0.5 meters. It consisted of two vertical lines, 22 mm. and 27 mm. in length and joined at the top by a horizontal line (12 mm. long). The longer line appeared on the left or the right with equal probability. Duration of the stimulus was variable, ranging from 17 to 200 ms. Following presentation of the stimulus, a mask consisting of two vertical lines 37 mm. long, shaped as lightening bolts, was presented for 300 ms. The inter-trial stimulus interval was 2 seconds. Participants were required to judge which one of two lines is the longest by pressing either the left or the right arrow keys on a keyboard.

A Parameter Estimation by Sequential Testing (PEST) procedure (Pentland, 1980, Findlay, 1978) was incorporated into which uses a staircase method to alter stimulus duration based on the subjects' previous response. The initial stimulus duration was 100 ms. for all participants. If a correct answer was given, stimulus duration time of the next trial was decreased, if an incorrect answer was given, stimulus duration of the next trial was increased. The amount of increase/decrease was dependent on the number of previous reversals of increase/decrease. Thus, after many reversals, increases/decreases on subsequent trials became smaller and the PEST procedure converged on the subjects' inspection time. The task ended when the PEST estimate had become sufficiently stable or as soon as the maximum number of trials is presented.

For each subject a cumulative normal function (mean = 0) was fitted post hoc to the stimulus duration times. The standard deviation of this curve is the stimulus onset asynchrony (SOA, in this case the inspection time) at which 84% accuracy (corrected for guessing) is achieved. The reciprocal of the standard deviation times 1000 can be interpreted as the number of inspections per second resulting in a correct judgement (Smith, 2000). To ensure accurate SOA's, a dynamic backward mask (Evans and Nettelbeck, 1993) was used. All instructions were given on a computer screen and the importance of accuracy over reaction time was stressed in the instruction.

Eriksen Flanker task

Inhibitory functioning, speed of premotor response selection, speed of motor response selection, and speed of response initiation were assessed with the Eriksen Flanker Task and simultaneous EEG recording. Two boxes with an upper and a

lower response button were attached on the left and right hand side in front of the monitor. The subjects put their index fingers of each hand on the lower "home" buttons, which started off each trial. Stimuli consisted of a horizontal stimulus array comprising five arrowheads. Arrowheads pointed to the right or the left. Subjects were instructed to respond with the left hand if the central arrowhead pointed to the left, and with the right hand if the central arrowhead pointed to the right. Responding meant pushing the upper "response" buttons. They were asked to respond as fast and accurate as possible and to ignore the flanking arrowheads. Visual feedback ("correct", "incorrect" and current points) was presented 1000 ms after the onset of the stimulus array, and lasted 1500 ms. They gained 1 point for each correct response and lost 5 points for each incorrect response. Responses were incorrect when subjects responded prematurely, released the wrong home button, pressed the wrong response button, or exceeded the maximum response time of 1000 ms.

There were four conditions each containing 30 trials: left congruent (<<<<<), right congruent (>>>>>), left incongruent (>><>>), and right incongruent (<<><<). Home button release time and response button presses were stored for all trials as well as codes for incorrect responses (wrong button, too early, too late). Performance measures were decision time and %incorrect, and these were averaged over left and right hand trials. Decision time (response initiation) was computed as the time interval between stimulus onset and home button release for congruent and incongruent trials. Incorrect responses were counted and converted to a performance score, again for the congruent condition and the incongruent condition, the latter was taken as a measure of frontal inhibitory functioning.

LRPs were computed for correct trials only. Per trial, the epoch used for data analysis started 250 ms preceding stimulus array onset, and ended 1000 ms after onset of the stimulus array. The mean amplitude in the 250 ms preceding the stimulus array was defined as the baseline. Epochs were discarded from further analyses if values exceeded 200 µV on the vertical or horizontal EOG channels, or values exceeded 80 µV on the EEG channels. A three-step subtraction method was performed to calculate the LRP waveforms. First, we subtracted the time series recorded from C4 from those recorded over C3 on each trial for the right hand responses. Second, we subtracted the time series recorded from C4 from those recorded over C3 on each trial for left hand responses. Third, the two difference waves for left and right hand responses were subtracted, which resulted in the LRP waveform. This method is also known as the double subtraction method:

$$\text{LRP} = (\text{C3} - \text{C4})_{\text{right hand}} - (\text{C3} - \text{C4})_{\text{left hand}}$$

Inclusion criteria for the LRP onset and peak latency was that the LRP waveform had to be based on at least 30 trials. LRP-peak latency (premotor and motor response selection processing) was determined by searching the most negative value in the

350-900 ms post stimulus window. LRP-onset (premotor response selection processes) was calculated by a single-subject based regression procedure with 1 degree of freedom (Mordkoff and Gianaros, 2000). This method fits a linear regression to the LRP slope using the individually fixed LRP peak negativity. The intercept with the x-axis denotes LRP onset.

Extended twin design

A main aspect of the sample used in the present thesis is the inclusion of non-twin siblings, in addition to the inclusion of monozygotic (MZ) and dizygotic (DZ) twins. Such an extended twin design is not only optimal for future gene detection (Dolan, Boomsma and Neale, 1999), but also for the estimation of heritability/environmental influences. All twins were recruited from the Netherlands Twin Registry (Boomsma, 1998), and most of them had previously participated in one of three studies in which zygosity was assessed by blood group polymorphisms and DNA typing (Boomsma, 1992; Snieder, 1996; van Beijsterveldt, 1996; Rijsdijk, 1997). Boomsma's study included a sample of 160 twin pairs aged between 14 and 21 years at the time of the study (1985-1992) (129 subjects returned for the present study). Snieder's study included 213 twin pairs aged 34 –63 years at the time of the study (1992-1996) (216 subjects returned for the present study). Van Beijsterveldt and Rijsdijk both used the same sample, which included 213 twin pairs aged 16 in 1993-1994 (96 subjects returned for the present study). Apart from these three datasets, we approached a group of 100 twin families (71 families agreed to participate) that did not participate in any of the above studies.

Twins were sent an invitational letter, and were then approached by phone to ask for participation. Twins were asked if they had additional siblings and permission was asked to approach the siblings as well.

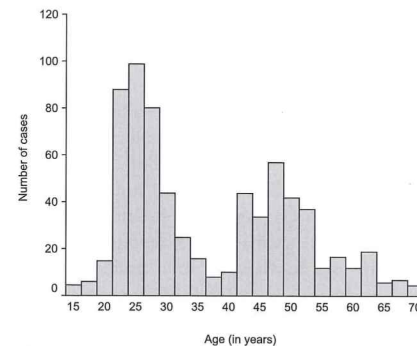

Figure 1.1
Age distribution showing two age cohorts (N = 688).

If subjects were willing to participate, an appointment was made, which could be during the day, but also in evenings or during weekends. One week before the subjects were expected in the laboratory they were sent additional information on the study, an informed consent form and details concerning the location of the lab. A total of 688 family members from 271 extended twin families participated. Figure 1.1 depicts the age distribution of the complete sample showing it actually consisted of two cohorts: a young adult cohort with a mean of 26.2 (SD 4.19) years of age and an older adult cohort with a mean around 50.4 (SD 7.51) years of age. Allocation of a family member to one of the two cohorts (young cohort under 36 years of age, older cohort above 36 years of age) was based on the age of the twins. There was a slight overlap in age of the non-twin siblings between the two cohorts.

Tables 1.2a and 1.2b list the complete sample configuration in families (Table 1.2a) and in subjects (Table 1.2b). For example, in the young cohort 20 MZ families consisting of a complete MZ pair and one additional sibling participated.

Participating family members ranged from one to eight with an average of 2.5 subjects per family. In the young cohort 171 males and 210 females participated, in the older cohort 135 and 172 respectively.

Table 1.2a
*Family configuration (in **families**) in the sample according to zygosity, age cohort, and number of additional non-twin siblings.*

		Number of additional siblings						
		0	1	2	3	4	6	
Young cohort								
MZ	twin pair	31	20	2	1	-	-	Total MZ pairs: 54
	single twin	1	3	-	-	-	-	
DZ	twin pair	16	24	7	-	-	-	Total DZ pairs: 47
	single twin	1	4	1	-	-	-	
DOS	twin pair	11	12	2	1	-	-	Total DOS pairs: 26
	single twin	2	4	1	-	1	-	
no twins		-	2	2	-	-	-	
Total Young		62	69	15	2	1	-	Total additional siblings: 109
Older cohort								
MZ	twin pair	26	16	4	1	-	1	Total MZ pairs: 48
	single twin	2	3	-	-	1	-	
DZ	twin pair	20	15	1	-	-	-	Total DZ pairs: 36
	single twin	3	1	2	-	-	-	
DOS	twin pair	11	8	2	-	1	-	Total DOS pairs: 22
	single twin	2	1	-	-	-	-	
no twins		-	1	-	-	-	-	
Total Older		64	45	9	1	2	1	Total additional siblings: 80
Total	271	126	114	24	3	3	1	

Table 1.2b
*Family configuration (in **subjects**) in the sample according to zygosity, age cohort, and number of additional non-twin siblings.*

		Number of additional siblings					
		0	1	2	3	4	6
Young cohort							
MZ	twin pair	62	60	8	5	-	-
	single twin	1	6	-	-	-	-
DZ	twin pair	32	72	28	-	-	-
	single twin	1	8	3	-	-	-
DOS	twin pair	22	36	8	5	-	-
	single twin	2	8	3	-	5	-
no twins			2	4	-	-	-
Total Young		120	192	54	10	5	-
Older cohort							
MZ	twin pair	52	48	16	5	-	8
	single twin	2	6	-	-	5	-
DZ	twin pair	40	45	4	-	-	-
	single twin	3	2	6	-	-	-
DOS	twin pair	22	24	8	-	6	-
	single twin	2	2	-	-	-	-
no twins		-	1	-	-	-	-
Total Older		121	128	34	5	11	8
Total	688	241	320	88	15	16	8

Note for Tables 1.2a and 1.2b: MZ = monozygotic twins, DZ = dizygotic same sex twins, DOS = dizygotic opposite sex twins.
Example: in the young cohort 24 families consisting of a full DZ pair and one additional sibling participated (72 subjects). In the complete sample 114 families consisting of one additional sibling and either a complete or an incomplete twin pair participated.

Table 1.3 lists the specific distribution of sex, age, educational level and zygosity groups within the two cohorts. The Dutch classification system for education level (Standaard Onderwijs Indeling, 1998) follows the International Standard Classification of Education (ISCED, 1997). The Dutch standard has 7 categories, ranging from primary education (category 1) until tertiary education (category 7). The average SOI educational level was 4.21 (SD 1.05), meaning that on average subjects received schooling until 16 years of age, which is compatible with the general Dutch population (CBS, 2000).

Table 1.3
Descriptives (age, education) of the two cohorts by zygosity and sex

	Subjects	Age range (years)	Mean age (SD)	Education (SOI categories)
Young cohort				
MZM	50	22.4 - 33.9	26.0 (3.07)	4.6 (1.14)
MZF	62	22.5 - 33.9	25.5 (3.42)	4.1 (0.93)
DZM	38	21.8 - 30.0	26.0 (2.13)	4.5 (0.76)
DZF	62	22.5 - 33.4	25.8 (2.72)	4.7 (0.92)
DOS	60	18.8 - 31.8	25.4 (2.87)	4.4 (0.85)
Add. sibs - males	54	13.9 - 42.6	27.3 (6.67)	4.0 (1.02)
Add. sibs - females	55	16.7 - 39.3	27.3 (5.85)	4.5 (1.03)
Total young cohort	381	13.9 - 42.6	26.2 (4.19)	4.4 (0.95)
Older cohort				
MZM	48	36.0 - 69.1	49.1 (6.92)	4.3 (1.09)
MZF	53	42.2 - 67.4	52.5 (7.80)	3.8 (0.96)
DZM	26	42.7 – 64.1	52.4 (5.07)	4.3 (1.37)
DZF	52	42.1 - 62.7	50.5 (6.21)	3.7 (1.09)
DOS	47	41.6 – 71.0	49.8 (7.98)	4.2 (1.09)
Add. sibs - males	37	37.0 - 68.4	50.8 (8.48)	4.3 (1.09)
Add. sibs - females	44	29.1 - 70.9	48.3 (8.50)	3.6 (0.97)
Total older cohort	307	29.1 – 71.0	50.4 (7.51)	4.0 (1.11)

Note: SOI = Dutch standard classification system; Add. sibs = additional non-twin siblings

The subjects in the young cohort had a significantly higher average education category (mean 4.4; SD 1.03) than subjects in the older cohort (mean 4.0; SD 1.04). The same was true for males (mean 4.3; SD 1.04) and females (mean 4.1; SD 1.03). This pattern was also compatible with males/females of different age cohorts in the general Dutch population (CBS, 2000).

Chapter 2

Genetic variation

'Genetic variability explains 70% of the population variation in a certain trait' is an often-heard outcome of studies in the field of quantitative genetics. However, it does not imply that the specific genes that influence the trait have already been identified. Given the rapid advancements made in molecular biology (Nature Genome Issue, February 15 2001; Science Genome Issue, February 16, 2001) and the development of sophisticated statistical genetic methods (e.g. Fulker, *et al.*, 1999; Zhao, 2000; Terwilliger and Goring, 2000), the identification of specific genes, even for complex traits, becomes a realistic goal of quantitative genetic analyses. Knowledge of transmission of genes, how they affect traits and how they interact with environmental factors, may help to identify these genes.

Prerequisite to understand phenotypic variance are two fundamental principles: the law of segregation and the law of independent assortment. The law of segregation states that for each trait there are two discrete elements of inheritance that separate during gameto-genesis and recombine randomly at fertilization, such that offspring receive one element from each parent (Mendel, 1865). The law of independent assortment states that the elements of inheritance for one character assort independently of the elements for other characters. Since the discovery of DNA (Watson and Crick, 1953a, 1953b) it is known that the element of inheritance consists of a deoxyribonucleic acid (DNA), which has the form of a double helix. DNA is organized in *chromosomes*, and each individual has two copies of a chromosome. Humans have 23 pairs of chromosomes. The unit of inheritance is the *gene*, characterized by a specific location on a chromosome. A gene may have different forms of appearance called *alleles*. Each individual has two copies, that is two alleles of a gene. During transmission each offspring receives one allele from each parent. If a gene exists in only one form (i.e. there is only one allele for this gene) there is no genotypic variation in this gene and there will not be any phenotypic variation associated with this gene.

Mendel observed discrete phenotypes such as a yellow or green seed colour and concluded on the basis of his experiments that discrete units of inheritance must be responsible for the existence of discrete phenotypes. Although the unit of inheritance is in fact 'discrete', the study of complex traits is not concerned with traits that have readily discernible phenotypes, but instead concerns traits that show a continuous range of variation. Fisher (1918) extended Mendel's single / two locus system to a multi-locus system and analytically showed how the discrete segregation of alleles can lead to a continuous range of measured traits by summing over the genetic effects of all contributing loci, and possibly also including interaction between loci (Fisher, 1918; Philips, 1998).

Continuous, observed variation may be attributed to genetic variation and environmental variation. Environmental variation results in variation in the phenotype when different aspects of the environment have differential effects on that phenotype. Genotypic variation causes phenotypic variation when different alleles of a gene differentially affect the phenotype. Human individuals differ from one another by about one base pair per thousand. Only 1.1% to 1.4% of the total genome is sequence that codes for protein. Differences within coding regions ultimately cause phenotypic variation in a trait. Quantifying the effects of DNA differences, or more specifically, the differential effects of different alleles of the same gene, is the basis of the biometrical model that underlies quantitative genetic analysis.

Studies in human quantitative genetics, including the chapters of this thesis, describe the decomposition of observed, phenotypic variance into sources of genetic origin and sources of environmental origin. These two sources of variance can be separated using a design that includes subjects of different degrees of genetic relationship. Resemblance between relatives is a function of the degree to which phenotypic expression is determined by shared genes, shared environment and random environmental factors (Lynch and Walsh, 1998).

A popular and widely used design in quantitative genetics is the twin design: the phenotypic resemblance of a certain trait between identical twins is compared to the phenotypic resemblance on that trait between fraternal twins. Since identical twins living at home share 100% of their family environment and 100% of their genes (but see Martin, Boomsma, and Machin, 1997), any resemblance between them is attributed to these two sources of resemblance. The extent to which identical twins do not resemble each other is ascribed to factors that identical twins do not share, the so-called unique or non-shared environmental factors, which also include measurement error. Resemblance between fraternal twins is also ascribed to the sharing of the family environment between fraternal twins (100%), and to the sharing of genes. However, fraternal twins share on average only 50% of their segregating genes, so any resemblance between them due to genetic influences will be lower than for MZ's. The extent to which fraternal twins do not resemble each other is due to non-shared environmental factors and to non-shared genetic influences.

Genetic effects at one single locus can be additive (i.e. the effect of one allele is added to the effect of another allele) or dominant (the two alleles show an interaction effect), or a combination. The total genetic influences on a trait are the result of the summed effects at multiple loci, plus the interaction effects between multiple loci (epistasis; Bateson, 1908, 1909). Depending on the mode of gene action, the expectation for the phenotypic resemblance between fraternal twins due to genetic influences will differ. If all contributing loci act additively and there is no interaction between them, the similarity for genetic effects for fraternal twins is on average 50%. However, if some loci act in a dominant way the genetic similarity is 25% for dominant genetic influence and 50% for additive genetic influences. The presence of dominant gene action thus reduces the expected phenotypic resemblance in fraternal twins. Epistasis reduces this similarity even further, and to what extent depends on the number of loci involved and their relative effect on the phenotype. In Table 2.1 the similarities for different types of familial relationships are given in terms of

additive genetic influences, dominant genetic influences, shared environmental influences and non-shared environmental influences.

Table 2.1
Similarities for additive genetic influences, dominant genetic influences, shared environmental influences and non-shared environmental influences, for different familial relationships.

	Additive genetic	Dominant genetic	Shared environment	Non-shared environment
MZ twins RT	100%	100%	100%	0%
DZ twins RT	~50%	~25%	100%	0%
Sib-pairs RT	~50%	~25%	100%	0%
MZ twins RA	100%	100%	0%	0%
DZ twins RA	~50%	~25%	0%	0%
Sib-pairs RA	~50%	~25%	0%	0%
Adopted siblings	0%	0%	100%	0%
Parents-children	50%	0%	0%	0%
Grandparents-grandchildren	~25%	0%	0%	0%

~ on average; MZ = monozygotic; DZ = dizygotic; RT = reared together ; RA = reared apart.

Employing a design including for example MZ and DZ twins reared together, allows decomposition of the phenotypic variance into components of additive genetic variance, dominant genetic variance or shared environmental variance, and non-shared environmental variance. Dominant genetic influences and shared environmental influences cannot be estimated at the same time in a twin design (i.e. including MZ and DZ twins).

Similarity between two (familiarly related) individuals can be quantified by covariances or correlations. A first estimate of the proportion of additive genetic influences of the total variance is given by twice the difference between the MZ and DZ correlations. The proportional contribution of the dominant genetic influences is obtained by subtracting four times the DZ correlation from twice the MZ correlation. An estimate of the proportional contribution of the shared environmental influences to the phenotypic variation is given by subtracting the MZ correlation from twice the DZ correlation. The proportional contribution of the non-shared environmental influences can be obtained by subtracting the MZ correlation from 1. This intuitively simple principle is described in every textbook on quantitative genetics and can be understood without knowledge of the relative effects and location of the actual genes that influence a trait, that is the genotypic effects on phenotypic means. However, knowledge of the underlying biometrical model becomes crucial when one wants to move beyond heritability estimates derived from twin correlations.

Genotypic effects on phenotypic means

Within a population many different alleles may exist for a gene (e.g. Lackner *et al.*, 1991), but for simplicity a gene with only two possible alleles, allele *A1* and allele *A2*, is considered. By convention, allele *A1* has a frequency p, while allele A2 has frequency q, and $p + q = 1$. With two alleles there are three possible genotypes: *A1A1*, *A1A2*, and *A2A2* with genotypic frequencies p^2, $2pq$, and q^2 respectively. The genotypic effect on the phenotypic trait (i.e. the genotypic value) of genotype *A1A1* , is called "a" (which by convention is the increasing effect), the effect of genotype *A1A2* "d", and the effect of genotype *A2A2* "$-a$". If allele *A1* is completely dominant over allele *A2*, effect d equals effect a. If the two alleles produce three discernable phenotypes of the trait, d is unequal to a. (see Figure 2.1 and Table 2.2).

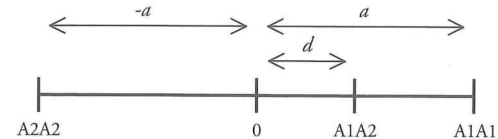

Figure 2.1
Graphical illustration of the genotypic values for a diallelic locus.

Table 2.2
Frequencies and genetic values of three genotypes A1A1, A1A2, A2A2.

Genotype	A1A1	A1A2	A2A2
Frequency	p^2	$2pq$	q^2
Genotypic value	a	d	$-a$
Frequency x value	p^2a	$2pqd$	$-q^2a$

The genotypic contribution to the population mean of the trait is the sum of the products of the frequencies and the genotypic values of the three different genotypes (Falconer and Mackay, 1996):

$$\text{Mean effect} = a\,p^2 + 2pqd - a\,q^2 = a\,(p-q) + 2pqd \qquad \textit{(Equation 2.1)}$$

The contribution of this locus to the population mean consists of two components: $a(p-q)$, which is the contribution of the homozygotes, and $2pqd$, which is the contribution of the heterozygotes. If there is no dominance, i.e. d equals zero, the second component is zero and the mean is a direct function of the allele frequencies. If d equals a, which is defined as complete dominance, the population mean becomes

a function of the square of the allele frequencies; substituting *d* for *a* gives $a(p-q) + 2pqa$, which simplifies to $a(1-2q^2)$.

Complex traits such as cognition, are assumed to be influenced by the effects of multiple loci. Assuming additive combination effects of all of these loci, the expectation for the population mean (μ) is the sum of the contributions of the separate loci, and is formally expressed as (see Falconer and Mackay, 1996);

$$\mu = \sum a(p-q) + 2\sum dpq \qquad \text{(Equation 2.2)}$$

To quantify the transmission of genetic effects from parents to offspring, and ultimately to decompose the observed variance in the offspring generation into genetic and environmental components, the concepts *average effect* and *breeding value* are needed. Parents transmit alleles to their offspring and not their genotypes. Therefore, they cannot transmit the values *a, d,* and *–a* to their offspring directly.

Average effects are a function of genotypic values and allele frequencies within a population. The average effect is defined as " .. *the mean deviation from the population mean of individuals which received that allele from one parent, the allele received from the other parent having come at random from the population*" (Falconer and Mackay, 1996). To calculate the average effects α_1 and α_2 of alleles A1 and A2 respectively, the frequency of the A1 or A2 alleles in the genotypes of the offspring coming from a single parent, need to be determined. Again assume a single locus system with two alleles for simplicity. If there is random mating between gametes carrying the A1 allele and gametes from the population, the frequency with which the A1 gamete unites with a gamete containing A1 (producing an A1A1 genotype in the offspring) equals *p*, and the frequency with which the gamete containing the A1 gamete unites with a gamete carrying A2 (producing an A1A2 genotype in the offspring) is *q*. The genotypic value of the genotype A1A1 in the offspring is *a* and the genotypic value of A1A2 in the offspring is *d*, as defined earlier. The mean value of the genotypes that can be produced by a gamete carrying the A1 allele equals the sum of the products of the frequency and the genotypic value. Or in other terms, it is $pa + qd$. The average genetic effect of allele A1 (α_1) equals the deviation of the mean value of all possible genotypes that can be produced by gametes carrying the A1 allele from the population mean. The population mean has been derived earlier as $a(p-q) + 2pqd$ (Equation 2.1). The average effect of allele A1 is thus (see Falconer and Mackay, 1996):

$$\begin{aligned}\alpha_1 &= pa + qd - [a(p-q) + 2pqd] \\ &= q[a + d(q-p)] \qquad \text{(Equation 2.3)}\end{aligned}$$

Similarly the average effect of the A2 allele is;

$$\begin{aligned}\alpha_2 &= pd - qa - [a(p-q) + 2pqd] \\ &= -p[a + d(q-p)] \qquad \text{(Equation 2.4)}\end{aligned}$$

For a single diallelic locus it is more convenient to express the average effects of alleles A1 and A2 in terms of the *average effect of gene*[1] *substitution*, which is the effect on the genotypic values of changing randomly chosen A1 alleles into A2 alleles. The *average effect of gene substitution* (α) can be obtained by calculating the difference of the average effects of the A1 and A2 alleles (Falconer and Mackay, 1996). Thus,

$$\begin{aligned}\alpha &= \alpha_1 - \alpha_2 \\ &= [a + d(q-p)] \qquad \text{(Equation 2.5)}\end{aligned}$$

Consequently, α_1 and α_2 can be expressed in terms of α; $\alpha_1 = q\alpha$ and $\alpha_2 = -p\alpha$.

The *breeding value* is the value of an individual derived from the mean value of its offspring, and it is equal to the sum of the average effects of the alleles it carries. Thus, the breeding value for an individual with genotype A1A1 is $2\alpha_1$ (or $2q\alpha$) , of individuals with genotype A1A2 it is $\alpha_1 + \alpha_2$ (or $(q-p)\,\alpha$) , and of individuals with genotype A2A2 it is $2\alpha_2$ (or $-2p\alpha$).

As the breeding value is expressed in terms of the average allele effects which are in turn expressed in terms of deviations from the population mean, the mean breeding value (that is the sum of the products of the genotypic frequencies and the breeding values) is by definition zero.

The breeding value is usually referred to as the *additive effect* of an allele, and the differences between the genotypic effects (in terms of *a, d* and *–a,* for genotypes A1A1, A1A2, A2A2 respectively) and the breeding values ($2q\alpha$, $(q-p)\,\alpha$, $-2p\alpha$, for genotypes A1A1, A1A2, A2A2 respectively), reflect the dominance deviations. In other words, the genotypic value consists of the breeding value and the dominance deviations.

The breeding values are, as stated earlier, expressed in terms of deviations from the population mean. This can also be done for the dominance deviations and for the genotypic values. As the dominance deviation is the difference between the genotypic value and the breeding value, it is convenient to calculate the genotypic value in terms of deviations from the population mean and then to subtract the breeding

[1] Falconer and Mackay (1996) use the term 'average effect of *gene* substitution'. Their use of 'gene' equals our use of 'allele', and it would be more logical to use the term 'average effect of *allele* substitution'. However, as others have adapted Falconer and Mackay's use of the term, we choose to adhere to their term.

value from the genotypic value to obtain the dominance deviations in terms of deviations from the population mean.

For genotype A1A1 the genotypic effect was assigned to be a. The population mean was calculated to be $[a(p-q)+2dpq]$ (Equation 2.1). The genotypic effect of genotype A1A1 in terms of a deviation from the population mean is thus;

$$\text{Genotypic effect (A1A1)} = a - [a(p-q) + 2dpq] = 2q(a-dp) \qquad \textit{(Equation 2.6)}$$

Similarly, the genotypic effect of genotype A1A2 in terms of a deviation from the population mean becomes

$$\text{Genotypic effect (A1A2)} = d - [a(p-q) + 2dpq] = a(q-p) + d(1-2pq) \qquad \textit{(Equation 2.7)}$$

And for genotype A2A2;

$$\text{Genotypic effect (A2A2)} = -a - [a(p-q) + 2dpq] = -2p(a+dq) \qquad \textit{(Equation 2.8)}$$

When the a in Equations 2.6 to 2.8 is substituted by $\alpha - d(q-p)$, the genotypic effects can also be written in terms of the average effect of gene substitution: for A1A1 this becomes $2q(\alpha - dq)$, for A1A2 this becomes $(q-p)\alpha + 2pqd$, and for A2A2 $-2p(\alpha + dp)$. Subtracting the breeding values from these terms gives the dominance deviations in terms of deviations from the population mean.

The above is summarized in Table 2.3

Table 2.3
Summary of genotypic values, frequencies, breeding values and dominance deviation for three genotypes A1A1, A1A2, and A2A2.

Genotype	A1A1	A1A2	A2A2
Genotypic value	a	d	$-a$
Frequency	p^2	$2pq$	q^2
Deviation from the population mean	$2q(a-dp)$	$a(q-p)+d(1-2pq)$	$-2p(a+dq)$
Deviation from the population mean in terms of breeding value	$2q(\alpha-dq)$	$(q-p)\alpha+2dpq$	$-2p(\alpha+dp)$
Breeding value	$2q\alpha$	$(q-p)\alpha$	$-2p\alpha$
Dominance deviation	$-2q^2d$	$2dpq$	$-2p^2d$

Adapted from Table 7.3 in Falconer and Mackay (1996).

Decomposition of phenotypic variance

So far the genotypic contributions to the population mean have been discussed, and effects of the environment on the population mean have been ignored. In reality the phenotype (P) is a function of genetic (G) and environmental effects (E); $P = G + E$, where E refers to the environmental deviations (Falconer and Mackay, 1996). This equation does not include the term GxE, and thereby assumes no interaction between the genetic effects and the environmental effects (see section on GxE interaction and GE-correlation).

The variance of the phenotype, which is defined by G + E, is given by $V_P = V_G + V_E + 2cov_{GE}$. V_P represents the variance of the phenotypic value, V_G represents the variance of the genotypic values, V_E represents the variance of the environmental deviations, and $2cov_{GE}$ represents twice the covariation between G and E, and reflects the presence of a gene-by-environment (GE)-correlation. As GE-correlation can only be modelled either in a parent-offspring design (Fulker, 1988) or when the specific genetic and environmental factors have been measured (see also section on GxE interaction and GE-correlation), for simplicity it is assumed that $V_P = V_G + V_E$. The total genetic variance (V_G) can be obtained by using the standard formula for the variance :

$$\sigma^2 = \sum f_i (x_i - \mu)^2 \qquad \textit{(Equation 2.9)}$$

where f_i denotes the frequency of genotype i, x_i denotes the corresponding mean of that genotype and μ denotes the population mean. The deviation from the population mean for each genotype of a diallelic locus was calculated earlier (Table 2.3). The expression for the total genetic variance is

$$V_G = p^2[2q(a-dp)]^2 + 2pq[a(q-p) + d(1-2pq)]^2 + q^2[-2p(a+dq)]^2 . \qquad \textit{(Equation 2.10)}$$

As the genetic effect consists of the additive effect and the dominance effect (G = A + D), the total genetic variance is a function of the additive genetic variance, the variance of the dominance deviations and twice the covariance between them.

$$V_G = V_A + V_D + 2cov_{AD} \qquad \textit{(Equation 2.11)}$$

It can be shown that the cov_{AD} equals zero (by summing over the products of the frequency, the breeding value and the dominance deviations as given in Table 2.3). Thus $V_A + V_D$ are calculated separately, and are summed to obtain V_G, giving a simpler expression for V_G:

$$V_G = V_A + V_D$$
$$= 2pq[a + d(q - p)]^2 + (2pqd)^2 \quad \textit{(Equation 2.12)}$$

which is derived from Equations 2.13 and 2.14 (below).

The genetic variance V_A is the variance of the breeding values, which are already expressed in terms of a deviation from the population mean (i.e. the mean of the breeding values is zero). Thus, squaring these, multiplying them by their corresponding frequency, and summing over all genotypic categories gives the contribution of that locus to the 'additive genetic variance' (V_A) of the trait;

$$V_A = p^2(2q\alpha)^2 + 2pq[(q - p)\,\alpha]^2 + q^2(-2p\alpha)^2$$
$$= 2pq\alpha^2 \quad \textit{(Equation 2.13)}$$

As α equals $[a + d(q - p)]$, V_A can also be expressed in terms of *a* and *d*, as $2pq[a + d(q - p)]^2$.

The genetic variance V_D is the variance of the dominance deviations.

$$V_D = p^2(4q^4d^2) + 2pq(4p^2q^2d^2) + q^2(4p^4d^2)$$
$$= (2pqd)^2 \quad \textit{(Equation 2.14)}$$

Combining Equations 2.13 and 2.14 gives Equation 2.12. If the phenotypic value of the heterozygous genotype lies midway between A1A1 and A2A2 (i.e. the effect of *d* equals zero), the total genetic variance simplifies to $2pqa^2$. If *d* is not equal to zero, the 'additive' genetic variance component contains the effect of *d*. Even in the absence of *a* (i.e. $a = 0$), but in the presence of *d*, V_A is greater than zero (except when $p = q$). Thus, although V_A represents the variance due to the additive influences, it is not only a function of *p*, *q*, and *a*, but also of *d*, as it is derived from the *breeding values*, which were shown to be a function of both *a* and *d*.

The consequences are that, except in the rare situation where all contributing loci are diallelic with $p = q$ and $a = 0$, V_A can never be zero when V_D is greater than zero. Models that decompose the phenotypic variance into components of V_D and V_E only, are therefore biologically implausible.

When more than one locus is involved and it is assumed that the effects of these loci are independent (i.e. no epistasis), the V_G's of each individual locus may be summed to obtain the total genetic variances of all loci that influence a trait (Fisher, 1918; Mather, 1949).

In quantitative genetic studies, results are often reported in terms of the heritability of a trait. The heritability is the proportion of the genetic variance relative

to the phenotypic variance. There are two different meanings of heritability: when the total genetic effects on a trait are measured, heritability is expressed as the ratio of V_G to V_P, or ($V_A + V_D$) to V_P (broad sense heritability). However, the variation of the effects expressed in V_D is not transmitted from parents to offspring and therefore some authors prefer to report heritability as the ratio of V_A to V_P (sometimes termed 'heredity', but more often referred to as narrow sense heritability).

To decompose the observed variance into components of V_A, V_D, and V_E a genetically informative design is needed, such as the twin design. By comparing the observed resemblance in MZ twins and DZ twins, V_A, V_D, and V_E can be separated. MZ twins are genetically identical and the expectation for their covariance is:

$$COV_{MZ} = V_A + V_D \quad \textit{(Equation 2.15)}$$

The expectation for DZ twins is less straightforward: as DZ twins share on average half of their alleles, they share half of the genetic variance that is transmitted from the parents, i.e. ½ V_A. As V_D is not transmitted from parents to offspring it is less obvious to determine the coefficient of sharing for the dominance deviations. If two members of a DZ twin pair share both of their alleles at a single locus they will have the same coefficient for *d*. If they share no alleles or just one parental allele they will have no similarity for the effect of *d*. In other words, the probability that two members of a DZ pair have received the same alleles from both parents is the coefficient of similarity for *d* between them. There is a probability of ½ that two siblings (or DZ twins) receive the same allele from their father, and there is a probability of ½ that they have received the same allele from their mother.

Thus, the probability that they have received the same two ancestral alleles is ½ x ½ = ¼, and the expectation for the covariance in DZ twins is

$$COV_{DZ} = \tfrac{1}{2} V_A + \tfrac{1}{4} V_D \quad \textit{(Equation 2.16)}$$

GxE interaction and GE-correlation

In the above the absence of genes x environment interaction was assumed. GxE interaction occurs when the effects of the environment are conditional on an individual's genotype, such as when some genotypes are more sensitive to environment influences than other genotypes. Genetic studies on crops and animal breeding experiments have shown that GxE interaction is extremely common (see summary in Lynch and Walsh, 1998. pages 657 - 686). However, in general GxE interaction accounts for less than 20% of the variance of a trait in the population (Eaves *et al.*, 1977; Eaves, 1984). In theory (and in practice in animal and crop breeding experiments) GxE interaction can be measured by studying the same trait in

two environments. A genetic correlation between the two measurements of the trait that is less than one indicates the presence of GxE interaction (Falconer, 1952). (The reverse, however, a genetic correlation equal to one does not need to imply the absence of GxE interaction; see Lynch and Walsh, 1998). In human quantitative genetic analyses it is often not possible to control the environmental or genetic influences, unless the specific genotype and specific environmental factors are explicitly measured (see Kendler and Eaves, 1986; Dick *et al.,* 2001; Rose *et al.,* 2001). The presence of GxE interaction may be explored by correlating the MZ intra-pair differences and MZ pair sums (Jinks and Fulker, 1970). Assuming that MZ twin similarity is purely genetic, a relation between MZ means and standard deviations suggests the presence of GxE interaction.

GxE interaction is often not included in quantitative genetic models. However, if the true world does include GxE interaction, assuming its absence may lead to biased estimates of G and E (Eaves *et al.*, 1977). For example if GxE interaction was truly gene by non-shared environment interaction, a model without GxE interaction will result in overestimation of the effects of the non-shared environment. If, however, GxE interaction was interaction between genes and shared environmental influences, assuming its absence will result in overestimation of the effect of genes on the phenotype, as well as in overestimation of the influence of the shared environmental on the phenotype. The separate detection of these two biased effects in the presence of genes by shared environmental interaction necessitates the inclusion of twin pairs reared apart (Jinks and Fulker, 1970; Eaves *et al.*, 1977).

The absence of a genes - environment *correlation* was also assumed in the above. GE-correlation occurs when the genotypic values and environmental values are correlated. Three different forms of GE-correlation have been described (Plomin, DeFries and Loehlin, 1977; Scarr and McCartney, 1983). Active GE-correlation is the situation where subjects of a certain genotype actively select environments that are correlated with that genotype. Reactive GE-correlation refers to the effects of reactions from the environment evoked by an individuals' genotype. The presence of GE-correlation leads to an increase in the phenotypic variance. It is difficult to measure GE-correlation, however, as active and reactive GE-correlation necessitate the direct measurement of these influences (Falconer and Mackay, 1996). Falconer and Mackay (1996) state that GE-correlation is best regarded as part of the genetic variance because ".. the non-random aspects of the environment are a consequence of the genotypic value ..".

Passive GE-correlation refers to the situation where parents transmit both their genes and an environment (cultural transmission) which both influence a certain trait (Eaves *et al.*, 1977). Effects of cultural transmission can be measured using a parent-offspring design (Fulker, 1988).

Assortative mating may also lead to GE-correlation. Assortative mating occurs when mate selection is based on a certain phenotype (P; which in turn is a function

of G and E), and tends to increase the resemblance between offspring which leads to an increase in the additive genetic variation (and therefore in the overall phenotypic variation) (Lynch and Walsh, 1998). Statistically, it may conceal the presence of non-additive genetic effects and overestimate the influence of additive genetic factors (Carey, 2002). Assortative mating is known to exist for intelligence (Vandenberg, 1972). In the present thesis parents were not included in the sample, and the possible effects of assortative mating were not included in the models.

Path analysis and Structural Equation Modelling

The expectations for the resemblance between MZ twins and DZ twins or sib pairs reared together (Equations 2.15 and 2.16) can be summarized in a path diagram (Wright, 1921). For simplicity, V_E reflects the non-shared environment, and does not include the variance due to the shared environmental influences. As stated earlier, influences of dominant genetic effects and shared environmental effects are confounded in the twin design. It was chosen to depict path diagrams including additive genetic influences (A), dominant genetic influences (D) and non-shared environmental influences (E). The dominant genetic influences may be substituted by shared environmental influences in the path diagram by substituting 0.25 (for the DZ correlation for dominant genetic influences) for 1.00 (for the DZ correlation for shared environmental influences).

The latent factors A, D, and E have a variance of 1.00. x, y, and z represent the respective path coefficients from A, D, and E to the phenotype P (x, y, and z are sometimes denoted by a, d and e. To avoid confusion with the genotypic values a and d, it is chosen to adhere to x, y and z). The path coefficients are standardized regression coefficients, similar to the factor loadings in factor analysis. The phenotypic variance (V_P) for the trait (the same for both members of a twin pair) equals $x^2 + y^2 + z^2$ which equals $V_A + V_D + V_E$.

Sewall Wright introduced path analysis to genetic analyses (1921, 1934) and - applying the tracing rules of path analysis - the covariance between DZ twins (and sib pairs) is traced as $0.50x^2 + 0.25y^2$, which equals ½ V_A + ¼ V_D (see Equation 2.16). The covariance between MZ twins is traced as $x^2 + y^2$ which equals $V_A + V_D$ (see Equation 2.15). Path analysis is directly related to matrix algebra and to structural equation modelling, and the latter can be implemented in statistical software for covariance structure analysis to estimate the variance components from real data in twin or family designs.

To rewrite the model depicted in Figure 2.2 in terms of matrix algebra we introduce three matrices X, Y, and Z of dimensions 1 x 1, containing the path coefficients x, y, and z, respectively. The matrix algebra notation for V_P is XX' + YY' + ZZ', where ' denotes the transpose of the matrix (and corresponds to tracing forwards through a path, see Neale and Cardon, 1992). The expectation for the MZ

covariance is XX' + YY' and the expectation for the DZ covariance is 0.5XX' + 0.25YY'. Including additional siblings in this design is straightforward: the expectation for sib pair covariance is also 0.5XX' + 0.25YY' (note: it can be tested whether the sib pair covariation really equals the DZ covariation: see below).

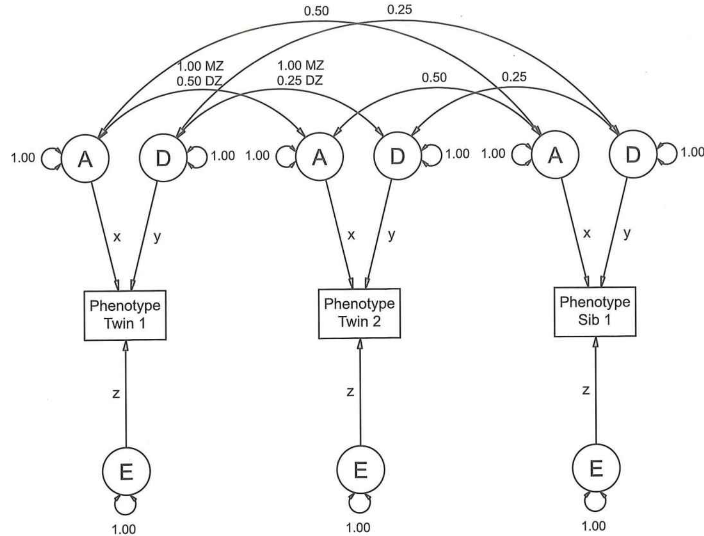

Figure 2.2
Path diagram representing the resemblance between MZ or DZ twins and one additional sibling, for additive genetic influences (A), dominant genetic influences (D) and non-shared environmental influences (E).

Multivariate Structural Equation Modelling

The model can also easily be extended to a multivariate model where more than one measurement per subject is available (Eaves and Gale, 1974; Martin and Eaves, 1977); Figure 2.3 is a path diagram for a bivariate design (two measurements per subject; four measurement for a pair). The corresponding matrix algebra terms for the expected variance and MZ or DZ covariances are the same as for the univariate situation, except that matrices X, Y, and Z are lower triangular and of dimensions n x n (where n is the number of variables assessed on a single subject). The subscripts of

the path coefficients correspond to matrix elements, i.e. x_{ij} denotes the matrix element on the i-th row, j-th column of matrix X. The path coefficients subscripted by $_{21}$ reflect the variation that both measured phenotypes have in common. For example, if the path denoted by x_{21} is not equal to zero, this suggests that there are some genes that influence both phenotypes.

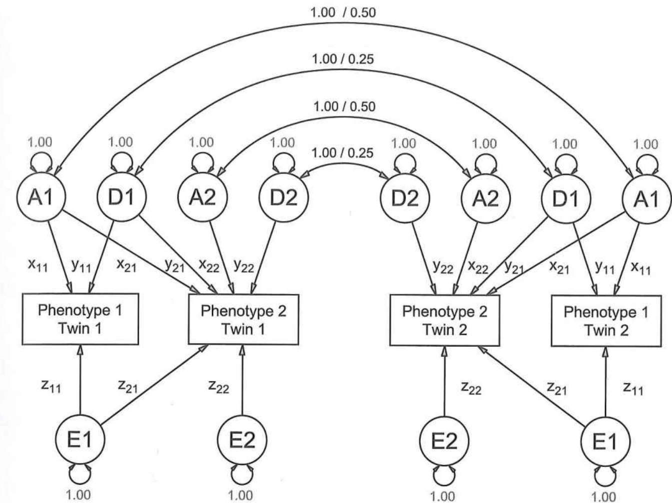

Figure 2.3
Path diagram representing the resemblance between MZ or DZ twins for additive genetic influences (A), dominant genetic influences (D) and non-shared environmental influences (E) in a bivariate design.

Thus, multivariate genetic designs allow the decomposition of an observed correlation between two variables into a genetic and an environmental part. This can be quantified by calculating the genetic and environmental correlations and the genetic and environmental contributions to the observed correlation.

In matrix algebra, matrices A (the product of matrix X and its transpose X'), D (the product of matrix Y and its transpose Y'), and E (the product of matrix Z and its transpose Z') can be calculated. Matrices A, D, and E are thus symmetric. This is also known as a Cholesky factorisation of matrices A, D and E which assures that these matrices are positive definite. The latter is necessary as matrices A, D and E are

variance-covariance matrices. They contain the additive genetic, dominance, and non-shared environmental variances respectively on the diagonals for variables 1 to n.

The *genetic correlation* between variables i and j (r_{gij}) is derived as the genetic covariance between variables i and j (denoted by element ij of matrix A; a_{ij}) divided by the square root of the product of the genetic variances of variables i (a_{ii}) and j (a_{jj});

$$r_{gij} = \frac{a_{ij}}{\sqrt{a_{ii} \times a_{jj}}} \qquad \textit{(Equation 2.17)}$$

Analogously, the *environmental correlation* (r_{eij}) between variables i and j is derived as the environmental covariance between variables i and j divided by the square root of the product of the environmental variances of variables i and j;

$$r_{eij} = \frac{e_{ij}}{\sqrt{e_{ii} \times e_{jj}}} \qquad \textit{(Equation 2.18)}$$

The phenotypic correlation r is the sum of the product of the genetic correlation and the square roots of the standardized genetic variances (i.e. the heritabilities) of the two phenotypes and the product of the environmental correlation and the square roots of the standardized environmental variances of the two phenotypes.

$$r = \underbrace{r_{gij} \times \sqrt{\frac{a_{ii}}{(a_{ii}+e_{ii})}} \times \sqrt{\frac{a_{jj}}{(a_{jj}+e_{jj})}}}_{\textit{genetic contribution}} + \underbrace{r_{eij} \times \sqrt{\frac{e_{ii}}{(a_{ii}+e_{ii})}} \times \sqrt{\frac{e_{jj}}{(a_{jj}+e_{jj})}}}_{\textit{environmental contribution}} .$$

(Equation 2.19)

The genetic contribution to the observed correlation between two traits is a function of the genetic correlation between the two sets of genes that influence the traits and the correlation between these sets of genes. A large genetic correlation does not imply a large phenotypic correlation, as the latter is also a function of the heritabilities. If these are low, the genetic contribution to the observed correlation will also be low.

If the genetic correlation is 1, the two sets of genes are identical. If the genetic correlation is less than 1, at least some genes are a member of both sets of genes. A large genetic correlation however, does not imply that the overlapping genes have effects of similar magnitude on each trait. The overlapping genes may even act additively for one trait and show dominance for the second trait. A genetic correlation less than 1 therefore cannot exclude that all of the genes are overlapping between the two traits (Carey, 1988). Similar reasoning applies to the environmental correlation.

Being a true 'correlation', genetic correlations do not provide information on the direction of causation. In fact, genes may influence one trait that in turn influences the second trait. Or, there may be genes that act in a pleiotropic way, that is they influence both traits but neither trait influences the other. Genetic correlations do not distinguish between these situations, but merely provide information on the nature of the causes of covariation between two traits.

Using the expectations for the variances and MZ / DZ covariance in terms of algebraic equations a structural equations / matrix algebra interpreter can be used to estimate the contributions of additive genetic, dominant genetic or shared environmental influences to the variation. A matrix algebra interpreter such as LISREL (Jöreskog and Sörbom, 1986) or Mx (Neale, 1997) uses structural equation modelling optimisation procedures to estimate population parameters from the observed data. A widely used optimisation procedure is *maximum likelihood*, where those parameter estimates are obtained that make the observed data most likely.

Structural equation modelling (SEM) has several advantages over merely comparing the MZ and DZ correlations (Eaves, 1969; Jinks and Fulker, 1970). Besides being less influenced by systematic differences in the variances of a trait by zygosity status, age or sex, SEM allows parameter *estimation*, while the correlational method merely allows parameter *calculation*. SEM thus also allows determination of confidence intervals and of standard errors of parameter estimates and quantifies how well the specified model describes the data. SEM also allows specification of (non)-scalar sex-limitation models, specification of (non)-scalar age-limitation models, and the inclusion of fixed effects of subpopulation e.g. according to SES or medication.

This is important as heritability is a characteristic of the (sub)population and may differ across sex or age. As heritability is defined as the ratio of genetic variability to total variability, it is also dependent on changes in the environmental variation. For example, if the absolute genetic contribution to the variance remains equal, but the absolute environmental contribution to the variance decreases (i.e. the environment may become more homogeneous), the heritability will increase (see for example Heath *et al.*, 1985). Across (sub)populations there may be different allele frequencies (Cavalli-Sforza, Menozzi, Piazza, 1994), different genotypic values, or different environmental contributions to the variance. Across sexes, ages or ethnicity there may be different loci that influence the same trait. Four kinds of models for populations in general and age and sex in particular may be evaluated using SEM: *i)* models including effects on the means as a function of e.g. sex or age; *ii)* scalar sex or age limitation models for the genetic or environmental variance or *iii)* scalar sex or age limitation models for the relative contributions of genetic or environmental variance to the phenotypic variance, or iv) non-scalar sex or age limitation models

The first effect can be tested by including sex and age as a fixed effect on the observed scores of a trait in the model, and then testing whether these effects are statistically different from zero. The second effect can be evaluated by testing whether the elements in matrices A, D, and E are equal across sexes or age cohorts. The third effect can be evaluated by equating the relative contributions of A, D, and E to the phenotypic variance across sexes and age cohorts. A difference in the relative contribution of for example additive genetic influences may exist as a result of a difference in the absolute contribution of these influences, or as a result of a difference in the absolute contributions of the other variance components. The last effects can be evaluated by comparing the opposite sex twins' correlation to the same-sex twins' correlation (for sex limitation), or by a smart extension of a crossectional design (Snieder, van Doornen and Boomsma, 1997). The data set used for this thesis exists of two age cohorts and includes both males and females, allowing all four effects of age and sex, except the non-scalar age limitation effect, to be evaluated.

SEM and linkage analyses

In addition to the above advantages, SEM can also easily include effects of measured genes or environmental factors. The effects of single loci may be detected using association or linkage analyses (Zhu *et al.* 1999; Neale *et al.*, 1999; Neale, 2000).

In association analyses the effects of alleles on a trait are tested, and are incorporated in the model as fixed effects on the means (Zhu *et al.* 1999; Neale *et al.*, 1999; Neale, 2000; Moxley *et al.*, 2002). Linkage analysis depends on the *co-segregation* (i.e. a violation of Mendel's law of independent assortment) of alleles of a marker and a trait locus (Ott, 1999). Non-parametric linkage analyses can be conducted using pairs of siblings of DZ twins, that share some regions of the genome, but not all. Sib-sharing of marker alleles can be quantified in terms of identical-by-descent (IBD) status for a sib pair, which denotes the number of alleles that have descended from the same ancestral allele and can be 0, 1, or 2 (Sham, 1998). IBD is distinct from identical-by-state (IBS), which denotes the number of alleles that two sibs have in common that are physically identical, but that have not necessarily been inherited from the same parent. MZ twins are by definition IBD 2 on every locus.

Table 2.4
IBD / IBS status from all possible sib pairings from parental mating type A1A2 (father) x A1A2 (mother).

		Sib 1			
		A1A1	A1A2	A2A1	A2A2
Sib 2	A1A1	2 / 2	1 / 1	1 / 1	0 / 0
	A1A2	1 / 1	2 / 2	0 / 2	1 / 1
	A2A1	1 / 1	0 / 2	2 / 2	1 / 1
	A2A2	0 / 0	1 / 1	1 / 1	2 / 2

Table 2.4 describes how all possible sib pairings in the offspring are related IBD / IBS. If a marker is linked to a trait locus, sib pairs of IBD status 2 are expected to show more phenotypic resemblance than sib pairs of IBD status 1, which in turn show more phenotypic resemblance than sib pairs of IBD status 0, assuming additive gene action (Haseman and Elston, 1972).

As IBD status is not always unambiguously known and must be estimated probabilistically from the specific allele pattern across chromosomes (haplotype) of two individuals, for each marker across the genome three probabilities per sib pair can be estimated: the probability that they are IBD status 0 (p_{IBD0}), the probability that they are IBD status 1 (p_{IBD1}), and the probability that they are IBD status 2 (p_{IBD2}). The sum of these probabilities equals 1. The proportion of alleles shared IBD at a particular marker site equals the correlation between the breeding values of that marker site for sib 1 and sib 2. The probability that a sib pair has IBD status 2 equals the correlation between the dominance values of that marker site between sib 1 and sib 2. The breeding values (or dominance deviations) of the marker are a function of the recombination fraction between the marker and the trait locus and the breeding values (or dominance deviations) of the trait locus. As the recombination fraction is unknown, this cannot be incorporated in a model. The path coefficients v and w (Figure 2.4) and the relative contribution of the factors *Am* and *Dm* to the phenotypic variation are thus a function of the recombination fraction between the marker and the trait locus and the magnitude of the genetic effects of the trait locus. Relatively small effects of the factors *Am* and *Dm* can thus either reflect a situation with small effects at the trait locus and a small recombination fraction (close to zero) or may reflect large effects at the trait locus in combination with a large (close to 0.5) recombination fraction between the marker and the trait locus.

The proportion of alleles shared IBD is called π, and the estimate of π is referred to as $\hat{\pi}$, which can be calculated as (Sham, 1998);

$$\hat{\pi} = \tfrac{1}{2}\, p_{IBD1} + p_{IBD2}, \qquad \textit{(Equation 2.21)}$$

We call the correlation between the dominance values of the marker site between sib 1 and sib 2 δ, the estimate of δ is referred to as $\hat{\delta}$ which can be calculated as

$$\hat{\delta} = p_{IBD2}, \qquad \textit{(Equation 2.22)}$$

This can be incorporated in a path diagram (Figure 2.4).

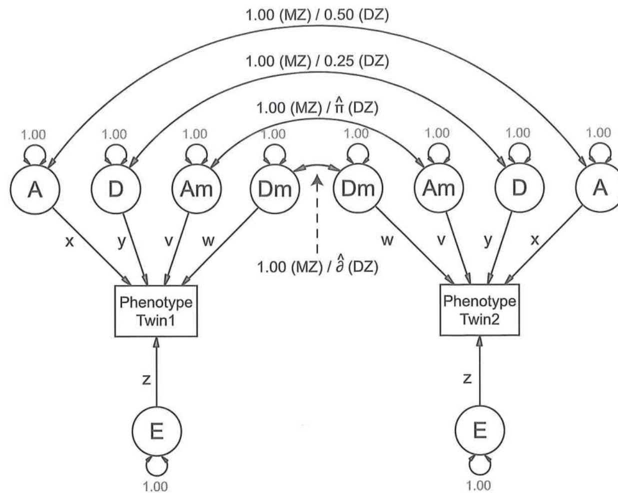

Figure 2.4
Path diagram representing the resemblance between MZ or DZ twins for background additive genetic influences (A), background dominant genetic influences (D), additive genetic influences due the marker site (Am), dominant genetic influences due to the marker site (Dm), and non-shared environmental influences (E) in a univariate design.

The expectation for the variance is in algebraic terms; $x^2 + y^2 + v^2 + w^2 + z^2$, the expectation for the covariance among MZ twins is $x^2 + y^2 + v^2 + w^2$, and for the covariance among DZ twins is $\frac{1}{2} x^2 + \frac{1}{4} y^2 + \pi v^2 + \delta w^2$. Dolan, Boomsma and Neale (1999) showed that a linkage design including sibship sizes greater than two was more powerful than designs including sib pairs (or DZ twins) only.

Extended twin design

A final advantage of SEM, most relevant to this thesis, is the easy extension from a univariate model to multivariate models, and the handling of missing data structures. The latter enables the relative easy incorporation of data from other family members, without the need to have the same number of family members available for each family. The inclusion of non-twin siblings (if available) will have positive effects on statistical power (see also Chapter three). As some families may consist of a twin pair

and six additional siblings and other families may consist of twins only or twins and one additional sibling, the correlational method cannot be applied to estimate genetic and environmental contributions to the variance. Fortunately, such non-rectangular data structures can be handled with ease using a SEM approach. The presence of non-twin siblings gives the opportunity to test all sorts of assumptions, such as whether the covariance between DZ twins equals the covariance between non-twin siblings (which is often assumed, but can now be tested), whether the means and variances in twins are similar to the means and variances observed in siblings, or whether twin-sib covariance is different from sib-sib covariance, across males and females.

In conclusion, in this thesis, the incorporation of the biometrical model underlying genetic variation into a SEM approach, allows the use of powerful extended twin designs in which parameter estimates of heritability and environmental influences can be evaluated. And ultimately, the specific effects of genes may be quantified in terms of *a*, *d*, and *–a*. Recently Fulker *et al.*, 1999 developed a method that allows the simultaneous analysis of linkage and association. This method has proven to be a most powerful tool in gene detection (e.g. Zhu *et al.*, 1999; Neale *et al.*, 1999). In Chapter eleven an extension of this model is incorporated within a SEM approach using the extended twin design.

Chapter 3

Statistical power in extended twin designs[1]

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A Note on the Statistical Power in Extended Twin Designs

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The power to detect sources of genetic and environmental variance varies with sample size, study design, effect size and the statistical significance level chosen. We explored whether the power of the classical twin study may be increased by adding non-twin siblings to the classical twin design. Sample sizes to detect genetic and shared environmental variation were compared for kinships with only twins, kinships consisting of twins and one additional sibling, and kinships with twins and two additional siblings. The effect of adding siblings to the classical twin design was considered for univariate and bivariate analyses.

For the univariate case, adding one non-twin sibling resulted in a decrease in sample size needed to detect additive genetic influences in the presence of environmental influences. However, adding two additional siblings did not decrease the number of subjects as compared to the classical twin design. The sample size required to detect common environmental factors was also greatly decreased by adding one non-twin sibling. Adding two non-twin siblings resulted in a small additional decrease. In models including additive genetic, dominant genetic, and unique environmental effects, adding one sibling to a twin family decreased the required sample size to detect dominant genetic influences. Adding two siblings to a twin family resulted in only a slight additional decrease in sample size.

In the bivariate case a similar pattern of results was found, in addition to the observation that the overall required sample size, as expected, was lower than in the univariate case. The decrease in sample size from bivariate testing was more pronounced in a design with one or two additional siblings, as compared to a design with twins only. It is concluded that a well considered choice of family design, i.e. including families with twins and one or two additional siblings increases the statistical power to detect sources of variance due to additive and non-additive genetic influences, and common environment.

KEY WORDS: Sample size; heritability; methodology; sibship size; twin study.

INTRODUCTION

Recent advances in molecular genetics have made it possible to partition genetic variance into sources due to particular genetic loci (quantitative trait loci's; QTL's) and sources due to background genetic variance (Fulker, Cherny, & Cardon, 1995; Fulker, Cherny, Sham, *et al.,*1999; Nance and Neale, 1989; Boomsma and Dolan, 1998). A necessary first step in mapping complex traits to QTL's is to establish the amount of genetic variation that underlies the phenotypic variation of the trait. If phenotypic variation in a trait is found to be caused in part by genetic sources, linkage and/or association studies can be conducted in order to characterize the effects of specific genetic loci on the phenotypic variation. If phenotypic variation is not found to be heritable, the search for effects of specific genetic loci will not be initiated. However, in some cases it may be concluded that phenotypic variance in

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a trait can not be ascribed to genes because the statistical power to detect sources of genetic variation is insufficient (Svikis, Velz & Pickens, 1994; Pickens, Svikis, McGue, Lykken, *et al.,* 1991). This will preclude further searching for effects of QTL's on that particular trait, even though such QTL's may be present.

The statistical power of quantitative genetic studies is influenced by the size of the effect (e.g. heritability), the sample size, the probability level (α) chosen, and the homogeneity of the sample (Neale and Cardon, 1992; Cohen, 1992; Tanaka, 1987). Increasing the sample size is the most common way to increase the statistical power of a study, but is often limited by resources of time and money. Another means to increase statistical power is the use of multivariate testing. In the context of structural equation modeling the statistical power to detect genetic effects rises as a (non-linear) function of multivariate testing under the condition that the measures are correlated (Schmitz, Cherny, and Fulker, 1998). In the context of partitioned twin analyses it has been shown that choosing a different (e.g. other than 1 to 1) MZ to DZ ratio influences statistical power such that an MZ to DZ ratio of 1 to 4 is optimal for partitioned twin analyses (Nance & Neale, 1989).

In the present paper we focus on increasing the statistical power of the classical twin study by adding non-twin siblings to MZ and DZ twin pairs. Since non-twin siblings share on average half of their segregating genes, just like DZ twins, adding non-twin siblings to the classical twin design may provide an efficient way to increase the power to detect sources of genetic and shared environmental variance. Adding two more siblings to a twin kinship provides five additional observed covariances, whereas adding a whole new family consisting of two siblings provides only one additional observed covariance. In the present paper we examine the effects of adding non-twin siblings to twin families on the estimated sample size needed to detect additive genetic (A) variance (V_a), dominant genetic (D) variance (V_d), and common environmental (C) variance (V_c), with a power of 80% in the context of structural equation modeling.

METHOD

We calculated covariance matrices for three experimental designs, which differed in family constitution. Design 1 included only MZ twins and DZ twins. Design 2 included families with MZ and DZ twins and one additional sibling. Design 3 included families with MZ and DZ twins and two additional siblings. For all three designs we calculated the sample size needed to detect an effect of interest with a power of 80%. The MZ twins to DZ twins ratio was 1 to 1 for all three designs (thus, the ratio MZ to 'non MZ sibpairs', is not 1 to 1 for all designs). It should be noted that we report sample size in subjects and not in twin pairs. The same number of subjects refers to different numbers of twin pairs and a different number of families for all three designs. We will use the terms 'highest power' and 'fewest subjects needed' to refer to an optimal design to detect sources of phenotypic variance.

All analyses were carried out using the statistical software package Mx (Neale, 1997). Estimation of parameters was obtained by normal theory maximum likelihood. Goodness of fit testing was based on the likelihood ratio tests. First univariate models were considered. In order to obtain the sample size needed to detect varying levels of additive genetic variance with a fixed power level of $(1 - \beta) = .80$, covariance matrices were calculated with sources of additive genetic variance (V_a) accounting for 10% to 90% of the phenotypic variance in the presence of sources of common environmental variance (V_c) accounting for 00%, 10%, and 20% of the variance. Remaining variance was attributed to unique environmental (E) sources of variance (V_e). To detect sources of V_c covariance matrices were calculated with V_c accounting for 10% to 90% of the phenotypic variance in the context of sources of V_a accounting for 00%, 10%, and 20% of the phenotypic variance. In addition, covariance matrices were calculated with sources of variation due to A, D (dominant genetic variance) and E. Only the situation in which dominance was 'complete' (V_a to V_d = 2 to 1; see appendix I) was considered. In the ADE-models the total genetic variance, i.e. V_a and V_d together accounted for 30% to 90% of the total phenotypic variance. For all situations, remaining variance was attributed to V_e.

Since non-twin siblings, like DZ twins, share on average half of their genes, expectations for non-twin sibling covariances were modeled similarly to expectations for DZ covariances.

In the ACE-models the expected phenotypic variance (σ^2) of twins and siblings is $V_a + V_c + V_e$, the expected MZ covariance $V_a + V_c$, and the expected DZ and sibling covariance $.5\ V_a + V_c$. In ADE-models, the expected phenotypic variance is $V_a + V_d + V_e$, the expected MZ covariance $V_a + V_d$, and the expected DZ and sibling covariance $.5\ V_a + .25\ V_d$.

It is known that the use of a multivariate phenotype, as opposed to a univariate phenotype, results in a gain of statistical power if the multivariate traits are correlated (Schmitz *et al.* 1998). To find out how much

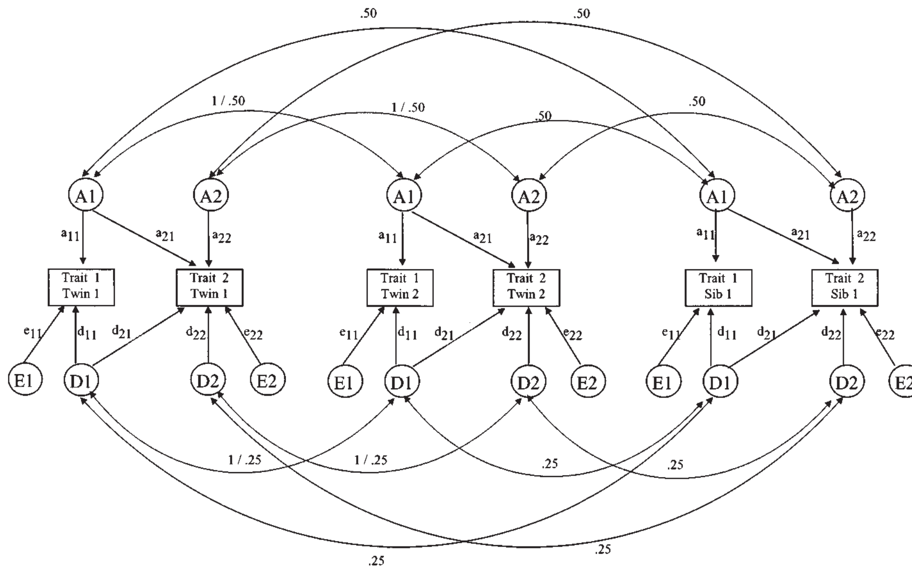


Fig. 1. Pathdiagram for the bivariate ADE-model, cholesky decomposition. Example for twins and one additional sibling, no unique environmental correlation (rE). The covariance between trait 1 and trait 2 is $(a_{11}*a_{21}) + (d_{11}*d_{21})$ and the correlation between trait 1 and trait 2 is $(a_{11}*a_{21}) + (d_{11}*d_{21})/\sqrt{(\sigma^2{}_1*\sigma^2{}_2)}$.

adding siblings *and* using a multivariate phenotype affects statistical power we also looked at several bivariate designs. We calculated covariance matrices for two traits with a phenotypic correlation of .50. Both traits could be influenced by A, C, and E or by A, D, and E. Total influences of sources of A, C or D, and E were uniform for each trait. The phenotypic correlation between the two traits could be due to additive genetic correlation (rA), dominant genetic correlation (rD), common environmental correlation (rC), or to unique environmental correlation (rE), depending on the specific situation that was considered. Figure 1 depicts the construction of covariance matrices for kinships consisting of twins and one additional sibling for a bivariate ADE-model (Cholesky decomposition) in which rE is absent and all phenotypic correlation is due to rA and rD. All latent variables have unit variance.

Power calculations were carried out by fitting the known model to the exact (population) covariance matrices as described in Neale and Cardon (1992). In models which contain a parameter which is known to be zero, the zero parameter can either be fixed at zero or freed (estimated) while computing the power to detect one of the other non-zero parameters. For example, when treating the ACE-model in which V_c is zero as an AE-model, the power to detect sources of variation due to A is significantly higher than when the ACE-model is treated as an ACE-model, i.e. with V_c estimated as a free parameter. In the power calculations the zero-parameter was estimated as a free parameter because we are interested in computing the power to detect V_a, in ACE-models, regardless of the value of V_c (and vice versa). The same reasoning applies to the bivariate calculations.

Constraining a certain set of parameters to zero and refitting the model provides the non-centrality parameter. From this non-centrality parameter the sample size required to reject the false model with a power of 80% and a significance level α of .05 can be calculated (Martin *et al.,* 1978; Hewitt and Heath, 1988) and is conveniently supplied by Mx.

RESULTS

Univariate Models

ACE-models

We fitted full univariate models with sources of variation due to additive genetic (A), common environmental (C) and unique environmental influences (E). Dropping either genetic or common environmental parameters and refitting the model provides the non-centrality parameter. With Mx (Neale, 1997) the corresponding number of subjects required to detect the parameter that was dropped with a power of 80% and α of 5% was calculated for 1 degree of freedom. Results concerning the estimated sample size (in subjects) needed to detect V_a in ACE-models for the three designs are depicted in Figure 2 (and appendix II). Figure 2a con-

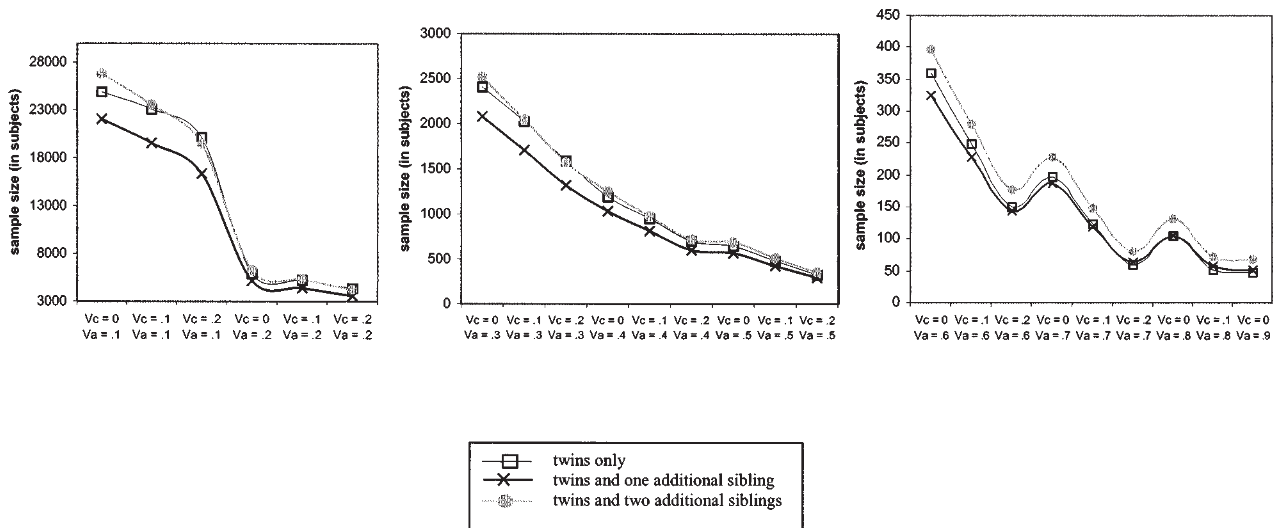


Fig. 2 a,b,c. Required sample size to detect sources of variance due to additive genetic effects in ACE models for three different family designs with a power of 80%. Design 1 = MZ and DZ twins only, Design 2 = MZ and DZ twins and one additional sibling, Design 3 = MZ and DZ twins and two additional siblings.

cerns low values of V_a (10% – 20%), Figure 2b concerns intermediate values of V_a (30%–50%), and Figure 2c concerns high values of V_a (60%–90%) accounting for the total phenotypic variance. All values of V_a are reported three times, i.e. in the context of values of V_c of 0%, 10%, and 20%.

As can be seen in Figure 2a, 2b, and 2c, for various values of V_a and V_c, design 2 (families consisting of MZ and DZ twins and one non-twin sibling) is the most optimal design to detect sources of variation due to A, i.e. with design 2 fewer subjects are required to achieve a power of 80% (see appendix II). The number of subjects needed to detect a fixed value of V_a is on average 9.3% more in the classical twin design (design 1) compared with a design with twins and one additional sibling. This can result in 2849 fewer subjects that are needed with design 2 to detect an additive genetic influence of 10% compared with the classical twin design.

Including families with twins and two additional sibs, is *less* powerful than including families with twins and one additional sibling, and also less powerful than including families with twins only for the detection of V_a; adding two siblings at the cost of the total number of MZ twins is disadvantageous, but adding one sibling is ideal.

Results for detecting common environmental influences are given in Figures 3a, 3b, and 3c, for low, moderate, and high values of V_c respectively (see also Appendix III).

Under various values of V_c and V_a, the power to detect sources of variation due to C rises substantially when one sibling is added to the classical twin design; on average 50.4% fewer subjects are needed as compared to the classical twin design (design 1). Adding two siblings decreases sample size even more, but not as dramatically as the decrease from no additional siblings to one additional sibling.

Many empirical studies suggest models in which sources of variation due to C are of less importance than sources of variation due to A (Plomin, DeFries, & McClearn, 1990). Therefore, we also calculated the sample size required to detect small values of V_c in the context of higher values of V_a. Figure 4 depicts the number of subjects needed to detect values of V_c of 10% and 20% in the context of values of V_a of 20% , 30%, 40% or 50% (Appendix IV).

As expected, sample size required to detect V_c with a power of 80% decreases as a result of higher values of V_c and higher values of V_a. Comparing the sample size required to detect sources of variation due to A (Figure 2b) with the sample size required to detect sources of variation due to C, shows that in the realistic situation where $V_a > V_c$ sources of variation due to C are very difficult to detect. Even if the sample size is large enough to detect sources of variation due to A, the small value of V_c may still go undetected. If for example the true model is an ACE-model with V_a = 50%, V_c = 20%, and V_e = 30%, and the total sample size 328

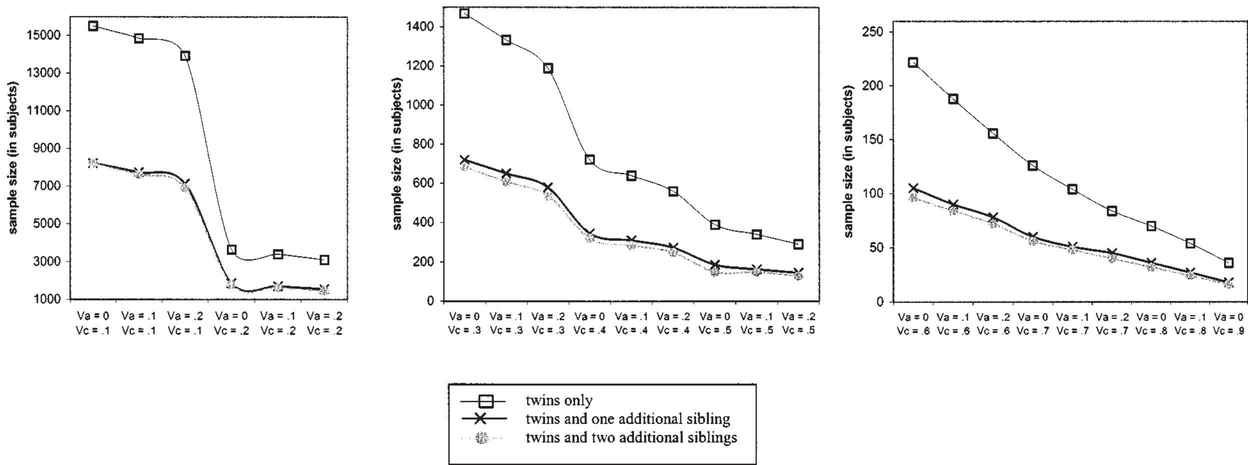


Fig. 3 a,b,c. Required sample size to detect sources of variance due to common environmental influences in ACE models for three different family designs with a power of 80%.

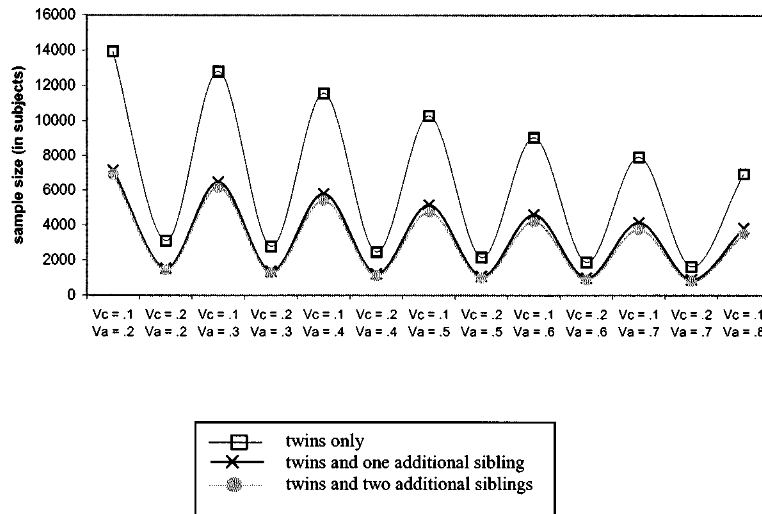


Fig. 4. Required sample size to detect sources of variance due to common environmental influences in ACE models where $V_a > V_c$, for three different family designs with a power of 80%.

(just enough for design 1 to detect V_a of 50%, with power of 80%), V_c will not be detected and the AE-model will be proposed as the most parsimonious model. This results in a biased estimate of V_a (in this case V_a is estimated to be 70%).

Adding siblings to the classical twin design decreases the sample size required to detect both V_a and V_c and has the largest effect on the sample size required to detect V_c (i.e. 50.4% fewer subjects needed for V_c, 9.3% fewer subjects needed for V_a). Therefore, the bias towards overestimating values of V_a as a result of not detecting V_c in situations where $V_a > V_c$, is less likely to be present in designs where siblings are added to the classical twin design.

ADE-Models

We also fitted full univariate models with sources of variation due to additive genetic (A), dominance (D) and unique environmental influences (E). Since a DE-model is unrealistic we report the sample size required to detect sources of variation due to A *and* D (2 df test) and to detect sources of variation due to D (1 df test) with a power of 80%. Results for detecting V_a and V_d, or V_d are given in Figures 5a and 5b (and appendix V).

Under various values of V_a and V_d, with fixed ratio of V_a to V_d is 2 to 1, adding one sibling to a twin family decreases the sample size required to detect V_d. Adding two siblings decreases sample size even more but less than the decrease due to adding one sibling. Absolute effects are slightly higher with increasing values of V_a and V_d. Figure 5a also emphasizes the very large sample size that is required to detect dominant genetic influences. Even the largest possible value of V_d under complete dominance with the most optimal design will go undetected if the sample is smaller than 1776 subjects.

Sample sizes required to detect both V_a and V_d simultaneously are considerably smaller as compared to sample sizes required to detect V_d. In contrast, however, adding siblings does not decrease sample size needed to detect V_a and V_d simultaneously. In fact, a design with one or two siblings requires somewhat more subjects to detect V_a and V_d with a power of 80%, as can be seen in Figure 5b. It should be noted however that the number of subjects needed to detect V_a and V_d at the same time is considerably less than the number of subjects needed to detect V_d only. This implies that if the sample size is large enough to detect V_d it will also be sufficient to detect V_a and V_d.

In conclusion, to optimize the power to detect V_d, a design with additional siblings, as compared to a design with twins only, is preferred.

Bivariate Models

ACE-Models

To detect sources of variance due to additive genetic influence (A), we calculated both the sample size required to detect all sources of V_a (df = 3; paths a_{11}, a_{21}, and a_{22} in Figure 1) and the required sample size to detect the common genetic pathway (df = 1; path a_{21}). We considered the test for the detection of the common pathway to be a test for the presence of a genetic correlation (rA). The following situations to detect sources of variance due to A were considered: a) The genetic correlation (rA) is 'moderate' and equal to the common environmental correlation (rC) and to the unique environmental correlation (rE). Variances due to A, C and E (uniform for both traits) are 40%, 10%, and 50% respectively of the phenotypic variance. b) rC is absent, rA is high (.80), and rE is small (.36), variances due to A, C and E are 40%, 10%, and 50% respectively.

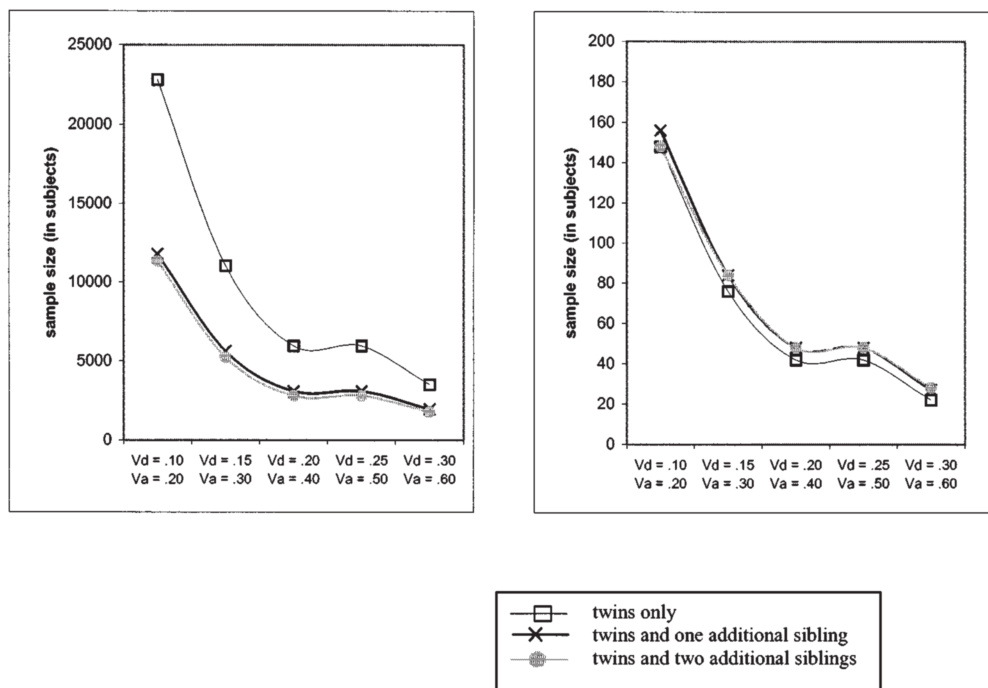


Fig. 5 a,b. Required sample size to detect sources of variance due to dominant genetic influences (a) and total genetic (dominant & additive influences)(b) influences in ADE models, for three different family designs with a power of 80%.

c) Variances due to C are absent. rA is .60, rE is .27, variances due to A and E are 70% and 30% respectively. As mentioned before, all parameters were estimated, as opposed to constraining these parameters, which were zero in the full model. It should also be noted that considering the tests for total V_a, total V_c, and total V_d to be 3 df-tests is a conservative approach, as it could be argued these are actually 2 df-tests, or tests with df's somewhere between 2 and 3. Testing, for example, whether either or both univariate genetic variances equal zero, implies that the genetic covariance is zero. If variances due to additive genetic influences for both traits equal zero, a correlation between these sources of variance is not possible. In other words, if the sample size required to detect each of the univariate variances due to additive genetic influences is insufficient, a correlation due to additive genetic influences can also not be detected. Therefore, considering the test for the power to detect 'total V_a' (i.e. both univariate variances due to additive genetic influences and the correlation due to additive genetic influences in the bivariate case) a 3 df test will provide an overestimation of the sample size needed for a power of 80%. Results of situation a, b, and c for the three different kinships, are given in Table I.

As can be seen in Table I the same pattern of results is found in the bivariate case as in the univariate case; a design with one additional sibling is optimal for the detection of V_a in ACE-models. In addition, significantly fewer subjects are needed in the bivariate case as compared to the univariate case. Depending on whether the phenotypic correlation is due to rA, rC, or rE, the sample size required to detect V_a may decrease and is lowest in cases where there is no influence of common environmental sources (i.e. statistical power is highest in these cases). However, when there are univariate common environmental influences but no common environmental correlation, the sample size required to detect variance due to additive genetic influences increases. Comparing situations a, b, and c leads to the conclusion that the power to detect sources of variance and covariance due to A (df 3) is highest (and the required sample size is smallest) when there is no univariate common environmental source of variation. However, if there are common environmental sources of variation, sources of variance due to A are easier to detect when there is also a correlation between these two univariate common environmental sources of variation, and again a design with one additional sibling is optimal.

To detect sources of common environmental sources of variation, we calculated both the power to detect all sources of variation due to C (df = 3) and the power to detect the common pathway (df = 1), which is a test to detect the environmental correlation (rC). We considered situations analogous to the situations in which power was calculated to detect sources of variation due to A; a) The common environmental correlation is 'moderate' and equal to the genetic correlation and to the unique environmental correlation, i.e. rC = rA = rE = .50. Uniform univariate variances due to A, C and E are 10%, 40%, and 50% respectively. b) rA is absent. rC is high (.80), and rE is small (.36), variances due to A, C and E are 10%, 40%, and 50% respectively, c) Variances due to A and rA are absent. rC is .60, rE is .27, variances due to C and E are 70% and 30% respectively. Again, for all situations the phenotypic correlation was .50. Results are given in Table II.

Although the results in the bivariate case resemble those in the univariate case (i.e. a design with two

Table I. Total samplesize (in number of subjects) needed to detect additive genetic influences in full bivariate ACE models under three different sibship sizes with power (1 − β) = .80 and α = .05

	V_a = 40% rA = .50 V_c = 10% rC = .50 V_e = 50% rE = .50		V_a = 40% rA = .80 V_c = 10% rC = .00 V_e = 50% rE = .36		V_a = 70% rA = .60 V_c = 00% rC = .00 V_c = 30% rE = .27	
	all V_a (df = 3)	rA (df = 1)	all V_a (df = 3)	rA (df = 1)	all V_a (df = 3)	rA (df = 1)
design 1	660	2392	782	884	156	270
design 2	564	1917	678	735	147	237
design 3	680	2260	820	876	180	284

Note: MZ/DZ ratio = 1/1; design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings.
'All V_a' refers to both univariate variances and the genetic correlation.
In order to calculate the total number of families needed, all cells concerning design 1 need to be divided by 2, all cells concerning design 2 need to be divided by 3, and all cells concerning design 3 need to be divided by 4.

Table II. Total samplesize (in number of subjects) needed to detect common environmental influences in full bivariate ACE models under three different sibship sizes with power $(1 - \beta) = .80$ and $\alpha = .05$

	V_a = 10% rA = .50 V_c = 40% rC = .50 V_e = 50% rE = .50		V_a = 10% rA = .00 V_c = 40% rC = .80 V_e = 50% rE = .36		V_a = 0% rA = .00 V_c = 70% rC = .60 V_e = 30% rE = .27	
	all V_c (df = 3)	rC (df = 1)	all V_c (df = 3)	rC (df = 1)	all V_c (df = 3)	rC (df = 1)
design 1	444	1498	518	560	100	156
design 2	213	774	249	279	48	96
design 3	48	760	44	268	16	108

Note: see table 1 for definitions.

additional siblings is optimal for the detection of V_c), the difference between design 2 and design 3 (i.e. adding one or two siblings) in the bivariate case is more substantial. Whereas in the univariate case only a small additional effect was found, in the bivariate case 4 to 5 times less subjects are needed with two additional siblings as compared to one additional sibling.

ADE-Models

We calculated covariance matrices for two traits that were influenced by A, D, and E in the context of complete dominance. Sources of variance due to A and D accounted for 40% and 20% respectively of the total phenotypic variance. We assumed that the ratio V_a to V_d remained equal over the two traits. This implies that rA = rD (see appendix I). Three situations were considered: a) rA = rD = .80,; b) rA = rD =.50; c) rA = rD = .30. For all three situations the phenotypic correlation was fixed at .50 by attributing all remaining covariance to rE. We report the total number of individual subjects needed to detect sources of total V_a and V_d due to A and D (df = 6), rA & rD (df = 2), total D (df = 3), and rD (df = 1) for a power of 80%. Results are given in Table III.

Analogous to the univariate case a design with two additional siblings is optimal for the detection of V_d and a design with twins only is optimal for the detection of V_a and V_d simultaneously. Comparison with the univariate results shows that in a design with twins only, fewer subjects are needed to detect sources of variance due to D as a result from bivariate testing. This effect, however, is stronger when a design consisting of twins and two additional siblings is used, suggesting that in addition to the decrease in sample size as a result from bivariate testing, adding siblings will decrease the sample size required to detect sources of variance due to D even further.

Designs Where Only Sibs of mz Twins are Included

In the previous analyses all families were of the same structure; consisting of MZ and DZ twins only, or with one or two additional siblings. For several reasons this may not always be realistic. For illustrative purposes, we included two other designs in which one (design 4) or two siblings (design 5) were added to MZ twin families, but not to DZ families. Analyses were run for a few 'standard' situations of the ACE-models and ADE-models for univariate testing only. Results for ACE and ADE models are given in Table IV.

Comparison of the results of designs 4 and 5 and the results of designs 2 and 3 shows that in ACE-models a design consisting of MZ twins and one additional sibling and DZ twins only (design 4) is optimal for the detection of V_a, and performs even better than design 2. For the detection of V_c in ACE-models design 3 and 5 are both optimal.

In the context of ADE-models, design 3 (MZ/DZ twins with two additional siblings), requires the smallest sample size and is more optimal than design 4 or 5 for the detection of sources of variation due to dominance.

CONCLUSION

We demonstrated that with a fixed power of 80%, a probablity level of 5% and under varying levels of heritability and common environmental influences, adding one sibling to the classical twin design significantly decreases the number of subjects that are needed to detect each of these sources of variation. Adding two siblings to a twin pair yields an additional decrease of sample size to detect sources of variation due to the common environment but is not optimal for the detection of additive genetic influences. If the trait is influenced by additive and non-additive genetic factors, adding one sibling to the classical twin design decreases the sample size needed to detect sources of variation

Table III. Total sample size (in subjects) needed to detect additive genetic influences and dominance in bivariate ADE models under under three different sibship sizes with power $(1 - \beta) = .80$ and $\alpha = .05$

	V_a = 40% rA = .80, V_d = 20% rD = .80, V_e = 40% rE = .05				V_a = 40% rA = .50, V_d = 20% rD = .50, V_e = 40% rE = .50				V_a = 40% rA = .30, V_d = 20% rD = .30, V_e = 40% rE = .80			
	all V_a + all V_d (df = 6)	rA&rD (df = 2)	all V_d (df = 3)	rD (df = 1)	all V_a + all V_d (df = 6)	rA&rD (df = 2)	all V_d (df = 3)	rD (df = 1)	all V_a + all V_d (df = 6)	rA&rD (df = 2)	all V_d (df = 3)	rD (df = 1)
design 1	62	64	8672	10042	54	184	7662	28054	32	548	4702	82356
design 2	69	78	4464	5121	60	228	3909	14073	39	681	2490	41544
design 3	72	88	4076	4660	60	264	3636	12872	40	784	2272	38436

Note: see table 1 for definitions.

Table IV. Total sample size (in number of subjects) needed to detect additive genetic, dominance and common environmental influences in univariate ACE-models and ADE-models for designs with including MZ and DZ twins and siblings added to MZ families only, a power of $(1 - \beta) = .80$, and significance level $\alpha = .05$

	V_a = 40% Vc = 10%	Vc = 40% V_a = 10%	V_a = 40% V_d = 20%	V_a = 40% V_d = 20%
effect detected→	V_a	V_c	$V_a + V_d$	V_d
design 4	705	338	83	6313
design 5	744	285	84	5313

Note: MZ/DZ ratio = 1/1: design 4 = Mz twins and one additional sibling, DZ twins only, design 5 = MZ twins and two additional siblings and DZ twins onl.

due to dominance. Adding two siblings decreases the number of required subjects somewhat more but the decrease is relatively small (compared to the decrease due to adding one sibling). These effects are more pronounced in the bivariate case than in the univariate case. An additional benefit of adding siblings is that these designs, as compared to the classical twin design, are less likely to result in an overestimation of additive genetic influences as a result of not detecting small sources of common environmental influences.

We modeled the sibling covariances under the assumption that age differences in heritability are not important. A more complex model would take into account age differences between non-twin siblings. It is known that for some measures heritability increases with age as a result of amplification of genetic effects across ages (e.g. intelligence; Boomsma, 1993), whereas for other measures heritability estimates may decrease with age (e.g. problem behaviour; Van der Valk *et al.,* 1998). Assuming that the same genes operate across the age span, adding siblings who are older than the twins will increase power when heritability increase with age, and will decrease power when heritability estimates decrease with age. Similarly, adding parents will increase power to detect genetic factors if heritability increases with age.

Schork (1993) noted the dramatic improvement in statistical power resulting from the use of larger sibships for the detection of QTL effects. In addition, Dolan, Boomsma and Neale (1999) demonstrated the value of adding non-twin siblings to two-sibling- (or DZ twin-) families for the detection of codominant QTL effects. Our aim was to determine whether the use of an extended twin design, as needed for the detection of QTL-effects, would also be useful for the detection of overall

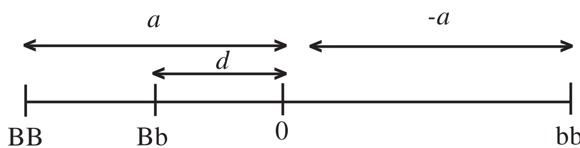
sources of variance (i.e. A, C, and D). Our calculations showed that without the need to increase total sample size, adding one sibling to the classical twin design improves the statistical power by a large extent to detect sources of variation due to common environmental influences, additive genetic influences and dominance. Adding siblings and using a bivariate phenotype results in gain of statistical power which can not only be ascribed to bivariate testing but also to the use of an extended twin design.

In conclusion, adding at least one sibling to the classical twin design, as opposed to a design with twins only, will provide a significant gain in statistical power to detects sources of variation due to A, C, and D. An attractive side-effect of a design with additional siblings is that it is also beneficial for the detection of QTL-effects.

APPENDIX I

Consider a biallelic trait with alleles B and b. Let *a* be the effect of genotype BB on the phenotypic mean, –*a* the effect of bb, and *d* the effect of Bb on the phenotypic mean. Assuming equal allele frequencies of B and b, the mean genotypic effect on the phenotypic mean is *1/2 d.* The total genetic variance ($\sigma^2 g$) equals $1/2\ a^2 + 1/4\ d^2$, $= V^a + V^d$

For complete dominance d = a. Substituting d for a in the formulae for the genetic variances, gives: $V_a = 1/2\ a^2$ and $V^d\ 1/4\ a^2$, thus $V^a = 2\ V^d$



Now consider a *bivariate* model with latent variances scaled to unity, (see figure 1) and

- uniform genetic influences over traits: $V_{a\,1} = V_{a\,2}$ and $V_{d\,1} = V_{d\,2}$
- assumption of uniform d to a ratio over traits $(a_{11})^2/(d_{11})^2 = (a_{21})^2/(d_{21})^2 = (a_{22})^2/(d_{22})^2$
- $rA = a_{11} * a_{21}/\sqrt{\{(a_{11})^{2} * [(a_{21})^2 + [(a_{22})^2]\}}$ which simplifies to $rA = a_{21}/a_{11}$
- $rD = d_{11} * d_{21}/\sqrt{\{(d_{11})^{2} * [(d_{21})^2 + [(d_{22})^2]\}}$ which simplifies to $rD = d_{21}/d_{11}$

This implies that the additive genetic correlation equals the dominant genetic correlation.

APPENDIX II

Sample size (in subjects) needed to detect additive genetic influences in full univariate ACE models under varying levels of variation due to common environmental sources for three different sibshipsizes.

MZ/DZ ratio = 1/1, significance level α = .05, power (1 – β) = .80, design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings.

In order to calculate the total number of families needed, all cells from design 1 need to be divided by 2, all cells from design 2 need to be divided by 3, and all cells from design 3 need to be divided by 4.

	$V_a = 10\%$			$V_a = 20\%$			$V_a = 30\%$			$V_a = 40\%$			$V_a = 50\%$			$V_a = 60\%$			$V_a = 70\%$			$V_a = 80\%$		$V_a = 90\%$
$V_c \rightarrow$	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	0%
design 1	24896	23084	20110	5908	5230	4332	2406	2026	1588	1192	950	700	644	482	328	360	248	150	198	124	60	104	52	48
design 2	22047	19557	16365	5151	4395	3540	2079	1707	1320	1032	813	600	567	426	294	324	228	144	186	120	63	105	57	51
design 3	26836	23560	19460	6256	5280	4208	2520	2048	1572	1252	976	716	688	512	356	396	280	176	228	148	80	132	72	68

APPENDIX III

Sample size (in subjects) needed to detect common environmental influences in full univariate ACE models under varying levels of variation due to additive genetic sources for three different sibshipsizes.

See Appendix II for definitions

	V_c = 10%			V_c = 20%			V_c = 30%			V_c = 40%			V_c = 50%			V_c = 60%			V_c = 70%			V_c = 80%		V_c = 90%
V_a →	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	0%
design 1	15504	14860	13934	3646	3398	3104	1468	1334	1190	722	640	560	390	340	290	222	188	156	126	104	84	70	54	36
design 2	8220	7746	7140	1860	1704	1542	720	651	579	345	309	273	186	162	144	105	90	78	60	51	45	36	27	18
design 3	8220	7628	6908	1808	1632	1448	684	608	536	320	284	248	148	148	128	96	84	72	56	48	40	32	24	16

APPENDIX IV

Sample size (in subjects) needed to detect common environmental influences in full univariate ACE models under varying levels of variation due to additive genetic sources in the realistic situation that sources of variation due to A are larger than sources of variation due to C for three different sibshipsizes.

See Appendix II for definitions

	V_a = 20%		V_a = 30%		V_a = 40%		V_a = 50%		V_a = 60%		V_a = 70%		V_a = 80%
V_c →	10%	20%	10%	20%	10%	20%	10%	20%	10%	20%	10%	20%	10%
design 1	13940	3104	12806	2786	11558	2466	10280	2158	9042	1876	7912	1628	6934
design 2	7143	1542	6471	1377	5790	1224	5151	1089	4590	978	4137	894	3822
design 3	6912	1448	6148	1276	5408	1120	4736	988	4172	884	3748	816	3496

APPENDIX V

Samplesize (in subjects) needed to detect additive genetic and dominance influences in ADE-models. *See Appendix II for definitions*

	$V_a = 20\%$ $V_d = 10\%$		$V_a = 30\%$ $V_d = 15\%$		$V_a = 40\%$ $V_d = 20\%$		$V_a = 50\%$ $V_d = 25\%$		$V_a = 60\%$ $V_d = 30\%$	
	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d
design 1	148	22808	76	11036	42	5958	42	5958	22	3518
design 2	156	11790	84	5631	48	3081	48	3081	27	1950
design 3	148	11328	84	5236	48	2784	48	2784	28	1776

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Chapter 4

Twin-singleton differences in intelligence ?[1]

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Twin–singleton differences in intelligence?

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The twin method has been criticised for its alleged non-generalisability. When population parameters of intellectual abilities are estimated from a twin sample, critics point to the twin–singleton differences in intrauterine and family environments. These differences are suggested to lead to suboptimal cognitive development in twins. Although previous studies have reported twin–singleton differences in intelligence, these studies had two major drawbacks: they tested young twins, and twins were compared with (genetically) unrelated singletons. To test accurately whether twin–singleton differences in intelligence exist, a group of adult twins and their non-twin siblings were administered the Dutch WAIS-III. The group was large enough to detect twin–singleton differences of magnitudes reported in earlier investigations. The data were analysed using maximum likelihood model fitting. No evidence of differences between adult twins and their non-twin siblings on cognitive performance was found. It is concluded that twin studies provide reliable estimates of heritabilities of intellectual abilities which can be generalised to the singleton population. *Twin Research* (2000) **3**, 83–87.

Keywords: twin study, intelligence, twins, singletons

Introduction

Classic behavioural genetic studies provide statistical estimates of heritabilities that form the first step in the search for genes for complex behaviour.[1,2] A large part of these behavioural genetic studies are based on twin samples. These samples have sometimes been criticised for their alleged non-generalisability; since twins are 'special' they may not be representative of singletons. Especially in the field of cognitive abilities twins are generally considered to be at a disadvantage compared with singletons.[3–6]

Twins share the womb at the same time and consequently share prenatal nutrition provided by the mother's dietary intake. When preparing for labour, twins compete for the best position. This suboptimal intrauterine environment may lead to prematurity, low birth weight and lower weight-for-gestational age,[7] which in turn in several cases have been associated with low childhood IQ.[8–12] Apart from a general suboptimal intrauterine environment for both twins, it is known that one of the two foetuses will suffer more from this suboptimal environment than the other.[13] It is usually the second-born twin that experiences the greatest adverse effects of sharing the womb.[14]

Beside these adverse effects of sharing the womb twins may suffer from twin-related stresses in the family environment in which they are reared. A multiple birth puts stress on a family which may have a negative effect on the (cognitive) development of a twin pair. In some studies it is argued that especially for monozygotic (MZ) twins, who are very much alike, limitation of resources and competition may lead to negative influences for at least one twin member.[3]

A relatively small number of studies has been devoted to detecting twin–singleton differences in cognition.[4,6,15] The one study that stands out was conducted by Record, McKeown and Edwards[6] who compared an impressive number of singletons, twins and even a few triplets. Verbal reasoning scores from the British eleven-plus examination were gathered from 48 913 singletons, 1082 twin pairs and eleven triplets. Standard verbal reasoning scores were significantly lower for twins (standard verbal IQ 95.7) than for singletons (100.1). Triplets performed even worse (91.6). The authors investigated whether this 4.4 standard points difference between twins and singletons could be attributed to effects of maternal age, birth weight, gestational age, zygosity and whether a twin was born first or second. None of these factors could explain the difference.

Record *et al*[6] also investigated whether twins of whom one had died shortly after birth differed from singletons; although for these 'twins' a slightly lower score than normal singletons (1.9 points) was found, this difference was much smaller than the 4.4 points difference between singletons and twins of which both members were still alive. Based on this observation the authors concluded that the difference of

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4.4 points between singletons and twins cannot be attributed to negative effects of sharing the womb, but instead must be sought in the environment in which twins are reared. However, since Record *et al*[6] did not control for any difference in twin families and singleton families, they could not rule out selection biases in the sampling of twin and non-twin families. Such biases may exist because twins as a group may have a slightly different genetic or social background than singletons.

Nathan and Guttman[16] tried to overcome selection bias in twin and singleton families by comparing twins and singletons (aged 8–13 years) who were reared in the same kibbutz. A kibbutz is an Israeli community in which children are collectively reared. So although the twins and singletons in this study did not have the same genetic background, they were accurately matched for family environment and childrearing practices. In this study dizygotic (DZ) twins performed worse than MZ twins and singletons. According to the authors, however, this difference could be totally ascribed to the relatively few years of schooling of the group of DZ mothers. Thus, in spite of the attempt to match twins and singletons this study is also an example of biased family sampling.

In addition to comparing twins with familially unrelated singletons, most previous studies have been conducted using young twins.[9,10,17–20] Because these studies show that twins recover any deficits in intellectual performance by 6–8 years of age,[18–20] the comparison of twins and singletons at ages below 8 years does not provide a good indication of adult twin–singleton differences. To the best of our knowledge studies comparing the IQ of adult twins and genetically related singletons have not yet been conducted.

In the present study mean scores of adult MZ and DZ twins on intellectual ability are compared with the mean scores of their non-twin siblings. Non-twin siblings make an ideal control group; both genetic background and early familial environments are perfectly matched.

Method

Subjects

The subjects were 358 family members from a total of 152 twin families who participated in a project investigating the genetics of adult brain function. The Dutch version of the Wechsler Adult Intelligence Scale-III (WAIS-III)[21] was administered when the participants visited the laboratory for a combined session of neuropsychological and electroencephalographic measurements. All subjects were recruited from the Netherlands Twin Registry. The twins had previously participated in one of two previously conducted studies in which zygosity was assessed by blood group polymorphisms and DNA typing.[22,23]

In total, 98 siblings, 101 MZ twins, 153 DZ twins and 9 triplets participated. Since the group of triplets was small, we discarded the data of the last born of the triplets and treated the remaining two members as if they were twins. This left 98 siblings and 260 twins. The study recruited twin pairs and at most two of their non-twin siblings. It also included single twins (co-twin refused participation) and siblings only (both twins refused). Thus, families consisted of at least one member and at most four members. Table 1 shows the number of families with a particular constitution, eg 27 MZ families consisting of two twin members and no siblings participated; siblings from nine families participated without the twins. Due to administrative errors five individual test scores are missing subtest *digit symbol-coding*, four individual test scores are missing subtests *block design* and *digit symbol-free recall*, and one individual test score is missing subtest *digit symbol-pairing* and subtest *letter-number sequencing*. Results are based on the available number of subjects per subtest (see Table 3).

Mean age and sex distribution per group are displayed in Table 2. Of the 98 non-twin siblings, 35 were younger than the twin from the same family, and 63 were older. Distribution of sex did not differ in the DZ twins and the siblings. Slightly fewer female MZ twins than male MZ twins participated.

Table 1 Sample configuration

		number of non-twin siblings			
		0	*1*	*2*	
mz twins	2 twins	27	18	3	total mz twin pairs: 48
	1 twin	–	4	1	
dz twins	2 twins	32	27	10	total dz twin pairs: 69
	1 twin	12	8	1	
no twin		–	7	2	
total non-twin siblings:			64	+ 34 = 98	

Table 2 Mean age and sex distribution per group

Group	*Male*	*Female*	*Total*	*Mean age in years* (sd)
mz twins	58	43	101	39.7 (*12.63*)
dz twins	70	89	159	37.3 (*11.87*)
sibs	46	52	98	37.1 (*12.02*)

sd = standard deviation

Procedure

Eleven subtests of the Dutch WAIS-III were administered in a fixed order. Subtests included *block design, letter–number sequencing, information, matrix reasoning, similarities, picture completion, arithmetic, vocabulary, digit symbol coding, digit symbol pairing and digit symbol free recall.* Age and sex normalised scores for the Dutch WAIS-III are not yet available; raw scores were used in the analyses throughout. All subjects were paid Dfl. 50.- for participation.

Statistical analyses

As can be seen from Table 1 the data were characterised by the varying number of participating family members; families consisted of one to four members which could be any combination of one or two twins and/or non-twin siblings. This variability in number of observations per family causes serious computational problems. In Mx[24] the handling of such 'incomplete' data is implemented by calculating twice the negative log-likelihood (–LL) of the raw data for each family, with the following formula:

$$-LL = -k \log (2\pi) + \log |\Sigma| + (x_i - \mu_i)' \Sigma^{-1} (x_i - \mu_i),$$

where k (k = 1, 2, 3 or 4) denotes the number of observed variables within a family, Σ (4×4) is the covariance matrix of family members, x_i (for i = 1, 2, 3, 4) is the vector of observed scores, μ_i is the column vector of the estimated means of the variables, and $|\Sigma|$ and Σ^{-1} are the determinant and inverse of matrix Σ, respectively.

When two models which provide –2LLs are nested, subtracting the two –2LLs from each other provides a $\Delta(-2LL)$ which has a χ^2 distribution. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model.

Four univariate nested models were fitted using this procedure. In the first model all means were estimated individually. The second model is the same as the first model with two extra equality constraints; one on the means of both members of the MZ twin pairs and another one on the means of both members of the DZ twin pairs. The third model is the same as the second model but further constrains the means of the MZ twin pairs and the DZ twin pairs to be equal. The fourth is the same as the third model but with an extra equality constraint on the means of all twins (mz and dz) and siblings.

Model 2 tests whether the means of first born twins and second born twins within zygosity groups are significantly different. The third model serves as a test of the assumption that the means in MZ twins and DZ twins do not differ. Model 4 tests whether the means of twins and siblings are significantly different.

For all models the variances of all twin members and all siblings were constrained equal, and all covariances of all twin sib pairs, the covariance of two sibs within one family and the covariance of the DZ twins were set equal.

Statistical power

We calculated the necessary sample size for each group (singletons and twins) based on the effect size as found in Record *et al*'s study.[6] A measure of effect size that is independent of scaling is Cohen's *d*, which is calculated as follows:

$$d = (\mu 1 - \mu 2)/\sigma$$

where $\mu 1$ is the mean of the first group (singletons), $\mu 2$ is the mean of the second group (twins) and σ is the common standard deviation.[25]

Record *et al*[6] found a 4.4 standard points difference between the two groups. The standard deviation of an IQ score is by definition 15. The effect size in the Record *et al* study was thus 0.29, which is considered a small effect. For a one-tailed test with $\alpha = 0.05$, $1-\beta = 0.80$, and two related samples, 70 individuals per group (singletons and twins) are needed to detect an effect of such small magnitude.[26] We had 260 twins and 98 non-twin siblings giving us the power to detect effect sizes well below 0.29.

Results

The observed means and standard deviations of WAIS-III subtests per group are displayed in Table 3.

Table 3 Observed means and standard deviations of WAIS-III subtests per group

subtest	*mz twin (N = 101)*	*dz twin (N = 159)*	*sibs (N = 98)*
Block design	26.20 (8.96)	25.72 (9.28)[a]	26.25 (8.85)[b]
LN sequencing	12.21 (3.42)	11.21 (2.61)	11.86 (2.90)[c]
Information	23.41 (6.32)	23.93 (6.00)	24.11 (6.54)
Matrix reasoning	19.36 (3.38)	19.16 (3.44)	19.40 (3.28)
Similarities	26.91 (5.58)	27.17 (5.43)	27.33 (5.58)
Picture completion	20.86 (2.55)	20.72 (2.60)	20.55 (3.18)
Arithmetic	13.86 (3.86)	13.75 (3.89)	14.70 (4.12)
Vocabulary	49.07 (11.60)	48.26 (10.55)	47.83 (13.54)
DS coding	76.09 (15.22)	77.66 (19.52)[d]	78.83 (15.86)[e]
DS free recall	7.63 (1.20)[f]	7.54 (1.12)[d]	7.54 (1.27)[c]
DS pairing	13.25 (4.25)	12.67 (4.19)	12.92 (4.02)[C]

[a]based on 157 observations
[b]based on 96 observations
[c]based on 97 observations
[d]based on 158 observations
[e]based on 94 observations
[f]based on 99 observations
LN = Letter-number
DS = Digit symbol

Table 4 Fit indices for nested sequence of models fitted to raw data of WAIS-III subtest scores of MZ twins, DZ twins and siblings

Subtest	*1. All means unequal*		*2. Means 1st born twins equal means 2nd born twins, within zygosity groups*		*3. Means mz twins equal means dz twins*		*4. Means twins equal means non-twin siblings*		*(4–1) All means equal against all means unequal*	
	−2LL	*df*	*−2LL*	*df*	*−2LL*	*df*	*−2LL*	*df*	χ^2	*(Δdf = 7)*[a]
Block design	2451.48	343	2453.90	345	2454.01	346	2459.56	350	8.08	n.s.
Letter-number sequencing	1738.22	346	1739.38	348	**1744.57**	**349**	1750.37	353	12.15	n.s.
Information	2194.37	347	2197.44	349	2197.87	349	2205.64	354	11.27	n.s.
Matrix reasoning	1842.22	347	1845.75	349	1845.93	350	1848.00	354	5.78	n.s.
Similarities	2150.00	347	2151.07	349	2151.21	350	2157.71	354	7.71	n.s.
Incomplete pictures	1681.34	347	1681.81	349	1681.85	350	1687.18	354	5.84	n.s.
Arithmetic	1919.46	347	1920.33	349	1920.44	350	**1930.52**	**354**	11.06	n.s.
Vocabulary	2675.27	347	2678.30	349	2678.60	350	2682.41	354	7.14	n.s.
Digit symbol coding	2964.08	342	2965.69	344	2965.99	345	2967.20	349	3.12	n.s.
Digit symbol free recall	1082.13	343	1082.29	345	1082.61	346	**1092.84**	**350**	10.71	n.s.
Digit symbol pairing	1988.00	346	1990.40	348	1991.25	349	1994.45	353	6.45	n.s.

df = degrees of freedom; −2LL = twice the negative log likelihood; n.s. = not significant: when the increase in $\chi2$ is not significant, the most restrictive model is accepted; [a]an increase in χ^2 of more than 14.07 for Δdf = 7 is significant at the 0.05 level.

To test whether the above differences in mean scores indicated true differences, univariate analyses in Mx using twice the negative log-likelihood were run. The results for these analyses are presented in Table 4, from which it can be seen that comparison of model 4, the most parsimonious model, with model 1 did not cause a significant worsening of the fit for any of the WAIS III subtests. In other words, for all subtests a model which estimates all means to be equal fits better than a model in which all means are estimated separately. There was no reason to believe that means of twins and singletons in our sample differed in IQ.

We did find, however, that comparison of model 4 (all means equal) with model 3 (separate means for twins and siblings) showed a significant worsening of the fit for subtests *arithmetic* and *digit symbol-free recall*, in the sense that on *arithmetic* singletons performed slightly better than both MZ and DZ twins, and on *digit symbol-free recall* MZ twins performed slightly better than both DZ twins and singletons. We also found that MZ twins performed significantly better than DZ twins on subtest *letter–number sequencing*.

Discussion

It has been suggested that twins have an intellectual disadvantage compared with singletons and that twin samples are not representative of the normal population. If true, this might influence generalisability of heritability estimates obtained in twin studies, for instance by a restriction of range of IQ scores. In the Record *et al*[6] study a standard IQ score difference of 4.4 points was found between twins and singletons. Our study had enough statistical power to detect an effect of at least the same magnitude on each of the individual IQ subtests. We found, however, no evidence of a twin–singleton difference. In fact, means and standard deviations in our study showed no differences at all between twins and singletons. In the Record *et al*[6] study, where these differences were found, *a priori* differences in social class or genetic background of twin families and singleton families could never be ruled out. Since our twins and singletons came from the same family, social class and genetic background were perfectly matched across twin families and singleton families.

Our results are in line with an earlier report by Kallman[27] who administered the Wechsler Bellevue Scale to 134 twin pairs (aged 60–89 years), and compared the scores of these twins to standardised scores based on a comparable group of singletons. Kallman concluded that there was no significant difference between twins and singletons in measures of intellectual performance.

Although in our study no evidence was found for twin–singleton differences in intellectual ability, one cannot necessarily generalise from this in respect of personality, lifestyle, disease susceptibility or mortality rates. However, recent comparisons of twins and singletons on problem behaviour,[28] mortality rates[29] and psychiatric symptoms[30] have not suggested twin–singleton differences in these fields either. All in all, significant disadvantages of twins in comparison with singletons seem to be implied rather than observed.

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Chapter 5

Twin-singleton differences in brain volumes?[1]

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Twin–singleton differences in brain structure using structural equation modelling

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Summary

Twin studies are important to investigate genetic influences on variation in human brain morphology in health and disease. However, the twin method has been criticized for its alleged non-generalizability due to differences in the intrauterine and family environment of twins, compared with singletons. To test whether twin–singleton differences complicate interpretation of genetic contributions on variation in brain volume, brains from 112 pairs of twins and 34 of their siblings with a mean (standard deviation) age of 30.7 (9.6) years were scanned using MRI. The influence of birth order, zygosity and twin–sibling differences on brain volume measures was analysed using maximum-likelihood model fitting. Variances were homogeneous across birth order, zygosity and twin–singleton status. Irrespective of zygosity, intracranial volume was smaller in second-born twins compared with first-born twins and compared with siblings. Grey matter volume was smaller in second-born twins compared with first-born twins. White matter was smaller in twins compared with siblings. Differences in grey and white matter between these groups were no longer significant after correction for intracranial volume. Total brain, and lateral and third ventricle volumes were comparable in twins and singletons. In conclusion, second-born twins have a smaller intracranial volume than their first-born co-twins and siblings. This suggests aberrant early brain development in second-born twins, which is consistent with the suboptimal pre- and perinatal environment related to birth order in twins. Since other brain volume measures were comparable between the groups, twin studies can provide reliable estimates of heritabilities in brain volume measures and these can be generalized to the singleton population.

Keywords: twins; structural equation modelling; MRI; brain; birth order

Abbreviations: DOS = dizygotic opposite-sex twins; DZ = dizygotic; DZF = dizygotic female twins; DZM = dizygotic male twins; MZ = monozygotic; MZF = monozygotic female twins; MZM = monozygotic male twins; SF = female sibling; SM = male sibling

Introduction

Twins studies are considered important to investigate genetic influences on variation in human brain morphology. Several studies have investigated quantitatively the contribution of genetic and environmental influences to individual differences in human brain structure (Bartley *et al*., 1997; Carmelli *et al*., 1998; Lohmann *et al*., 1999; Le Goualher *et al*., 2000; Pennington *et al*., 2000; Pfefferbaum *et al*., 2000; Baaré *et al*., 2001*a*). Moreover, twin studies have shown genetic and environmental influences on the interaction between brain structure and psychiatric disease (Reveley *et al*., 1982; Suddath *et al*., 1990; Baaré *et al*., 2001*b*).

The twin method has sometimes been criticized for its alleged non-generalizability due to differences in intrauterine and family environment of twins, compared with singletons. As foetuses, twins share the womb and prenatal nutrition, and compete for the best position during labour. The intrauterine environment may therefore be considered as suboptimal compared with that of singletons, with the greatest disadvantage for the second born of a (monozygotic) twin pair (Price *et al*., 1950). In addition, it has been argued that family environments in which twins are reared can be suboptimal compared with those of singletons. Some studies have shown

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that physical likeness, limitation of resources and competition may lead to negative influences on the cognitive development of at least one twin member (Hay *et al.*, 1983). However, in a large sample of 3-year-old twins, it was found that they had similar, or even lower, levels of behavioural and emotional problems than singletons (Van den Oord *et al.*, 1996).

Evidence for differences between twins and singletons has been suggested in a few studies for cognitive measures (Hay *et al.*, 1983; Nathan *et al.*, 1984), raising concerns regarding generalizations towards the singleton population (Vandenberg, 1984). However, these studies generally compared twins with genetically unrelated singletons, which complicates the generalizability of the findings. Recently, using an extended twin design (Posthuma and Boomsma, 2000; Posthuma *et al.*, 2000*a*), a study was completed in which monozygotic and dizygotic twins were compared with their own siblings on intellectual ability, providing perfectly matched genetic and familial environments (Posthuma *et al.*, 2000*b*). No evidence was found for differences in intellectual ability between twins and their siblings. This suggests that twin studies can provide reliable estimates of heritabilities, which can be generalized to the singleton population, at least with respect to intellectual abilities. Similarly, comparison of twins and their non-twin relatives showed no differences in psychiatric symptoms (Kendler *et al.*, 1995). However, it is not known whether twin studies provide reliable estimates of heritabilities of brain structure. Studies examining the relative contributions of genetic and non-genetic factors to structural brain volume in health and disease rely on the assumption that brains of twins are comparable with those of singletons. To test whether twin–singleton differences complicate interpretation of genetic contributions to variation in brain structure, brain volume measures from pairs of twins and their siblings were compared.

Methods

Subjects

A total number of 258 family members from 112 families participated in the study after written consent was obtained (Baaré *et al.*, 2001*a*). They consisted of 33 monozygotic (MZ) male (MZM), 17 dizygotic (DZ) male (DZM), 21 MZ female (MZF), 20 DZ female (DZF), 21 DZ opposite-sex (DOS) twin pairs and 19 male (SM) and 15 female (SF) full siblings. Twins were recruited from the (healthy) twin sample of the Department of Psychiatry of the University Medical Centre Utrecht, The Netherlands, and The Netherlands Twin Registry (Boomsma, 1998). DNA fingerprinting using either the polymorphic markers D06S474, D07S1804, D07S1870, D12S811, D13S119, D13S126, D13S788, D20S119, D22S683, DXS1001 and ELN, or D13S317, VWA, D74520, D35158, TH01, TPOX, CSF1P0 and D55818 determined zygosity. Except for one twin pair, all twins and their siblings were reared together. Two twin pairs were born by Caesarean section delivery. Subjects with severe medical diseases were excluded. Mental and physical health was assessed by means of the Family Interview for Genetic Studies (Nurnberger *et al.*, 1994) and a medical history inventory, respectively. Birth weight was traced in 219 subjects (in 96 first-born twins, in 95 second-born twins and in 28 siblings). It was based on the report of the mother and, when this was not available, on the subject's report. Subjects' consent was obtained according to the declaration of Helsinki. The Scientific and Ethical Committee of the University Medical Centre Utrecht, in which the study was performed, approved the study.

Brain imaging

MRIs were obtained on a 1.5 tesla Philips Gyroscan scanner at the University Medical Centre Utrecht. For volumetric analysis, a three-dimensional T_1-weighted, coronal FFE (spoiled gradient echo scan) of the whole head [TE (echo time) = 4.6 ms, TR (repetition time) = 30 ms, flip angle = 30°, 170–180 contiguous slices; $1 \times 1 \times 1.2$ mm^3 voxels], and a coronal DTSE (dual contrast turbo spin echo) of the whole brain (TE1 = 14 ms, TE2 = 80 ms, TR = 6350 ms, 120 contiguous slices; $1 \times 1 \times 1.6$ mm^3 voxels) were acquired.

Images were coded to ensure blindness for subject identification, zygosity and family membership. Image volumes were transformed into Talairach space (no scaling) and corrected for magnetic field inhomogeneities (Maes *et al.*, 1997; Sled *et al.*, 1998). Volumetric measurements were obtained using automated segmentation procedures and included intracranial, whole brain, grey and white matter of the cerebrum (excluding cerebellum and brainstem), and lateral and third ventricle volumes. Automatic segmentation software included histogram analysis algorithms, anatomical knowledge-based decision rules and series of mathematical morphological operators to connect all voxels of interest (Schnack *et al.*, 2001*a*, *b*). Intracranial volume was segmented on DTSE scans. Whole brain volume was segmented on the three-dimensional FFE scans using a binary image of the intracranial volume as a mask. A plane through the fourth ventricle and the aqueduct limited the cerebellum. In lateral ventricle segmentation, automatic decision rules bridged connections not detectable and prevented 'leaking' into cisterns. The third ventricle was limited by coronal slices that clearly showed the anterior and posterior commissures; the upper boundary was a plane through the plexus choroideus ventriculi tertii in the midsagittal slice perpendicular to this slice. Segmented intracranial, whole brain and lateral and third ventricle volumes were checked visually and edited if necessary. The segmentation procedures yielded highly reliable volume measurements, with inter-rater intraclass correlations all above 0.96.

Statistical analysis

Structural equation modelling with Mx software (Neale, 1997) was used to estimate the contribution of birth order of the twins to mean scores of and variance in brain volumes, to

Table 1 *Age and brain volumes in monozygotic (MZ), dizygotic (DZ) twins and their siblings*

	MZM 1st* (n = 33)	MZM 2nd (n = 33)	DZM 1st (n = 17)	DZM 2nd (n = 17)	DOS 1st male (n = 9)	DOS 2nd male (n = 12)	SM (n = 19)	MZF 1st (n = 21)	MZF 2nd (n = 21)	DZF 1st (n = 20)	DZF 2nd (n = 20)	DOS 1st female (n = 12)	DOS 2nd female (n = 9)	SF (n = 15)
Mean age in years	30.76	30.75	29.82	29.76	31.78	28.50	28.89	33.67	33.67	30.20	30.15	28.50	31.78	29.53
(standard deviation)	(9.71)	(9.69)	(7.04)	(7.06)	(14.75)	(11.39)	(4.79)	(11.80)	(11.80)	(8.55)	(8.55)	(11.38)	(14.75)	(4.90)
Brain volumes, mean (standard deviation) in ml														
Intracranium	1531.39	1515.93	1496.98	1426.42	1486.62	1502.56	1528.68	1354.37	1330.48	1339.63	1327.74	1379.65	1245.77	1376.90
	(109.89)	(118.61)	(72.00)	(81.85)	(95.51)	(99.93)	(111.15)	(108.64)	(127.83)	(104.74)	(118.77)	(88.14)	(83.28)	(115.73)
Total brain	1339.64	1330.73	1329.87	1265.28	1298.87	1313.96	1348.06	1183.31	1170.66	11.82.15	1178.94	1211.86	1118.23	1211.54
	(106.18)	(111.68)	(66.86)	(77.55)	(97.96)	(97.62)	(92.76)	(110.19)	(124.11)	(115.44)	(109.12)	(77.69)	(68.22)	(108.10)
Grey matter	676.17	670.41	663.77	629.22	667.07	681.10	681.55	612.90	599.19	614.31	612.72	639.68	580.96	624.42
	(64.59)	(69.36)	(43.85)	(41.15)	(44.39)	(73.16)	(51.66)	(68.76)	(77.48)	(59.17)	(53.44)	(55.49)	(54.78)	(58.83)
White matter	498.81	494.85	506.98	478.68	474.33	476.36	505.31	426.58	428.08	422.37	415.45	423.37	397.62	436.14
	(53.42)	(55.15)	(34.36)	(47.15)	(53.91)	(44.35)	(49.04)	(49.40)	(58.64)	(55.94)	(57.34)	(42.48)	(27.14)	(53.40)
Lateral ventricles	15.26	15.19	12.76	11.78	17.67	16.90	13.88	14.39	13.47	11.99	9.83	16.10	9.72	14.23
	(9.11)	(7.33)	(6.28)	(5.89)	(10.29)	(9.18)	(7.96)	(7.09)	(6.37)	(7.64)	(3.90)	(6.81)	(2.56)	(7.03)
Third ventricle	0.79	0.75	0.70	0.64	0.95	0.67	0.69	0.75	0.68	0.59	0.56	0.68	0.50	0.78
	(0.42)	(0.35)	(0.28)	(0.31)	(0.56)	(0.41)	(0.34)	(0.29)	(0.28)	(0.30)	(0.19)	(0.26)	(0.18)	(0.24)

*1st = first born; 2nd = second born.

test the assumption that the mean volumes (and variance) in MZ twins and DZ twins do not differ, and to test whether the mean volumes (and variance) of twins and siblings are significantly different. Models were fitted to the raw data using maximum-likelihood to estimate parameters. Hierarchic χ^2 tests were used to compare the fit of different models. Twice the difference between the log-likelihood of two models is distributed asymptotically as χ^2. The degrees of freedom for these tests are equal to the difference in parameters being estimated. Utilizing the principle of parsimony, the most restrictive model is accepted as the best fitting one in case the difference between a nested and a more comprehensive model is not significant (Neale and Cardon, 1992).

Four univariate nested models were fitted using this procedure (Posthuma *et al.*, 2000*a*). In the first model (the control model), the variances for brain volumes of all twin members and all siblings were constrained to be equal. In addition, all covariances of twin–sibpairs, the covariance of two sibs within one family and the covariance of the DZ twins were set to be equal. The second model (to test birth order effects) is the same as the first model, with two extra equality constraints; one on the means of both members of the MZ twin pairs and another on the means of both members of the DZ twin pairs. The third model (to test zygosity effects) is the same as the second model, but further constrains the means of the MZ twin pairs and the DZ twin pairs to be equal. The fourth model (to test twin–sibling differences) is the same as the third model, but with an extra equality constraint on the means of all twins (MZ and DZ) and siblings.

Univariate models were fitted on all variables, with the effects of age and sex corrected for by means of a linear regression on the observed values of each of the dependent variables.

Post hoc analyses were done with intracranial volume as a covariant, when effects for total brain, grey and white matter or ventricular volumes were found to evaluate the specificity of the finding. Moreover, in case of a significant finding for birth order, the influence of birth weight was tested, by adding birth weight as covariate to the analyses.

Results

Means and standard deviations of brain volume measures are shown in Table 1. The volume estimates in the control model are shown in Table 2. The tests for equality of variances showed no evidence of differences in variance according to birth order, zygosity or twin–singleton status. In addition, no differences were found in DZ covariance and sibpair covariances.

The results for the univariate analyses of birth order, zygosity and twin–sibling differences on the means of the brain volume measures using the Mx software are shown in Table 3.

Table 2 *Brain volume estimates (in ml) under the constraints that variances are equal for all family members (control model)*

	Grand mean MZ 1st*	Grand mean MZ 2nd	Grand mean DZ 1st	Grand mean DZ 2nd	Grand mean sibs	Age effect per year	Male deviation
Intracranium	1380.52	1361.80	1359.04	1328.90	1387.83	–0.57	158.59
Total brain	1263.92	1253.56	1248.11	1228.75	1272.47	–2.18	137.41
Grey matter	711.68	702.87	704.31	690.65	711.05	–2.86	52.02
White matter	397.91	396.07	393.03	384.55	407.25	0.93	69.31
Lateral ventricles	11.65	10.25	11.13	9.14	10.23	0.09	1.22
Third ventricle	0.39	0.34	0.36	0.30	0.40	0.01	0.09

*1st = first born; 2nd = second born.

Table 3 *Influence of birth order, zygosity and twin–sibling differences on brain volumes*

	Model 2* birth order		Model 3 zygosity		Model 4a twin–sibling		Model 4b twin–1st sibling		Model 4c twin–2nd sibling	
	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.	χ^2	Δd.f.
Intracranium	**11.986**	2	3.102	2	–	–	2.749	1	**9.321**	1
Total brain	5.85	2	1.363	1	3.488	1				
Grey matter	**7.233**	2	1.494	2	–	–	0.405	1	3.364	1
White matter	1.678	2	0.896	1	**4.263**	1				
Lateral ventricles	4.521	2	0.056	1	0.001	1				
Third ventricle	4.996	2	0.481	1	1.475					

*An increase in χ^2 of >3.841 for Δd.f. = 1 is significant at the 0.05 level; an increase in χ^2 of >5.991 for Δd.f. = 2 is significant at the 0.05 level; χ^2 values in bold indicate a significant influence of the factor (i.e. the model cannot be accepted); note that when the increase in χ^2 is not significant, the most restrictive model is accepted.

For intracranial volume, the more restrictive models revealed a significant difference in means due to birth order ($\chi^2 = 11.99$, Δd.f. = 2, $P < 0.05$). The second-born twins had smaller intracranial volumes than the first-born twins. Because birth order mattered, separate comparisons for first-born and second-born twins with siblings were made. The mean intracranial volume of the first-born twins did not differ from that of the siblings. However, the mean intracranial volume of the second-born twins was smaller than that of the siblings ($\chi^2 = 9.32$, Δd.f. = 1, $P < 0.05$). For grey matter volume, the more restrictive models revealed a significant difference in mean due to birth order ($\chi^2 = 7.23$, Δd.f. = 2, $P < 0.05$). The comparisons for first-born and second-born twins with siblings revealed no significant differences in mean grey matter volume, although grey matter volume of the second-born twins was smaller compared with that of the siblings. For white matter volume, the more restrictive models revealed a significant difference ($\chi^2 = 4.26$, Δd.f. = 1, $P < 0.05$). Mean white matter volume in twins, irrespective of birth order and zygosity, was smaller than in siblings.

Post hoc analyses revealed that the effects of grey and white matter in (second-born) twins compared with siblings were no longer significant after correction for intracranial volume.

For the total brain, and the lateral and third ventricular volumes, the more restrictive models caused no significant differences in χ^2. This means that the more restrictive models may all be accepted. Thus, the estimated means of first- and second-born twins, the estimated means of MZ twins and DZ twins, and those of twins compared with siblings were not significantly different with respect to total brain, and lateral and third ventricular volumes.

There was a significant correlation between birth weight and intracranial volume ($r = 0.23$, $P < 0.01$). Birth weight was lower in second-born twins (mean ± standard deviation birth weight 2455.3 ± 569.9 g) compared with first-born twins (2575.6 ± 567.8 g) ($\chi^2 = 6.404$, Δd.f. = 2, $P < 0.05$). Twins had a lower birth weight than siblings (3369.1 ± 591.2 g) ($\chi^2 = 59.124$, (d.f. = 1, $P < 0.0001$). When birth weight was added as covariant in the model, it did not influence the results, i.e. intracranial volume remained significantly smaller in the second-born compared with the first-born twins ($\chi^2 = 11.481$, Δd.f. = 2, $P < 0.05$).

Discussion

This study compared brain morphology between MZ and DZ twins with their non-twin siblings. No differences in (co)variances were found according to birth order, zygosity or twin–singleton status. However, a mean difference was found for intracranial volume, such that the second-born twins had a significantly smaller intracranial volume compared with the first-born twins and compared with their

siblings, whereas the intracranial volume of first-born twins did not differ from that of the siblings. Moreover, grey matter volume was smaller in the second-born twins compared with the siblings, and white matter was smaller in the twins, irrespective of birth order and zygosity, compared with the siblings. After correction for intracranial volume, the effects for grey and white matter were no longer significant. Mean values of total brain, and lateral and third ventricular volumes were not influenced by birth order or zygosity, and no evidence was found for twin–sibling differences.

The finding that, irrespective of zygosity, second-born twins had a smaller intracranial volume than first-born twins and their siblings suggests that brain growth is influenced by non-genetic factors during early brain development. Brain growth is thought to be the main factor influencing growth of the neurocranium in the first years of life (O'Rahilly and Müller, 1992; Sgouros *et al.*, 1999). No effect of birth order on head circumference was reported earlier, but that finding was based on the inclusion of 10 pairs of MZ twins only (Tramo *et al.*, 1998). Because no effects of zygosity were found, it is likely that environmental and not genetic factors resulted in the development of a relatively smaller head in second-born twins. Nutritional deficiency during the first trimester of gestation (Hulshoff Pol *et al.*, 2000), and birth complications (McNeil *et al.*, 2000; for a review, see Frangou and Murray, 1996), in schizophrenia as well as very preterm birth (Allin *et al.*, 2001) have all been related to decreased brain volume. Twin gestations have a significantly higher rate of complications compared with singleton gestations, particularly with regard to preterm labour, pregnancy-induced hypertension and foetal death (Kovacs *et al.*, 1989; Doyle, 1996), and the second-born twin seems to be particularly at risk. The overall mortality risk of second-born twins has been reported to be 8% greater than that of first-born twins. Mortality risks as a result of respiratory distress syndrome, intrauterine hypoxia and birth asphyxia, and congenital anomalies were 19–27% higher among second-born twins than among first-born twins (Fowler *et al.*, 1991). Moreover, second-born twins were found to have a lower birth weight compared with first-born twins in a sample of 193 twins where 55% of the second born twin were male (Daniel *et al.*, 2000) and in a sample of 2930 Dutch twins (Baal and Boomsma, 1998). Although, in our study, no direct comparison between obstetric complications and brain volume measures was made, it is likely that pre- and perinatal factors specific for second-born twins influenced intracranial volume in these subjects. Correcting for birth weight in the analysis did not change the finding that second-born twins had a smaller intracranial volume than the first-born twins. Because the effects were only found in the second-born twins and not in the first-born twins, suboptimal family environments that have been associated with twin rearing (Hay and O'Brien, 1983) are less likely to have influenced intracranial volume.

Twin–sibling differences were found for mean white matter volumes, with those of twins being smaller than their siblings, and grey matter volume, which was smaller in the second-born twins compared with siblings. However, after correction for intracranial volume, these effects on the means were no longer significant. This suggests that the difference in (second-born) twins compared with siblings is due predominantly to differences in intracranial volume and occurs early in brain development. Moreover, it implies that overall volumes of grey and white matter do not develop differently in twins and singletons.

No differences in mean values of total brain, and lateral and third ventricular volumes were found secondary to twin–sibling differences. Although total brain volume was somewhat smaller in twins compared with siblings, this finding did not reach significance. The comparability of both the variances and the means of brain volumes across twins and their siblings suggests that suboptimal pre-, peri- and possibly postnatal circumstances in twins and siblings do not differentially influence total brain, and lateral and third ventricle volumes in twins and singletons. Moreover, it suggests that twin studies can provide reliable estimates of heritability of these brain volumes and that these estimates can be generalized to the singleton population.

Whether the smaller intracranial volume in second-born twins has consequences for their subsequent cognitive and behavioural development remains to be determined. However, it is unlikely that the smaller intracranial volume in second-born twins implies such consequences. In a study that included a majority of the twins from this study, no differences in intelligence measured by the Wechsler Adult Intelligence Scale and birth order in twins were found (Posthuma *et al.*, 2000*b*). Earlier findings suggested that twins recover from deficits in intellectual performance by 6–8 years of age (Wilson, 1979). Indeed, by the age of 11 years, no evidence for a relationship between the order of delivery of twins on their intelligence quotient as measured by a verbal reasoning task was found (Record *et al.*, 1970). Finally, levels of behavioural and emotional problems were found to be similar, or even lower, in 3-year-old twins compared with singletons (Van den Oord *et al.*, 1996).

Our findings suggest that twin studies can provide reliable estimates of heritabilities of brain volumes that can be generalized to the singleton population. Whether twin–sibling differences occur in particular brain areas such as in limbic, diencephalic and basal ganglia structures remains to be established in future studies.

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Chapter 6

The genetic analysis of brain volumes[1]

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Multivariate Genetic Analysis of Brain Structure in an Extended Twin Design

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The hunt for genes influencing behavior may be aided by the study of intermediate phenotypes for several reasons. First, intermediate phenotypes may be influenced by only a few genes, which facilitates their detection. Second, many intermediate phenotypes can be measured on a continuous quantitative scale and thus can be assessed in affected and unaffected individuals. Continuous measures increase the statistical power to detect genetic effects (Neale *et al.,* 1994), and allow studies to be designed to collect data from informative subjects such as extreme concordant or discordant pairs. Intermediate phenotypes for discrete traits, such as psychiatric disorders, can be neurotransmitter levels, brain function, or structure. In this paper we conduct a multivariate analysis of data from 111 twin pairs and 34 additional siblings on cerebellar volume, intracranial space, and body height. The analysis is carried out on the raw data and specifies a model for the mean and the covariance structure. Results suggest that cerebellar volume and intracranial space vary with age and sex. Brain volumes tend to decrease slightly with age, and males generally have a larger brain volume than females. The remaining phenotypic variance of cerebellar volume is largely genetic (88%). These genetic factors partly overlap with the genetic factors that explain variance in intracranial space and body height. The applied method is presented as a general approach for the analysis of intermediate phenotypes in which the effects of correlated variables on the observed scores are modeled through multivariate analysis.

KEY WORDS: Extended twin study; methodology; structural equation modeling; intermediate phenotype; MRI.

INTRODUCTION

The study of the genetics of human behavior has long focused on actual observable behavior, such as smoking, alcoholism, or intelligence (e.g., Maes *et al.,* 1999; Heath *et al.,* 1999; Bouchard and McGue, 1981). Although there is now clear evidence of genetic influences on these behaviors, it has often proven difficult to locate the particular genes that account for these influences (e.g., Petrill *et al.,* 1997; Flint, 1999). The genetic influence on observable behavior is the outcome of a complex interplay between several genes which each may have unique but small effects on the observed behavior. Kosslyn and Plomin (2000) suggested that to increase one's chances of finding the actual genes influencing behavior, it might be wiser to look for genes that are linked to more basic traits (i.e., more directly under the influence of DNA) than behavior. The more basic traits have become known as intermediate phenotypes or endophenotypes (Boomsma *et al.,* 1997; Lander, 1988; Kendler, 1999).

Indices of brain function are already widely used as intermediate phenotypes in the study of behavior. Changes in serotonin neurotransmission may affect mood

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and memory (e.g., Fink *et al.,* 1999), and electrical activity of the brain has been linked to alcoholism, sensation seeking, and cognition (e.g., Schukit, 1986; Rodriguez, 1999; Glass and Riding, 1999; Zuckerman, 1990). Electroencephalographic θ and α oscillations of the brain have been linked to memory performance (for a review see Klimesch, 1999) and the P300 evoked potential has been linked to general IQ (for a concise overview see Detterman, 1994). In the genetics of psychopathology, another main intermediate phenotype is brain structure. Indices of brain structure have been associated with schizophrenia (e.g., Lawrie and Abukmeil, 1998; McCarley *et al.,* 1999), mood disorders (e.g., Drevets *et al.,* 1997), and dementia (e.g., Kaye *et al.,* 1997). Although an obvious intermediate phenotype, human brain structure and volume have received little attention from geneticists. The few studies that have reported on the heritability of brain structure in humans generally report on very specific structures of the brain (e.g., Carmelli *et al.,* 1998; Steinmetz *et al.,* 1994) or have been conducted on small sample sizes (e.g., Bartley *et al.,* 1997). Thus, while many studies report genetic influences on behavior, and a number of studies link behavior to brain structure, there are virtually no studies that report on the genetic or environmental influences on brain structure.

In the light of future investigations of the genetic influences on brain structure or other intermediate phenotypes, we illustrate in this paper how Mx (Neale, 1997) can be used to analyze the genetic and environmental influences on a particular brain structure: the cerebellum. The cerebellum is one of the larger structures of the brain and lies posterior to the brain stem. It is thought to be involved in the coordination of movement and motor functioning (Ghez, 1991). Since the measurement of cerebellar volumes or other structures with magnetic resonance imaging (MRI) is costly, an approach is needed that minimizes the required number of subjects without affecting statistical power. A powerful design to study the genetic and environmental influences on a measured intermediate phenotype like brain structure is the extended twin design (Posthuma and Boomsma, 2000). In this design nontwin siblings are added to the classical twin design (as opposed to recruiting more twin families), which yields increased statistical power to detect genetic and shared environmental influences on a measured variable. Extended twin designs, however, provide data characterized by families of variable size, i.e., some families may include twins and one nontwin sibling, while other families may include twins and two or three nontwin siblings. Analyzing families of variable size means having to deal with the nuisance of having missing data and requires a statistical package which efficiently handles variable pedigree sizes.

Intermediate phenotypes are often correlated with other observed variables. For example, age and sex are known to affect cerebellar volume (see, e.g., Luft *et al.,* 1999; Raz *et al.,* 1998; Passe *et al.,* 1997). Also, cerebellar volume is expected to covary with body height and intracranial space. These two types of "confounders" need to be addressed differently. In nongenetic designs, it is common practice to regress out the effects of body height and intracranial space on brain volumes. However, in the hypothetical situation where half of the phenotypic variance in cerebellar volume is due to genetic factors which are shared with genetic factors that influence both intracranial space and body height, such a regression approach will lead to the conclusion that phenotypic variance in cerebellar volume is low. Applying a multivariate approach would correctly show the heritability of cerebellar volume.

In the present paper an approach is illustrated that deals with these two issues simultaneously: correction for linear effects of age and sex on multivariate observed scores (of cerebellar volume, body height, and intracranial space) in an analysis that allows estimation of genetic and environmental (co-)variation of these multivariate phenotypes. This analysis is embedded in an extended twin design to maximize the statistical power.

METHODOLOGY

A Linear Regression Model for Causal Effects on Observed Scores

Both age and sex are associated with body height, intracranial space, and cerebellar volume. In order to correct for these effects we employ a linear regression model for a continuous trait Y_j ($j = 1, \ldots, m$, where m is the number of phenotypes) with observed values $y = (y_{ij}, \ldots, y_{nj}$, where n is the total number of subjects).

In the subsequent analyses two explanatory variables (x_1 and x_2; age and sex, respectively) have causal effects on the observed individual scores of height, intracranial space, and cerebellar volume. All variables were multivariately normal distributed conditional on the values of age and sex except for body height in additional siblings. This was totally explained by one very tall male additional sibling. Inclusion of this individual did not influence the results presented in this paper.

The linear regression model for individual i (i =1, . . ., n) and trait j (1, . . ., m) is

$$\mu_{ij} = \beta 0_j + \beta 1_j age_i + \beta 2_j sex_i$$

where μ_{ij} is the expected value of individual i on variable j, age_i is the individual value of the first (age, in years) explanatory variable, and sex_i is the individual value of the second (sex; 0 denotes female, 1 denotes male) explanatory variable. $\beta 0_j$ is the intercept (grand mean) of variable j, $\beta 1_j$ is the regression estimate of age for variable Y_j, and $\beta 2_j$ is the deviation of males on variable Y_j.

Trivariate Analysis

Simultaneously with the linear correction for age and sex, the covariance of cerebellar volume with body height and intracranial space is modeled, using a triangular decomposition of the (co-)variance matrix of these traits (Neale and Cardon, 1992). With this decomposition it is possible to investigate whether the observed covariance between traits is due to a common set of genes and/or due to a common set of environmental influences. For example, diet habits may influence both body weight and cholesterol levels, yielding a phenotypic correlation caused by a common environmental factor.

We used a trivariate, triangular decomposition model including regression on the observed scores, in which all latent variances which are part of the variance decomposition model have been scaled to unity. This must be distinguished from the variances of the definition variables (age and sex) which are part of the regression model; these definition variables concern individual observed values and therefore have no variance (N = 1 for each individual).

The triangular variance decomposition model can easily be redefined as a model in which the common pathways are recalculated into correlations by following the general tracing rules of path analysis (Wright, 1934; Neale and Cardon, 1992, Chap. 13) and applying the general formula for calculating a correlation. If the coefficients from the paths of A1 (the first latent additive generic factor) to body height (B) and intracranial space (I) are denoted a1b and a1i, respectively, and the coefficient of the path from A2 (the second latent additive generic factor) to intracranial space is denoted a2i, the genetic correlation between body height and intracranial space [r_g (B, I)] is obtained as follows:

$$r_g(\mathrm{B,I}) = \mathrm{a1b} \times \mathrm{a1i} \,/\, [\mathrm{a1b}\sqrt{((\mathrm{a1i})^2 + (\mathrm{a2i})^2)}]$$

The (non-)shared environmental correlation is calculated analogously.

Handling Variable Pedigree Sizes

Extended twin designs provide data characterized by families of variable size. Such "incomplete" data can be analyzed in Mx (Neale, 1997) via full information maximum likelihood, which uses the observed data. To obtain a measure of how well the specified model for means and covariances fits the observed values, the raw data option in Mx calculates the negative log-likelihood (–LL) of the raw data for *each* pedigree (Lange *et al.,* 1976), as

$$-\mathrm{LL} = -k \log(2\pi) + \log|\Sigma| + (y_i - \mu_i)' \, \Sigma^{-1} (y_i - \mu_i)$$

where k (k = 1, . . ., p; p = number of family members times number of phenotypes) denotes the number of observed variables within a family (and can vary over families), $\Sigma(pxp)$ is the expected covariance matrix of family members, y_i (for i = 1, . . ., p) is the vector of observed scores, μ_i the column vector of the expected values of the variables, and $|\Sigma|$ and Σ^{-1} are the determinant and inverse of matrix Σ, respectively.

Combining the expression of the −LL for each pedigree with a linear model for the expected scores as outlined previously gives a new expression for the −LL:

$$-\mathrm{LL} = -k \log(2\pi) + \log|\Sigma| + (y_i - \beta 0 - \beta 1 x1_i - \beta 2 x2_i)' \\ \Sigma^{-1} (y_i - \beta 0 - \beta 1 x1_i - \beta 2 x2_i)$$

Since the families are independent, their joint likelihood is simply the product of their individual likelihoods and the log of the joint likelihood is the sum of the log-likelihoods per family. Thus, summing the negative likelihoods (–LL's) of all families gives the –LL of the model. In Mx the –LL of the model is doubled because twice the difference between two models (2 [$-\mathrm{LL}_{full\ model}$ − ($-\mathrm{LL}_{nested\ model}$)]) is—under certain regularity conditions—asymptotically distributed as χ^2. Thus, two nested models (a nested model includes fewer parameters and does not introduce new parameters compared to the model under which it is nested), which provide −2LL's, may be subtracted to provide a $\Delta(-2\mathrm{LL})$ which has a χ^2 distribution. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model.

An example Mx job that can be used to conduct the trivariate analysis with a linear correction of age

and sex on the individual scores in a design with variable pedigree sizes is available at the Mx website, http://views.vcu.edu/mx/examples.html, in the brain section.

Subjects

Subjects were recruited from The Netherlands Twin Registry (Boomsma, 1998) (170 cases) and through the Utrecht Medical Centre Twin Sample (86 cases). All subjects underwent physical and psychological screening to exclude cases of pathology known to affect brain structure.

Subjects were 256 family members from a total of 111 twin families. In total, 34 siblings (aged 29.6 years; SD, 4.81 years; 15 female, 19 male), 32 MZ male twin pairs (aged 30.34 years; SD, 9.20 years), 17 DZ male twin pairs (aged 30.3 years; SD, 7.01 years), 21 MZ female twin pairs (aged 34.1 years; SD, 11.68 years), 20 DZ female twin pairs (aged 30.6 years; SD, 8.48 years), and 21 DZ opposite-sex twin pairs (aged 30.3 years; SD, 12.35 years) participated. Seventy-seven families consisted of a twin pair and 34 families consisted of a twin pair and one additional sibling.

Cerebellar volume and intracranial space were obtained by 1.5-T MRI as described by Baaré *et al.* (2000) and analyzed according to the method described by Staal *et al.* (2000) and Hulshoff Pol *et al.* (2000).

RESULTS

Descriptive Statistics

Significant correlations (corrected for the effects of age) were observed between body height and intracranial space (0.194 and 0.229 for males and females, respectively; see Table I) and between body height and cerebellar volume (0.280 and 0.194 for males and females, respectively). In addition, a substantial correlation of 0.593 for males and 0.575 for females was observed between intracranial space and cerebellar volume.

Twin- and sib-pair correlations, as given in Table II, suggest that cerebellar volume, as well as body height and intracranial space, is largely heritable. The low DZM correlation was due mainly to two DZM pairs with large intrapair differences. However, in these two pairs individual scores were in the normal range and there was no indication of environmental confounding, so they were included in the analyses.

Model Fitting

When using raw data, the fit (−2LL) of a model can merely provide information on how well a more parsimonious model fits the data relative to a more general model. To gain some insight into the fit of the ACE model, which is the basic model for nested models AE/CE and E, we report the −2LL of a saturated model. In this saturated model the means are modeled in a similar way as in the ACE models, while the variance/covariance structure is not modeled, and all variances and covariances in MZ and DZ twins are estimated.

First, univariate genetic models for height, intracranial space, and cerebellar volume were fitted to the data correcting for the effects of age and sex on the observed scores. The regression estimates of the linear regression models for the observed scores of body height, intracranial space, and cerebellar volume show that height, intracranial space, and cerebellar volume decrease with age in our sample and are larger in males

Table I. Means and Intercorrelations of Cerebellar Volume, Intacranial Space, and Height

	Mean	SD	Body height	Intracranial space
Body height (cm)				
Male	181.94	6.66	—	—
Female	168.50	6.55	—	—
Intracranial space (cm^3)				
Male	1504.10	107.01	0.194*	—
Female	1340.26	113.09	0.229*	—
Cerebellar volume (cm^3)				
Male	146.80	11.17	0.280**	0.593**
Female	133.56	12.15	0.194*	0.575**

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Table II. Twin and Sibling Correlations by Zygosity[a]

	MZM (32)[b]	MZF (21)[b]	DZM (17)[b]	DZF (20)[b]	DOS (21)[b]	TSM (15 + 11)[c]	TSF (8 + 11)[c]	TSOS (11 + 12)[c]
Body height	0.78	0.92	0.61	0.64	0.47	0.70	0.31	0.15
Intracranial space	0.90	0.92	0.33	0.70	0.40	0.67	0.62	−0.07
Cerebellar volume	0.85	0.93	−0.06	0.78	0.27	0.66	0.77	−0.12

[a] MZM/MZF—monozygotic male/female; DZM/DZF/DOS—dizygotic male/female/opposite sex; TSM/TSF/TSOS—twin-sib pair male/female/opposite sex.
[b] Pairs.
[c] Twin-sib correlations are calculated as the mean correlation of all "first" twins with their nontwin sibling and all "second" twins with their nontwin sibling. The number of pairs denotes the number of first twins with siblings and the number of second twins with siblings. Please note that for TSM and TSF, in all families except DOS families, the nontwin sibling provides two correlations: one with the first twin and another one with the second twin.

Table III. Regression Estimates of the Linear Regression Model on the Means of Body Height, Intracranial Space, and Cerebellar Volume

	β_0 (grand mean)	β_1 (effect of age; age entered in years)	β_2 (deviation of males)
Body height (cm)	172.20	−0.11	13.16
Intracranial space (cm^3)	1345.63	−0.33	169.82
Cerebellar volume (cm^3)	140.94	−0.23	12.70

than in females (Table III). This decrease with age may also reflect a cohort effect in our sample.

From the univariate regression analyses the expected value for an individual can be calculated. For example, the expected cerebellar volume (cm^3) for a male subject aged 30 is $140.94 - (0.23 * 30) + 12.70 = 146.74\ cm^3$.

Simultaneous with the correction for the effects of age and sex, the remaining phenotypic variance was decomposed into sources of variance due to additive genetic factors, shared environmental factors, and nonshared environmental factors. Comparison of the fit of the variance decomposition models with the saturated model shows that the ACE model describes the data reasonably (body height and intracranial space) to well (cerebellar volume). The most parsimonious model of the variance decomposition models for all three variables was a model in which additive genetic influences and unique environmental influences contributed to the phenotypic variance, whereas the influence of common environmental factors was nonsignificant (Table IV). Table IV includes the estimates and 95% confidence intervals for A, C, and E as found in the full ACE model. As expected, the observed variance in body height is highly heritable; 72% (47–92%) of the total variance is explained by genetic factors in the full ACE model. The heritabilities of intracranial space and cerebellum are also high; estimates for sources of variance due to genetic factors are 65% (40–91%) and 81% (54–92%), respectively.

In the multivariate analysis the influence of common environmental factors was, again, not significantly different from zero (dropping C from the ACE model caused an increase in −2LL of 4.546 for a gain of 6 df's). The fit of the multivariate ACE model was reasonable compared to that of a saturated model (Δ−2LL, 138.681; Δdf,72).

The regression coefficients in the multivariate analysis (AE model) are slightly different from the regression weights as estimated in the three univariate analyses. Figure 1 shows the unstandardized estimates in the triangular variance decomposition model. The unique environmental correlations between body height and intracranial space and between body height and cerebellar volume were nonsignificantly different from zero (Δdf = 2, Δ−2LL = 3.293) and were excluded from the models to which Table V refers. In Table V the genetic and unique environmental correlations (Table Va) and the standardized genetic contributions of body height and intracranial space to the total variance of cerebellar volume are given, as well as the unique genetic variance of cerebellar volume (Table Vb).

Table IV. Nested Sequence of Univariate ACE Models with Linear Regression of Age and Sex on the Observed Value of Body Height, Intracranial Space, and Cerebellar Volume Fitted to the Raw Data

	Saturated model		ACE model		CE model		AE model		Standardized estimates in full model (95% confidence interval)			Standardized estimates in best-fitting model (95% confidence interval)	
	−2LL	df	−2LL	df	−2LL	df	−2LL	df	A	C	E	A	E
Body height	1556.32	241	1574.34	250	1601.30	251	1575.69	251	72% (47–92)	17% (0–41)	11% (7–18)	89% (82–92)	11% (8–18)
Intracranial space	2991.761	241	3010.76	250	3037.76	251	3012.83	251	65% (40–91)	23% (0–47)	12% (8–19)	89% (12–92)	12% (8–19)
Cerebellar volume	1860.22	241	1863.76	250	1895.94	251	1863.94	251	81% (54–92)	7% (0–34)	12% (8–19)	89% (81–92)	12% (8–19)

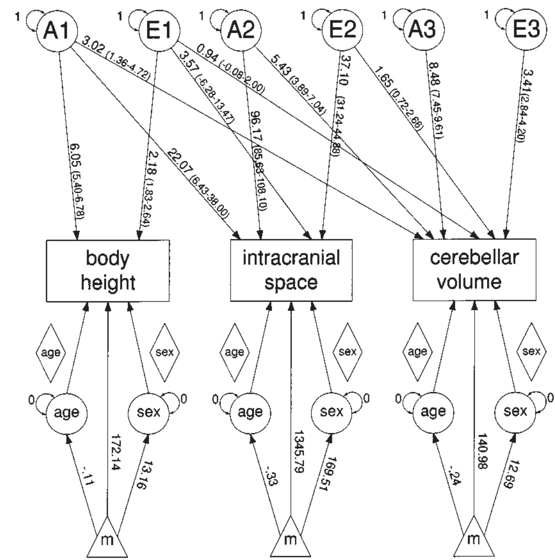

Fig. 1. Results of multivariate model fitting. The upper half shows the decomposition of the variance and covariance for body height, intracranial space, and cerebellar volume. Path coefficients are unstandardized; standardized estimates are given in Tables Va and b. The lower half represents the regression weights as estimated in the multivariate model, which may differ slightly from those estimated in the univariate analyses.

The genes that account for individual differences in body height also account to some extent for individual differences in both intracranial space and cerebellar volume (genetic correlations are 0.21 and 0.25, respectively). The genetic correlation of 0.57 between intracranial space and cerebellar volume indicates that some, but not all, of the genes that influence intracranial space are also important for cerebellar volume. Since the proportion of variance accounted for common environmental influences for each trait is relatively low, the common environmental correlation between intracranial space and cerebellar volume (0.44) can be misleading: although of medium size, it explains only a relatively small part of the total covariance between these two traits.

Six percent of the total variance in cerebellar volume is accounted for by genetic factors shared with body height, 24% is accounted for by genes that are shared with intracranial space, and 58% of the total variance in cerebellar volume is due to genetic factors that are unique to cerebellar volume.

DISCUSSION

Direct effects of age and sex on body height, intracranial space, and cerebellar volume were modeled

Table V

(a) Genetic (lower half) and unique environmental (upper half) correlations with 95% confidence intervals (in parentheses)

	Body height	Intracranial space	Cerebellar volume
Body height	—	n.s.	n.s.
Intracranial space	0.21 (0.06–0.36)	—	0.44 (0.21–0.63)
Cerebellar volume	0.25 (0.10–0.40)	0.57 (0.44–0.67)	—

(b) Standardized estimates in the multivariate approach of components of the genetic variance of cerebellar volume after correction for the effects of age and sex on the observed values

Cerebellar volume	Total genetic variance	Genetic variance due to genes that also influence body height	Genetic variance due to genes that also influence intracranial space	Remaining genetic variance unique to cerebellar volume
Estimate	88%	6%	24%	58%
95% confidence interval	81–92%	1–14%	14–36%	47–69%

simultaneously with a multivariate genetic model for the covariance between family members. For all three variables a slight decrease with age was found and a significant deviation for males, who were taller and had larger brain volumes than females. A trivariate genetic analysis was conducted on body height, intracranial space, and cerebellar volume, to dissect the pattern of covariance among these three variables and to determine the relative contributions of genetic and environmental influences to the remaining variance of each of these variables. For intracranial space and cerebellar volume, genetic factors accounted for 88% of the phenotypic variance. A large part of the genetic factors that are associated with cerebellar volume also controlled intracranial space (24%). Genetic factors that explain phenotypic variance in body height, however, accounted for only a small part of the genetic variation in both intracranial space and cerebellar volume. These findings suggest that studies using cerebellar volume as an intermediate phenotype will also need to consider the genetic covariance of cerebellar volume with intracranial space.

The causes of interindividual variation in human brain structure are largely unknown. This study shows that at least for one brain structure, cerebellar volume, interindividual differences are due largely to genetic variation between individuals. In mouse studies, several genes have already been implicated that influence development of the cerebellum. For example, Favor *et al.* (1996) showed that in mice, functioning of the *Pax2* locus, which has its counterpart in the human *PAX2* locus, is absolutely necessary for the normal development of the cerebellum. In addition, Millen *et al.* (1994) reported a reduction in cerebellar volume in mice due to dysfunctioning of the *En-2* locus.

Besides being of importance in its own right, a high heritability of human cerebellar volume in particular and brain structure in general may be of crucial importance in the study of causes of variation in complex behaviors. For example, correlations between brain size and psychometric IQ range between 0.38 and 0.45 [see Storfer (1999) for an overview of brain size–IQ relations], depending on which brain structure (i.e., gray matter volume, white matter volume, cerebral volume) is studied.

Quantitative intermediate phenotypes with high heritability are becoming more and more important in the field of behavioral genetics (e.g., Flint, 1999; Begleiter *et al.,* 1999; Boomsma *et al.,* 1997). These phenotypes are more “upstream,” as Kosslyn and Plomin (2000) put it, and it is possible that they are influenced by a smaller number of genes, which could facilitate detection of these genes. In addition, quantitative intermediate phenotypes can also be obtained from nonaffected individuals. Thus, if a strong relationship between some brain structure and a psychiatric trait exists, such as the association between a reduction in prefrontal cortex volume and uni- and bipolar depression (Drevets *et al.,* 1997), it might be wiser to put a continuous index of prefrontal cortex volume in a time-consuming search for genes than to use a measure of uni- or bipolar depression.

Although a strong phenotypic relationship between the brain and the behavior is prerequisite, an equally important requirement for the intermediate phenotype to be of use in linkage studies is that it has a high genetic correlation with the behavior. Therefore, the intermediate phenotypes and the target behavior need to be analyzed in a multivariate design. Such a design must allow for the correction of covariates such as age and sex. Finally, since intermediate phenotypes require psychophysiological measurements, they are usually more complex and costly than behavioral measures obtained from observation, interview, or questionnaires, which makes it crucial to use an optimal statistical design. The present study shows that all three requirements for the genetic analyses of intermediate phenotypes (i.e., multivariate genetic analysis, correcting for linear effects on the mean, and optimal statistical power) can be handled in a single statistical approach using the Mx statistical package.

An additional advantage of the approach used in this paper is that it can easily be generalized to association analysis of quantitative trait loci (QTL). Measured covariates are not limited to sex and age but can also include polymorphic markers or candidate genes (e.g., Neale *et al.,* 2000) which can be modeled directly (Zhu *et al.,* 1999) or more sophisticatedly via within- and between-family effects (Fulker *et al.,* 1999; Sham *et al.,* 2000).

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Chapter 7a

The association between brain volume and intelligence is of genetic origin[1]

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The association between brain volume and intelligence is of genetic origin

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To THE EDITOR—The recent study by Thompson and colleagues[1] reported high heritability of gray-matter volume in several cortical regions using voxel-based MRI techniques. Gray matter substantially correlated with general intelligence, or '*g*'. These findings prompt three major questions: (i) is the high heritability specific to gray-matter volume, (ii) is the correlation with *g* specific to gray-matter volume and (iii) is the correlation between gray-matter volume and *g* of genetic or environmental origin?

We addressed the first question in a large Dutch sample of twins and their siblings (258 Dutch adults from 112 extended twin families)[2]. We found high heritability for total brain gray-matter volume (Table 1), comparable to the estimate reported by Thompson and colleagues[1]. In addition, we found high heritability for total brain white-matter volume.

As stated in a commentary[3] on the recent report in *Nature Neuroscience*[1], high heritability of gray matter implies that interindividual variation in cell-body volume is not modified by experience. Because white matter reflects the degree of interconnection between different neurons, interindividual variance in white-matter volume might be expected to be more under the influence of experience and less under genetic control. Our results clearly suggest otherwise. Either environmental experience barely contributes to interindividual variation in white-matter volume or, alternatively, exposure to relevant environmental experience is under strong genetic control.

The subjects for whom MRI scans were available partly overlapped with a sample of extended twin families from a large study on cognition[4]. In the latter study, we found a heritability of *g* of 0.86, which is consistent with previous reports[5]. We have now confirmed a correlation between gray-matter volume and *g* (0.25; $p < 0.05$) and, in addition, have found a significant correlation between white-matter volume and *g* (0.24; $p < 0.05$). Thus, regarding the first two questions, we conclude that white-matter volume is also highly heritable and that *g* is related to the volumes of both gray and white matter.

In twin samples of sufficient size, the correlation between brain volume and *g* can be decomposed into genetic and environmental components[6]. Such analysis is based on the comparison of cross-trait/cross-twin correlations for monozygotic (MZ) and dizygotic (DZ) twins (or sibling pairs). If the correlation between brain volume of a twin and *g* in the co-twin is larger in MZ than in DZ twins, this indicates that the genes influencing brain volume partly overlap with the genes that influence *g*. The extent of the overlap is reflected by the magnitude of the genetic correlation. When the cross-trait/cross-twin correlations are similar for MZ and DZ twins, this suggests that environmental factors contribute to the observed phenotypic correlation between brain volume and *g*. Given a heritability of 0.85 for brain volume[2], a heritability of 0.80 for *g* (ref. 5) and a correlation between brain volume and *g* of 0.40 (ref. 7), at least 17 MZ and 17 DZ pairs are needed to detect a genetic correlation with 80% power (and $\alpha = 0.05$) that explains the observed correlation.

In 24 MZ pairs, 31 DZ pairs and 25 additional siblings, we decomposed the correlation between brain volumes and *g* into genetic and environmental components by using structural equation modeling for a multivariate genetic design (gray matter, white matter and g)[6]. This showed that the correlation between gray-matter volume and *g* was due completely to genetic factors and not to environmental factors. We obtained the same result for the correlation between white-matter volume and *g*. Thus, the answer to the third question is

Table 1. Heritability estimates from multivariate genetic analyses

	Heritability
Whole-brain gray matter	0.82
Whole-brain white matter	0.87
General intelligence (*g*)[a]	0.86
Working memory[a]	0.67

[a]WAIS-IIIR IQ test.
Details of brain imaging methods and subject characteristics of the MRI sample (258 subjects from 112 extended twin families) have been described previously[2], as have detailed characteristics of the WAIS-IIIR sample (688 subjects from 271 extended twin families)[4]. The overlapping dataset consisted of 135 subjects from 60 extended twin families (24 MZ pairs, 31 DZ pairs and 25 additional siblings).

Table 2. Observed (phenotypic) correlations, cross-trait/cross-twin correlations, genetic correlations and environmental correlations

	Correlation			
	Observed	MZ cross-trait/ cross-twin	DZ cross-trait/ cross-twin	Genetic
Whole-brain gray matter–*g*	0.25*	0.26*	0.14	0.29*
Whole-brain gray matter–Working memory	0.29*	0.32*	0.20*	0.38*
Whole-brain white matter–*g*	0.24*	0.22*	0.19	0.24*
Whole-brain white matter–Working memory	0.29*	0.27*	0.19	0.35*

*Significantly different from zero at the $\alpha = 0.05$ level.
MZ, monozygotic twins; DZ, dizygotic twins, including sibling pairs. The cross-trait/cross-twin correlations in MZ pairs showed evidence for a genetic mediation between brain volumes and *g* (and its working-memory component); the cross-trait/cross-twin correlation for MZ pairs was as high as the correlation between brain volume and *g* (or working memory) within the same person. In other words, the IQ of MZ twins was predicted equally well from the size of the brain of their co-twins as from the size of their own brain. All analyses were carried out using maximum likelihood estimation implemented in Mx software[11]. Mx is especially suited to handle incomplete data structures, as is the case when not all variables have been measured in all subjects. Effects of sex and age have been regressed out on the observed scores. Observed correlations and cross-trait/cross-twin correlations were estimated from a saturated model in which the (co-) variances are not decomposed, whereas the genetic and environmental correlations were estimated from a multivariate genetic design in which the (co-) variances are decomposed into genetic and environmental components. The 'genetic correlation' reflects the correlation between the set of genes that influences brain volume and the set of genes that influences intelligence. The 'genetic contribution to the observed correlation' can be derived as the product of the genetic correlation and the square roots of the heritabilities of the two phenotypes.

that the correlation between brain volumes and *g* is, as anticipated[8], mediated entirely by genetic factors (Table 2).

As our measure of *g* consisted of the IQ score on the WAIS-IIIR IQ test, we were also interested in whether a particular dimension of *g* correlated more highly with gray- and white-matter volume than any other dimension. The four standard WAIS-IIIR dimensions are Verbal Comprehension, Perceptual Organization, Processing Speed, and Working Memory. We found that the Working Memory dimension had the highest phenotypic and highest genetic correlation with brain volumes. This is perhaps not surprising, because working memory is considered a major component of *g* (ref. 9). Also, working memory is often 'localized' in the frontal lobes[10], which provides further convergence between our findings and those of Thompson and colleagues[1].

Establishing that the correlation between brain volumes and *g* is mediated by common genetic factors is only the first step in unveiling the relation between them. The next step will be to identify specific genes that influence both brain volume and *g*.

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We welcome short letters on matters arising from previous papers in *Nature Neuroscience* or on other topics of widespread interest to the neuroscience community. Because space in this section of the journal is limited, priority is given to short (fewer than 500 words), well-written letters addressing the most topical issues. Typically, new data are not presented in this section, although they may occasionally be allowed at the discretion of the editors. Letters concerning material previously published in *Nature Neuroscience* are usually sent to the authors of the original piece for their comments and/or a formal reply. Letters may be edited for brevity and clarity.

Chapter 7b

Brain volumes and intelligence[1]

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Genetic Correlations Between Brain Volumes and the WAIS-III Dimensions of Verbal Comprehension, Working Memory, Perceptual Organization, and Processing Speed

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We recently showed that the correlation of gray and white matter volume with full scale IQ and the Working Memory dimension are completely mediated by common genetic factors (Posthuma et al., 2002). Here we examine whether the other WAIS III dimensions (Verbal Comprehension, Perceptual Organization, Processing Speed) are also related to gray and white matter volume, and whether any of the dimensions are related to cerebellar volume. Two overlapping samples provided 135 subjects from 60 extended twin families for whom both MRI scans and WAIS III data were available. All three brain volumes are related to Working Memory capacity (r = 0.27). This phenotypic correlation is completely due to a common underlying genetic factor. Processing Speed was genetically related to white matter volume (r_g = 0.39). Perceptual Organization was both genetically (r_g = 0.39) and environmentally (r_e= –0.71) related to cerebellar volume. Verbal Comprehension was not related to any of the three brain volumes. It is concluded that brain volumes are genetically related to intelligence which suggests that genes that influence brain volume may also be important for intelligence. It is also noted however, that the direction of causation (i.e., do genes influence brain volume which in turn influences intelligence, or alternatively, do genes influence intelligence which in turn influences brain volume), or the presence or absence of pleiotropy has not been resolved yet.

Two independent studies recently quantified the contribution of genetic and environmental factors to interindividual differences in brain volumes (Baaré et al., 2001; Thompson et al., 2001). Baaré et al. (2001) used magnetic resonance imaging (MRI) to measure intracranial space, total brain volume, total white matter volume, total gray matter volume, and lateral ventricle volume in 258 subjects belonging to 112 (extended) twin families. They reported very high heritabilities for all volumes (ranging from 80 to 90%) except ventricular volume (no genetic influences). Thompson et al. (2001) used voxel based MRI techniques on 10 MZ twin pairs and 10 DZ twin pairs and reported high heritability of gray matter volume in several cortical regions. Results from previous studies also suggested that genetic factors are much more important than environmental factors for inter-individual differences in brain volumes (Bartley et al., 1997; Carmelli et al., 1998; Carmelli et al., 2002; Pennington et al., 2000; Reveley et al., 1984).

Since the second half of the 19th century positive correlations between head size (as measured with a measuring tape around the head) and psychometric intelligence have been observed. Correlations generally range around 0.20 (Jensen, 1994; Posthuma et al., 2001a), but can be as high as 0.44 (van Valen, 1974). MRI provides a more accurate measure of the size of the brain, as head size includes both brain volume and thickness of the skull. Several studies have correlated MRI-brain volumes with measures of intelligence, and, on average, brain volume as measured with MRI and IQ correlate around 0.40 (e.g., Andreasen et al., 1993; Egan et al., 1994; Raz et al., 1993; Storfer, 1999; Wickett et al., 2000; Willerman et al., 1992).

Three multivariate genetic studies of brain volume and intelligence have investigated the nature of the correlation between brain volume and intelligence (Pennington et al., 2000; Thompson et al., 2001; Wickett et al., 1997), but did not have the optimal design (Pennington et al., 2000; Wickett et al., 1997) or enough statistical power (Thompson et al., 2001) to decompose the observed correlation into genetic and environmental components. Using a dataset consisting of 24 MZ pairs, 31 DZ pairs, and 25 additional siblings (135 individuals from 60 families) for whom both data on brain volume and intelligence were available, we recently showed that the correlations between gray or white matter volume to full scale IQ (WAIS III) and its Working Memory dimension are completely mediated by an underlying set of genes that influences both brain volumes and IQ (Posthuma et al., 2002).

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Using the same sample of 135 individuals from 60 families we now investigate whether three different brain volumes (total white matter of the cerebrum, total gray matter of the cerebrum, total volume of the cerebellum) are differentially correlated to each of the other standard WAIS III dimensions: Verbal Comprehension, Perceptual Organization, and Processing Speed. We expect to find differential correlations between each of the three brain volumes and each of the four WAIS III dimensions. For example, the WAIS III dimension Processing Speed is an index of the speed of central nervous system processing (WAIS III, 1997), and is therefore expected to be related strongest to white matter volume, as white matter reflects the degree of interconnectiveness between neuronal cells.

The three brain volumes were obtained by using MR imaging in a large Dutch sample (258 Dutch adults from 112 extended twin families) of twins and their siblings (Baaré et al., 2001). Using structural equation modelling on the combined MRI and WAIS III datasets, we will test whether the correlation between these brain volumes and WAIS III dimensions is genetically or environmentally mediated.

Methodology

Subjects — WAIS III Sample

Six hundred eighty-eight family members from 271 extended twin families participated in an ongoing study on the genetics of adult brain function (Posthuma et al., 2001a, 2001b; Posthuma, 2002; Wright et al., 2001) until December 2000. All participants were obtained from the Netherlands Twin Registry (Boomsma, 1998). Zygosity was determined by DNA fingerprinting. The complete sample consisted of two age cohorts: a young adult cohort with a mean of 26.2 years of age (*SD* = 4.19) and an older adult cohort with a mean around 50.4 years of age (*SD* = 7.51). Participating families consisted of one to eight siblings (including twins). On average 2.5 subjects per family participated. In the young cohort 171 males and 210 females participated, in the older cohort 135 and 172 respectively. The young cohort included 54 MZ pairs, 73 DZ pairs, 18 single twins and 109 additional siblings. The older cohort included 48 MZ pairs, 58 DZ pairs, 15 single twins, and 80 additional siblings (for a detailed description of the sample characteristics see Posthuma et al., 2001b). The study was approved by the scientific and ethical committee of the Vrije Universiteit Amsterdam. Subjects were paid NLG 50 (23 EUROS) for participation.

Subjects — MRI sample

The MRI sample was obtained from a large study on the genetics of brain volumes (Baaré et al., 2001; Posthuma et al., 2000). For this second dataset, subjects were recruited from the (healthy) twin sample of the department of Psychiatry of the University Medical Center Utrecht, the Netherlands, and from the Netherlands Twin Registry. One hundred and twelve pairs of twins (112 families), 33 MZ male (MZM), 17 DZ male (DZM), 21 MZ female (MZF), 20 DZ female (DZF), and 21 DZ opposite-sex (DOS), and 19 male (SM) and 15 female (SF) full siblings participated in the study. Zygosity was determined by DNA fingerprinting. Subjects were required not to have any severe medical diseases. Mental and physical health was assessed by means of the Family Interview for Genetic Studies (Nurnberger et al., 1994), and a medical history inventory, respectively. All subjects gave written informed consent to participate in the study after full explanation of the study aims and procedures. The study was approved by the scientific and ethical committee of the University Medical Center Utrecht. Subjects were paid NLG 75 (34 EUROS) for participation.

Overlap Between the Two Samples

The combined dataset consisted of 808 subjects from 322 families. For 135 subjects from 60 families data on both IQ and MRI-scans were available. This "overlapping" dataset consisted of 16 families from which MZ twins (without additional non-twin siblings) participated, 8 families from which the MZ twins and one additional sibling participated, 21 families from which the DZ twins (without additional non-twin siblings) participated, 10 families from which the DZ twins and one additional sibling participated, two families from which one twin and one non-twin sibling participated, and three families from which only one member participated. In other words, the overlapping dataset consisted of 24 MZ twins and 69 DZ twins/sib pairs. The mean age in the overlapping dataset was 29.2 (*SD* = 7.34). There were 57 females and 78 males.

To obtain the most accurate estimates of means and variances of IQ scores and brain volumes, the combined dataset of 808 subjects was used in the analyses, as opposed to using only the subset with data on both MRI and WAISIII scores. In Mx (Neale, 1997) such incomplete datasets can be handled easily (see also Statistical Analyses).

The average time between the MRI scans and the IQ measurements was 13 weeks, ranging from –1.5 years (IQ measurement before MRI scans) to + 3.3 years (MRI scan before IQ measurement). Age at time of IQ measurement was included as an effect on the IQ scores while age at time of the MRI scans was included as an effect on brain volumes.

Intelligence Testing

Psychometric IQ was measured with the Dutch adaptation of the WAIS III (WAIS-III, 1997). As we previously showed (Posthuma et al., 2001b) that our IQ sample is representative of the Dutch population and the present sample size exceeds the WAIS III standardization sample, we report unstandardized raw IQ scores, and explicitly model the effects of sex and age in the multivariate analysis. Individual scores for each subtest except digit-symbol substitution were calculated by weighting the observed score by the maximum possible score on that subtest times 100 (i.e., percentage correct on each subtest). For digit-symbol substitution the number of correct items per 60 seconds was calculated. Nine subtests were used to calculate the four dimensions according to the WAIS III guidelines (1997); Verbal Comprehension (VC; the mean percentage correct of subtests information, similarities, and vocabulary), Working Memory (WM; the mean percentage correct of subtests arithmetic and letter-number sequencing), Perceptual Organization (PO; the mean percentage correct of subtests block design, matrix reasoning, and picture

completion), and Processing Speed (PS; the number of correct items per 60 seconds of subtest digit-symbol substitution). The validity of these four dimensions was recently confirmed by a reanalysis of the WAIS manual data by Deary (2001).

MR Image Acquisition and Processing

MR images were obtained on a 1.5 Tesla Philips Gyroscan scanner at the University Medical Center Utrecht. For volumetric analysis a three dimensional (3D) T1 — weighted, coronal, spoiled gradient echo scan (FFE) of the whole head (TE = 4.6 ms, TR = 30 ms, flip angle = 30^0, 170-180 contiguous slices; $1 \times 1 \times 1.2$ mm^3 voxels), and a coronal dual contrast turbo spin echo (DTSE) of the whole brain (TE1 = 14 ms, TE2 = 80 ms, TR = 6350 ms, 120 contiguous slices; $1 \times 1 \times 1.6$ mm^3 voxels) were acquired.

Images were coded to ensure blindness for subject identification, zygosity and family membership. Image volumes were transformed into Talairach space (no scaling) (Talairach & Tournoux, 1988) and corrected for magnetic field inhomogeneities. Volumetric measurements were obtained using automated segmentation procedures and included intracranial, whole brain, gray and white matter of the cerebrum (excluding cerebellum and brain stem), and lateral and third ventricle volumes (Schnack et al., 2001a; Schnack et al., 2001b). Automatic segmentation software included histogram analysis algorithms, anatomical knowledge based decision rules and series of mathematical morphological operators to connect all voxels of interest. Intracranial volume was segmented on DTSE scans. Cerebral gray and white matter volumes were obtained after cerebellar and brain stem tissue was removed. The segmentation procedures yielded highly reliable volume measurements with inter-rater intraclass correlations all above 0.96.

The present study included gray matter volume of the cerebrum, white matter volume of the cerebrum and cerebellar volume. Cerebellar volume was not separated into white and gray matter volumes as the location of the cerebellum complicates the reliable separation of cerebellar white and gray matter volume (i.e., at the edges of the coil artefacts will influence this separate detection). However, the detection of total cerebellar volume does not suffer from these artefacts and can be reliably measured.

Statistical Analyses

As the sample consisted of unbalanced pedigrees and had some missing data, models were fitted to the raw data instead of covariance matrices. This was accomplished by using the rectangular data file option in Mx (Neale, 1997).

We previously determined whether interindividual variation in each of the four WAIS III dimensions could be explained by additive genetic influences (A), dominance genetic influences (D), shared environmental influences (C), or non-shared environmental influences (E). We found that shared environmental influences on each of these dimensions were non-significantly different from zero, and that all four dimensions were highly heritable (ranging from 66% to 83%) (Posthuma et al., 2001a), in line with estimates from previous studies (Bouchard & McGue, 1981; McClearn et al., 1997). Heritability estimates did not differ across males and females, but cohort differences existed for Working Memory. In the young cohort the genetic variation was mainly due to dominance genetic variation whereas in the older cohort the genetic variation was additive. The broad heritability estimates of the Working Memory dimension, however, were homogeneous across cohorts.

For the MRI measures as well as for the IQ measures shared environmental influences were non-significantly different from zero (Baaré et al., 2001; Posthuma et al., 2000; Posthuma et al., 2001a) and a model that decomposed the variance in genetic variance (A) and non-shared environmental variance (E) fitted well for all brain volumes and WAIS III dimensions. Therefore, the (co-)variances in the multivariate genetic models were decomposed into two possible latent sources of variance: genetic variance (A), and non-shared environmental variance (E). The latter also includes all sources of variance due to measurement error. For DZ twin pairs (and sibpairs) similarity of addixtive genetic influences was set at 50%, and no similarity in non-shared environmental influences. For MZ twin pairs, similarity of additive genetic variance was set at 100% and similarity in non-shared environmental influences was fixed at zero.

Using Structural Equation Modelling to Decompose the (co-)variance into Genetic and Environmental Components

Decomposition of the variances and covariances into genetic (A) and environmental (E) components was obtained using structural equation modelling with maximum likelihood estimation. Environmental factors incorporate those factors in the environment that are not shared by siblings. Let matrices A and E be symmetric and of dimensions 7×7 (for seven variables; total gray matter, total white matter, cerebellar volume, Verbal Comprehension, Working Memory, Perceptual Organization, and Processing Speed). Matrix A denotes the genetic component while matrix E denotes the environmental component. The diagonal elements of matrix A denote the genetic variances of each of the seven variables. For example, element a_{11} is the genetic variation in gray matter volume. The off-diagonal elements of matrix A represent the genetic covariance between variables. Analogously, the diagonal elements of matrix E denote the environmental variances of the seven variables, and the off-diagonal elements denote the covariances due to environmental influences.

As matrices A and E are covariance matrices, they are restricted to be positive definite. This is accomplished by calculating matrix A and E as the product of a triangular matrix and its transpose. Thus, matrix A is calculated as $X \times X'$, where X is triangular and of dimensions 7×7 (for seven variables). Analogously, matrix E is $Z \times Z'$. This is also known as a Cholesky factorization of matrices A and E.

The decomposition of variances and covariances into genetic and environmental components necessitates the use of a genetically informative design, such as the twin design. The correlation between the genetic component that influences the phenotype of one twin and the genetic component that influences the phenotype of the co-twin is 1 for MZ twins and 0.5 for DZ twins/sibling pairs. The correlation between the environmental component that influences the phenotype of one twin and the environmental component

that influences the phenotype of the co-twin is zero for both MZ and DZ twins/sibling pairs.

The variance is formally represented as

$$A + E = X \times X' + Z \times Z'.$$

The covariance is formally represented as

$$A = X \times X' \quad \text{for MZ twins,}$$

$$0.5 \times A = 0.5 \times X \times X' \quad \text{for DZ twins.}$$

The *genetic correlation* between variables i and j (a_{ij}) is derived as the genetic covariance (a_{ij}) between variables i and j divided by the square root of the product of the genetic variances of variables i (a_{ii}) and j (a_{jj}):

$$r_{gij} = \frac{a_{ij}}{\sqrt{a_{ii} \times a_{jj}}}.$$

Analogously, the *environmental correlation* (r_{eij}) between variables i and j is derived as the environmental covariance between variables i and j divided by the square root of the product of the environmental variances of variables i and j:

$$r_{eij} = \frac{e_{ij}}{\sqrt{e_{ii} \times e_{jj}}}.$$

The phenotypic correlation (r) is the sum of the product of the genetic correlation and the square roots of the genetic variances of the two phenotypes and the product of the environmental correlation and the square roots of the environmental variances of the two phenotypes. Or, in other words, the phenotypic correlation is composed of a genetic contribution and an environmental contribution.

$$r = r_{gij} \times \sqrt{\frac{a_{ii}}{(a_{ii} + e_{ii})}} \times \sqrt{\frac{a_{jj}}{(a_{jj} + e_{jj})}} + r_{eij} \times \sqrt{\frac{e_{ii}}{(a_{ii} + e_{ii})}} \times \sqrt{\frac{e_{jj}}{(a_{jj} + e_{jj})}}.$$

Results

Descriptives

Means, and standard deviations of the WAIS III scores clearly show that males generally have a higher score on the WAIS III dimensions, except on Processing Speed, where females are faster than males (Table 1a). These effects are evident in both the young and the older cohort. Subjects in the older cohort generally have a lower score on all four WAIS III dimensions (see Table 1a).

The MRI-data set was not divided in two age cohorts as the median of age was 28.6 years and 83% of the sample were younger than 36. Thus, for brain volumes, age (at time of MRI-scan) was included as a linear effect on the mean volumes. A sex difference in brain volume is evident in each of the three total volumes; males generally have larger volumes than females (see Table1b).

Table 2 presents the phenotypic correlations on the variables adjusted for the effects of sex, age or cohort.

The Working Memory dimension of the WAIS III consistently and significantly correlated to all three brain volumes. The Verbal Comprehension dimension did not correlate significantly with any of the brain volumes. Perceptual Organization correlated significantly with gray matter volume and cerebellar volume, but not with white matter volume. Processing speed correlates significantly with white matter volume, and the correlation with gray matter volume was almost significant ($p = 0.07$).

A seven-variate Cholesky decomposition of gray matter volume, white matter volume, cerebellar volume, Verbal Comprehension, Working Memory, Perceptual Organization, and Processing Speed was conducted to estimate the contributions of genetic factors and non-shared environmental

Table 1a

Descriptives of WAIS III Dimensions

	Age	VC	WM	PO	PS
Young Females					
Mean	26.0	63.6	62.2	79.9	44.0
N	210	210	210	210	208
SD	4.0	11.2	13.0	11.0	6.7
Young Males					
Mean	26.3	67.5	66.1	83.9	39.4
N	171	171	171	171	168
SD	4.4	12.6	13.2	9.4	6.4
Older Females					
Mean	50.5	58.1	54.2	66.1	35.7
N	172	172	172	172	172
SD	7.7	13.2	13.5	12.2	8.4
Older Males					
Mean	50.3	65.0	64.0	69.8	35.3
N	135	135	135	135	135
SD	7.3	13.0	12.4	12.7	6.3

Note: VC = verbal comprehension; WM = working memory; PO = perceptual organization; PS = processing speed. *N* = number of subjects; *SD* = standard deviation.

Table 1b

Descriptives of Brain Volumes

	Age	GMV (in cm^3)	WMV (in cm^3)	CBV (in cm^3)
Females				
Mean	31.7	612.4	422.9	133.6
N	118	118	118	118
SD	10.2	63.0	51.9	12.2
Males				
Mean	30.6	668.2	493.8	147.0
N	140	140	140	140
SD	9.0	60.1	50.1	11.2

Note: GMV = gray matter volume; WMV = white matter volume; CBV = cerebellar volume. *N* = number of subjects; *SD* = standard deviation.

Table 2

Pearson Correlations Between Gray Matter Volume, White Matter Volume, Cerebellar Volume, Verbal Comprehension, Working Memory, Perceptual Organization and Processing Speed. Individual Scores on Each Variable Are Adjusted for the Effects of Sex, Age and Cohort

	GMV	WMV	CBV	VC	WM	PO
WMV	0.59**					
CBV	0.47**	0.49**				
VC	**0.06**	**0.01**	**0.03**			
WM	**0.27****	**0.28****	**0.27****	0.54**		
PO	**0.20***	**0.08**	**0.18***	0.49**	0.51**	
PS	**0.16**	**0.25****	**0.11**	0.28**	0.40**	0.34**

Note*:* Intra-domain correlations Printed in normal text, Inter-domain correlations are printed in bold.

* significant at the 0.05 level; ** significant at the 0.01 level. (*N* = 258 for brain volumes, *N* = 135 for inter–domain correlations; *N* = 688 for WAIS III dimensions).

factors to the phenotypic correlations. Analyses included simultaneous correction for the effects of sex, age or cohort on the individual scores. Table 3a lists the genetic (below diagonal) and environmental correlations from the full AE-Cholesky model, Table 3b gives the heritabilities of each variable — these are not thoroughly discussed as they have been discussed previously (Posthuma et al., 2001a; Posthuma et al., 2000; Baaré et al., 2001). As an illustration, Figure 1 shows MRI scans of four individuals belonging to an MZ and a DZ twin pair. Table 4 provides the path coefficients as estimated.

All inter-domain environmental correlations are statistically non-significantly different from zero (as judged from the 95% CIs) and do not contribute to the observed correlation, except for the environmental correlation (–0.71) between Cerebellar volume and Perceptual Organization. The contribution of environmental factors to the phenotypic correlation is $\sqrt{0.13} \times -0.71 \times \sqrt{0.32} = -0.14$, whereas the contribution of genetic factors to the phenotypic correlation is $\sqrt{0.87} \times 0.35 \times \sqrt{0.68} = 0.27$. Thus the maximum likelihood estimate of the phenotypic correlation is $-0.14 + 0.27 = 0.13$.

Table 3a

Genetic (Below Diagonal) and Environmental (Above Diagonal) Correlations and 95% Confidence Intervals (in Brackets) from the AE Full Cholesky Model

	GMV	WMV	CBV	VC	WM	PO	PS
GMV		**0.00**	**0.08**	**–0.14**	**–0.13**	**0.19**	**0.00**
		(–0.24–0.25)	(–0.17–0.32)	(–0.43–0.18)	(–0.38 –0.15)	(–0.10–0.46)	(–0.27–0.28)
WMV	**0.69**		0.35	**0.07**	**0.03**	**–0.22**	**–0.17**
	(0.58–0.79)		(0.10–0.56)	(–0.35–0.49)	(–0.30–0.38)	(–0.50–0.18)	(–0.50–0.19)
CBV	**0.49**	**0.47**		**–0.23**	**–0.05**	**–0.71**	**0.26**
	(0.35–0.62)	(0.33–0.59)		(–0.58–0.22)	(–0.34–0.29)	(–0.84––0.35)	(0.09–0.54)
VC	**0.15**	**0.05**	**0.03**		**0.19**	**0.11**	**0.09**
	(–0.09–0.37)	(–0.18–0.28)	(–0.19–0.24)		(0.01–0.36)	(–0.07–0.29)	(–0.09–0.27)
WM	**0.40**	**0.33**	**0.30**	**0.66**		**0.12**	**0.04**
	(0.14–0.61)	(0.08–0.55)	(0.07–0.51)	(0.57–0.76)		(–0.05–0.29)	(–0.12–0.22)
PO	**0.10**	**0.01**	**0.35**	**0.61**	**0.72**		**0.04**
	(–0.17–0.36)	(–0.24–0.28)	(0.11–0.57)	(0.51–0.70)	(0.60–0.82)		(–0.13–0.22)
PS	**0.25**	**0.39**	**0.09**	**0.35**	**0.62**	**0.51**	
	(–0.02–0.50)	(0.12–0.63)	(–0.15–0.31)	(0.23–0.47)	(0.49–0.74)	(0.37–0.64)	

Note: Intra-domain correlations in normal text inter-domain correlations in bold.

Table 3b

Maximum Likelihood Estimates of Heritabilities of Brain Volumes and WAIS III Dimensions.

	Heritability
Whole brain gray matter volume	0.82
Whole brain white matter volume	0.87
Cerebellar volume	0.87
Verbal Comprehension	0.84
Working Memory	0.65
Perceptual Organization	0.68
Processing Speed	0.63

The observed correlation between perceptual Organization and Gray matter volume consisted of a genetic contribution (0.07) and an environmental contribution (0.05), both did not reach significance. The other inter-domain phenotypic correlations were all completely explained by an underlying common genetic factor.

Verbal Comprehension is not genetically associated with any of the three brain volumes, whereas Working Memory is genetically associated with all three brain volumes. Processing Speed is genetically related with white matter volume, but not with gray matter volume or cerebellar volume.

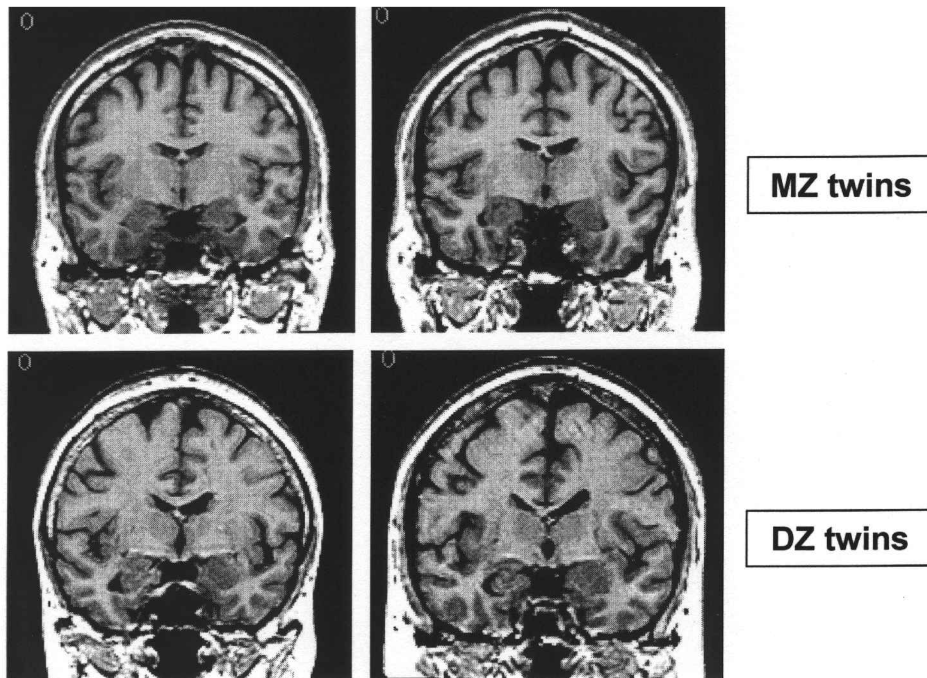

Figure 1

MR images of the brains of a same sex monozygotic twin pair (MZ twins, upper row) and a same sex dizygotic twin pair (DZ twins, lower row).

Table 4

Unstandardized Genetic and Non-shared Environmental Path Coefficients.

	GMV	WMV	CBV	VC	WM	PO	PS
Unstandardized Genetic Path Coefficients.							
GMV	47.93						
WMV	31.55	33.18					
CBV	5.21	1.86	8.98				
VC	1.63	–0.78	–0.44	10.96			
WM	4.19	0.86	1.11	6.60	6.97		
PO	0.91	–0.75	3.38	5.65	3.66	5.11	
PS	1.36	1.64	–0.58	1.84	2.44	1.55	3.58
Unstandardized Non-shared Environmental Path Coefficients.							
GMV	22.54						
WMV	0.04	17.94					
CBV	0.31	1.42	3.76				
VC	–0.71	0.35	–1.28	4.66			
WM	–1.01	0.20	–0.40	1.23	7.58		
PO	1.19	–1.42	–4.36	–0.19	0.78	4.14	
PS	0.01	–0.71	1.43	0.85	0.15	1.53	3.37

Discussion

Phenotypic correlations between gray and white matter volume, and cerebellar volume on the one hand, and the four WAIS III dimensions (Verbal Comprehension, Working Memory, Perceptual Organization, and Processing Speed) on the other hand, indicated that part of the interindividual variance in IQ dimensions is shared with interindividual variance in brain volumes. The most consistent correlation was found between brain volume and Working Memory, which is generally considered a central part of intelligence (Kyllonen & Chrystal, 1990). Gray and white matter and cerebellar volume relate equally strong to Working Memory. The correlations between Working Memory and all three brain volumes were completely mediated by a common underlying genetic factor. Seventeen per cent of the genetic variation in Working Memory can be accounted for by genes influencing these three brain volumes.

Intriguingly, the Verbal Comprehension dimension, which was the most heritable of all four WAIS III dimensions, did not correlate to any of the three brain volumes. Results from lesion and neuroimaging studies indicate that left temporal and frontocortical regions predominantly influence tasks that tap verbal comprehension. Thompson et al. (2001) reported the highest heritabilities (ranging from 95–100%) for gray matter density in these *linguistic* regions. They specifically reported a higher heritability in the left temporal parietal region — comprising Wernicke's regions thought to be involved in language processing — than in the right linguistic region (Thompson et al., 2001). For the present study broad volumes were available, as opposed to voxel based data. We therefore choose not to incorporate hemispheric effects, also because we previously demonstrated the high correlation (> .9) between left-right broad volumetric measurements and the absence of differential heritabilities for the two hemispheres (Baaré et al., 2001). In the present study, the absence of a (genetic) association of Verbal Comprehension with global volumes of white and gray matter does not preclude the existence of a genetic association with more localized volumes of the brain, such as Wernicke's area or the dorsolateral prefrontal areas (Brodmann areas 9 and 46; Rajkowska & Goldman-Rakic, 1995).

Processing Speed was genetically related to white matter volume. White matter volume includes all myelinated axons in the cerebrum. Thickness of the myelin sheath is related to nerve conduction velocity and therefore its relation to Processing Speed seems intuitively appealing. We previously showed that genetic variation in *perceptual* speed as indexed by inspection time accounted for only 10% of the genetic variance in Verbal IQ but for 22% of the genetic variance in Performance IQ (Posthuma et al., 2001b). As perceptual speed is likely to depend on axonal myelination, it can be hypothesized that part of the genes that influence IQ are common to the genes that influence myelination of axons by oligodendrocytes. A candidate gene known to be involved in myelination is the *Plp* gene (Boison & Stoffel, 1994; Griffiths et al., 1995, Ikenaka & Kagawa, 1995; Lemke, 1993). Other genes implicated to be important for myelination from knock out mouse studies are the *cgt*-gene (Stoffel and Bosio, 1997), the *MAG* gene (Fujita et al., 1998, Sheikh et al., 1999; Bartsch, 1996 for a review), and the tn-r gene (Weber et al., 1999).

Part of the genes responsible for cerebellar volume are also responsible for Perceptual Organizational ability, as reflected by the genetic correlation between these two measures. Although traditionally the cerebellum has been viewed as a neural substrate mainly involved in motor control (e.g., Ito, 1984), the presence of a correlation between cerebellar volume and components of intelligence has been reported since the 1980s (e.g., Leiner et al., 1986, 1993). Functional neuroimaging studies have shown that the cerebellum is involved in both motor and non-motor cognitive operations, such as working memory (Klingberg et al., 1996), complex problem solving (Kim et al., 1994), attentional activation (Allen et al., 1997), and semantic association (Petersen et al., 1989; Martin et al., 1995). The involvement of the cerebellum in higher cognitive functions may not be surprising from a biophysiological point of view, as the human cerebellum contains more neurons than the remainder of the brain combined (Williams & Herrup, 1988), and has axonal connections with all major subdivisons of the central nervous system.

The genetic association of cerebellar volume with both Perceptual Organization and Working Memory is indicative of its general role in cognition. Recently, Airey et al. (2001) reported linkage of five quantitative trait loci for cerebellar size in mice, and proposed a set of candidate genes lying within the linkage regions. For example, the Pax2 gene on chromosome 19 plays a critical role in early development of the cerebellum. Human homologous chromosomal regions of the five QTL's in mice as reported by Airey et al. (2001), are 1q23-43, 10q11-23, 9q13-q24, 11q12-q13, 10q23-qter, 16q12-22. These regions may also contain candidate genes for cognition.

The recent advances in morphometric/imaging techniques will enable future research to investigate function-volume relations on a voxel based manner (e.g., Ashburner & Friston, 2000; Hulshoff Pol et al., 2001). This may eventually lead to a detailed map of the human brain as it relates to specific cognitive abilities. The present study has made a first step in this direction by determining that the long-known relation between brain size and intelligence, even if using relatively unrefined measures such as total gray matter, total white matter, or total cerebellar volume, is of genetic origin.

Some caution in interpretation of a "genetic association" must be taken: determining that the association between different brain volumes and WAIS III-IQ dimensions is of genetic origin, as opposed to being of environmental origin, does not resolve the direction of causation between these two domains of measures. Four scenarios may underlie the established genetic association: 1) pleiotropy — there is a set of genes that influences both brain volumes and scores on the WAIS III dimensions; 2) unidirectional causation — there is a set of genes that influences variation in brain volumes and this variation in turn leads to variation in WAIS III dimension scores; 3) reversed unidirectional causation — there is a set of genes that influences variation in WAIS III dimension scores and this variation in turn leads

to variation in brain volumes; 4) reciprocal causation — a combination of scenarios 2 and 3.

Which of these four scenarios is most plausible has not been resolved yet, we merely established that the association follows a genetic pathway. Future studies will need to resolve the direction of causation to understand the plasticity of the brain and its role in cognition.

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Chapter 8

Alpha peak frequency and intelligence[1]

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Are Smarter Brains Running Faster? Heritability of Alpha Peak Frequency, IQ, and Their Interrelation

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It has often been proposed that faster central nervous system (CNS) processing amounts to a smarter brain. One way to index speed of CNS processing is through the assessment of brain oscillations via electroencephalogram (EEG) recordings. The dominant frequency (peak frequency) with which neuronal feedback loops in an adult human brain oscillate in a relaxed state is around 10 cycles/sec, but large individual differences exist in peak frequencies. Earlier studies have found high peak frequencies to be associated with higher intelligence. In the present study, data from 271 extended twin families (688 participants) were collected as part of a large, ongoing project on the genetics of adult brain function and cognition. IQ was assessed with the Dutch version of the Wechsler Adult Intelligence Scale (WAIS-IIIR), from which four dimensions were calculated (verbal comprehension, working memory, perceptual organization, and processing speed). Individual peak frequencies were picked according to the method described by Klimesch (1999) and averaged 9.9 Hz (*SD* 1.01). Structural equation modeling indicated that both peak frequency and the dimensions of IQ were highly heritable (range, 66% to 83%). A large part of the genetic variance in alpha peak frequency as well as in working memory and processing speed was due to nonadditive factors. There was no evidence of a genetic correlation between alpha peak frequency and any of the four WAIS dimensions: Smarter brains do not seem to run faster.

KEY WORDS: Neural speed; intelligence; twin study; electroencephalogram (EEG).

INTRODUCTION

The idea that faster central nervous system (CNS) processing may amount to a smarter brain has been proposed in earlier studies (e.g., Vernon, 1987) and has recently been supported by studies reporting positive relations between inspection time and IQ (Luciano *et al.,* 2001; Posthuma *et al.,* 2001). An alternative way to index speed of CNS processing is through the assessment of brain oscillations via electroencephalogram (EEG) recordings. Rhythmic activity measured with EEG scalp recordings derives from the summed synchronized synaptic activity of large populations of neurons (Steriade *et al.,* 1990). The dominant frequency (peak frequency) of this rhythmic activity in a relaxed state in adults is around 10 Hz, but large differences exist in individual peak frequencies (Lykken *et al.,* 1974; van Beijsterveldt and Boomsma, 1994; Klimesch, 1999; Osaka *et al.,* 1999). Previous studies have attempted to relate peak frequency to intelligence, arguing that a faster oscillating brain reflects rapid information processing, which in turn is associated with higher intelligence (e.g., Vogel and Broverman, 1964; Anokhin and Vogel, 1996; Osaka *et al.,* 1999), but this theory has long been debated (e.g., Ellingson, 1966; Ellingson and Lathrop, 1973; Vogel and Broverman, 1964).

In the past decade, experimental evidence has increased our understanding of the underlying physiological mechanisms responsible for brain oscillations, particularly in the alpha frequency range (Steriade *et al.,* 1990; Lopes da Silva, 1991). Generally, the alpha

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rhythm, as measured from the scalp, is defined to range between 8 to 13 Hz, occurs during wakefulness, and can be measured particularly over the occipital cortex. It appears when the eyes are closed and disappears when the eyes are opened (Berger, 1929). Alpha waves have been shown to be generated in thalamocortical feedback loops of excitatory and inhibitory nerve cells (Steriade *et al.,* 1990; Lopes da Silva, 1991). In the visual cortex, the alpha rhythm can also be generated by cortico-cortical networks involving layer V pyramidal neurons (Lopes da Silva and Storm van Leeuwen 1977; Steriade *et al.,* 1990). The specific alpha peak frequency of an individual is determined by the intrinsic membrane properties of the thalamic neurons projecting to the cortex (Steriade *et al.,* 1990).

Lebedev (1990, 1994) has proposed a functional role for the human alpha rhythm in stating that "cyclical oscillations in an alpha rhythm determine the capacity and speed of working memory. The higher the frequency the greater the capacity and the speed of memory" (Lebedev, 1994). In addition, Klimesch (1997) has argued that thalamo-cortical feedback loops oscillating within the alpha frequency range allow searching and identification of encoded information. He speculated that faster oscillating feedback loops would correspond to faster access to encoded information. These theories are supported by the results of some recent studies; Klimesch (1997) found that the alpha peak frequency of good working memory performers lies about 1 Hz higher than that of poor working memory performers. A study by Lehtovirta *et al.* (1996), comparing Alzheimer's patients with controls, found that alpha peak frequency of Alzheimer's patients was significantly lower than that of controls. This was explained in terms of cognitive slowing due to cholinergic deficits characteristic of Alzheimer's disease. It is also known that peak frequency tends to decrease with normal aging (Köpruner *et al.,* 1984). In summary, a theoretical neurophysiological framework as well as empirical evidence support the existence of a link between peak alpha frequency and (working) memory processes. Because working memory is a central component of intelligence (Daneman and Merikle, 1996; Engle *et al.,* 1999; Kyllonen and Christal, 1990; Necka, 1992), it seems reasonable to expect that alpha peak frequency is important to intelligence.

Metaphorically, the peak frequency of thalamocortical alpha activity can be hypothesized to determine the speed of encoding (and accessing) of information just like the processor speed of a microprocessor is determined by its basic clock cycle. High alpha peak frequency then is expected to be associated with high IQ. Only very few studies, however, have related alpha peak frequency successfully to measures of IQ. Anokhin and Vogel (1996) reported a correlation of 0.35 between alpha peak frequency and verbal abilities, but thus far this result has not been replicated (e.g., Jausovec and Jausovec, 2000). Also, no large study has provided a heritability estimate for alpha peak frequency (only a few small studies have appeared that reported twin correlations, e.g., Christian *et al.,* 1996). Although it has been speculated that the relation between alpha peak frequency and IQ is due to a genetic basis (e.g., Vogel, 2000), to our knowledge there have been no multivariate genetic studies reporting on the genetic correlation of alpha peak frequency with measures of IQ.

In the present study, we investigated whether and to what extent individual differences in alpha peak frequency can be attributed to genetic or environmental factors. In addition, the possible association between alpha peak frequency and each of the four dimensions of the WAIS-IIIR is decomposed into genetic and environmental components. An extended twin design (i.e., including families consisting of twins and additional siblings) is used to maximize statistical power to detect genetic and environmental influences (Posthuma and Boomsma, 2000).

METHODS

Subjects

Subjects were recruited from the Netherlands Twin Registry (Boomsma, 1998) as part of a large ongoing project on the genetics of cognition and adult brain function (Posthuma *et al.,* 2001; Wright *et al.,* 2001). Adult twins and their non-twin siblings were asked to participate in a 4.5-hour testing protocol. In one-half of the protocol, psychometric intelligence, inspection time, and reaction times were assessed; in the other half EEG activity was measured. The EEG registration included two noncognitive tasks that were analyzed for the present paper: 3 min resting EEG with eyes closed (EC) and 3 min resting EEG with eyes open (EO). The order of the two halves of the protocol was randomized across family members.

A total of 688 family members from 271 extended twin families participated in the study until December 2000. The complete sample consists of two age cohorts: a young adult cohort with a mean age of 26.2 years (*SD* 4.19) and an older adult cohort with a mean age of 50.4 years (*SD* 7.51). Participating families consisted of one

to eight siblings (including twins). On average, 2.5 subjects per family participated. In the young cohort, 171 males and 210 females participated, in the older cohort 135 and 172, respectively. The young cohort included 54 MZ pairs, 73 DZ pairs, 18 single twins, and 109 additional siblings. The older cohort included 48 MZ pairs, 58 DZ pairs, 15 single twins, and 80 additional siblings (for a detailed description of the sample characteristics see Posthuma *et al.,* 2001).

Intelligence Testing

IQ was measured with the Dutch adaptation of the WAISIII-R (WAIS-III, 1997). Dutch standardization norms for this version are currently being finalized, so it is not yet possible to report standard IQ scores. Individual scores for each subtest, except digit-symbol substitution, were calculated by weighting the observed score by the maximum possible score on that subtest times 100 (i.e., percentage correct on each subtest). For digit-symbol substitution the number of correct items per 60 sec was calculated. Nine subtests were administered. Subtest *information* measures general knowledge and information gathered from daily life. In subtest *similarities,* the subject is asked to describe in which aspect two verbally presented concepts are similar. In subtest *vocabulary,* the subject is asked to verbally describe the meaning of a specified term. Subtest *arithmetic* requires the subject to solve arithmetic questions within a certain time limit without paper and pencil. In subtest *letter-number sequencing,* the subject is asked to repeat a random sequence of up to eight numbers and letters and to put them in numerical and alphabetical order. In subtest *block design,* the subject needs to copy within a certain time limit a red and white pattern using red and white blocks. Subtest *matrix reasoning* requires the subject to decide which of five alternatives is most reasonably the missing part from a logical sequence. In subtest *picture completion,* the subject needs to state which essential part has been omitted from a given picture. In *digit-symbol substitution,* the subject needs to replace numbers with specified symbols as quickly and accurately as possible.

According to the WAIS guidelines (1997), the following four dimensions were calculated: Verbal Comprehension (VC; the mean percentage correct of subtests *information, similarities,* and *vocabulary*), Working Memory (WM; the mean percentage correct of subtests *arithmetic* and *letter-number sequencing*), Perceptual Organization (PORG; the mean percentage correct of subtests *block design, matrix reasoning,* and *picture completion*), and Processing Speed (PSPD; the number of correct items per 60 seconds of subtest *digit-symbol substitution*). The validity of these four dimensions was recently confirmed by a re-analysis of the WAIS manual data by Deary (2001).

EEG Administration

The EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (Enschede, The Netherlands). Signals were continuously represented online on a Nec multisync 17-in. computer screen using POLY 5.0 software (POLY, 1999) and stored for offline processing. Standard 10–20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, T7, P3, Pz, P4, T8, O1, and O2 (Jasper, 1958). Software-linked earlobes (A1 and A2) served as a reference. The vertical electrooculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes placed on the outer right canthus and 1 cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed 1 cm left from the left eye and 1 cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 KΩ; impedances of the EOG electrodes below 10 kΩ. The EEG was amplified (0.05–30 Hz), digitized at 250 Hz and stored for offline processing. Dynamic regression analysis in the frequency domain (Brillinger, 1975) was used to minimize eye artifacts, especially rolling of the eyes in the eyes closed (EC) condition. During the EEG measurements, the subjects were seated in a comfortable reclining chair in a dimly-lit, sound-attenuated, electrically shielded room. A computer screen was placed 80 cm in front of them. Subjects were instructed to close their eyes, relax, and minimize movement during the 3-min EEG recording of the EC task. During the 3-min recording of the eyes open (EO) task subjects were instructed to fixate on the dot presented at the center of the computer screen and to avoid blinking.

Determination of Individual Alpha Peak Frequency (IAF)

Alpha peak picking is usually conducted on EEG recording of an EC condition by finding the maximum power within a certain frequency range. It is sometimes argued, however, that the “real” alpha peak occurs at that frequency which is most depressed by opening of

the eyes (e.g., Klimesch, 1999). In the present paper, the latter criterium was used to obtain accurate localization of the individual alpha peak frequency.

A power density spectrum was calculated by using a Fast Fourier Transform applied to 4-sec epochs of the 3-min recordings of each condition. This yielded 44 epochs (epoch 45 was not used for computational reasons) and a 0.25 Hz resolution in the power spectra. Because the occipital-parietal alpha rhythm can best be detected at occipital leads (depressed by opening of the eyes; Berger, 1929), O1 and O2 were chosen to calculate the power density spectra and the individual alpha peak frequencies (IAF). In the first 100 subjects, correlation of alpha peak frequency between O1 and O2 was found to be very near to 1, so one of the two occipital leads (O2) will be reported on only.

The peak frequency in the EC condition was determined as the highest peak in a window of 7 to 14 Hz in the EC power spectrum, irrespective of the shape of the spectrum. Visual inspection was conducted for peak frequencies occurring at the boundaries of the search window. Final localization of the correct IAF was based on an automated comparison between the peak frequency, as determined in the EC condition and the frequency at which alpha power was most depressed by opening of the eyes (i.e., finding the peak frequency in the spectrum obtained by subtracting the EO spectrum from the EC spectrum). If these two methods of peak detection yielded an identical peak frequency, this was taken as the IAF.

If the two methods yielded different peak frequencies (which occurred in 21% of the sample), the spectra were visually inspected in order to determine the real alpha peak frequency. For example, in cases where the EC spectra showed two peaks of approximately the same magnitude, that peak was taken at which alpha depression was highest.

Spectra with very low power (i.e., below 1.5 μV/Hz) and spectra with less than 44 epochs were removed from further analysis.

Statistical Analysis

Because the sample consisted of unbalanced pedigrees and had some missing data, models were fitted to the raw data instead of covariance matrices. This was accomplished by using the rectangular data file option in Mx (Neale, 1997). Four bivariate saturated models of IAF with each of the four WAIS dimensions were fitted in order to determine the fit of the four more restrictive bivariate variance decomposition models. The saturated models included a linear regression effect of age within each cohort and a deviation for males from the females within each cohort. The significance of these effects on the means were estimated in the saturated models. In addition, it was tested whether there was evidence for: (1) heterogeneity of variances across MZ twin pairs, DZ twin pairs, and siblings, across males and females, and across cohorts; (2) heterogeneity of correlations across MZM pairs and MZF pairs, and across DZM pairs, DZF pairs, DOS pairs, and sib-sib male/female pairings; (3) heterogeneity of DZ correlations and sib-sib correlations; (4) differences in means between MZ twin pairs, DZ twin pairs, and siblings; and (5) differences in means between age cohorts. The resulting most parsimonious saturated model is the model against which the bivariate variance decomposition models are tested.

In the bivariate variance decomposition models, the observed variance was decomposed in three of four possible latent sources of variance: additive genetic (A), shared environment (C) or non-additive (D), and nonshared environment (E) following Neale and Cardon (1992). For DZ twin pairs (and sib pairs if the saturated models indicated no difference in correlation between DZ twin pairs and sib pairs), similarity in shared environmental influences was fixed at 100%, similarity of additive genetic influences at 50%, similarity of nonadditive genetic influences at 25%, and no similarity in nonshared environmental influences. For MZ twin pairs, similarities of additive genetic, nonadditive genetic, and shared environmental influences were fixed at 100% and no similarity in nonshared environmental influences.

RESULTS

Of the complete sample of 688 subjects, 27 took the IQ test at home and did not participate in the EEG measurement session. Data from 12 subjects contained too many recording errors to be included in the peak picking procedure. In 18 cases, the IAF could not be picked due to very low-voltage power spectra. This left 631 subjects with an IAF. The mean IAF of the complete sample was 9.9 Hz (*SD* 1.01). Subjects with IQ test data, but without an IAF, were still included in the analyses.

Saturated Model Fitting Results and Descriptives

The saturated model fitting procedures indicated that for the individual alpha peak frequency and the four WAIS dimensions: (1) the variances were homogenous across sexes and across zygosities; (2) the MZF and

MZM correlations were homogenous; (3) the DZM, DZF, and DOS correlations were homogenous; and (4) the DZ correlations and sib pair correlations were homogenous. In addition, no differences in means were found between MZ twins, DZ twins, and sibs. However, the total variances and twin correlations across age cohorts were found to be statistically significant.

Table I shows the estimates of descriptive statistics in the most parsimonious saturated model, including significant effects on the means.

Males score higher than females in both age cohorts on all four WAIS dimensions except for Processing Speed. In contrast, males have a slower IAF, compared with females, in both the young and the older cohort. Please note that for IAF, the grand mean (10.03) represents the female mean (because no significant effects of age cohort and age within cohorts were found); the general mean, including females and males, was 9.9 Hz as previously stated.

All significant regression effects of age within the older cohort are negative, indicating that IAF and IQ scores decrease with age. In the young cohort, only the effect of age on Verbal Comprehension was statistically significant. The positive sign indicated an increasing score with age within the younger cohort.

The scores on the four WAIS dimensions in the older cohort are lower than the scores in the young cohort. From Table I it can be computed, for example, that for a male of average age (i.e., 26.18 years) in the young cohort the expected score for Verbal Comprehension is $51.39 + 0.47 * 26.18 + 3.86 = 67.55$, whereas for a male of average age (50.39 years) in the older cohort the expected score is $51.39 + 22.50 - 0.28 * 50.39 + 5.92 = 65.70$.

The phenotypic correlation between IAF and each of the four WAIS dimensions is calculated simultaneously with modeling the effects of age and sex on the observed scores. The correlations between IAF and each of the four WAIS dimensions were homogeneous over sex and ranged from −0.04 to 0.15. With one exception, none of the phenotypic correlations was statistically significant. The correlation of 0.15 between IAF and Working Memory in the older cohort was significant at an α of .05 ($\Delta\chi^2 = 4.96$, $\Delta df = 1$, $p = .026$), but was not significant at the Bonferroni corrected α of .006, correcting for multiple testing. The correlations were not dropped from the variance decomposition models, however, because a significant genetic correlation and a significant environmental correlation acting in opposing ways may result in a phenotypic correlation that is not different from zero.

The pattern of MZ and DZ correlations as estimated by maximum likelihood (ML) from the most parsimonious saturated model (Table II) suggests mainly genetic influences on IAF and the four WAIS dimensions. For IAF and Processing Speed in both cohorts, Working Memory in the young cohort, and Perceptual Organization in the older cohort, the MZ correlation is more than twice as high as the DZ correlation, suggesting nonadditive genetic influences. ADE models were fitted for these variables. For all other variables, ACE models were fitted.

Table I. Estimates of Descriptive Statistics of Individual Alpha Peak Frequency (IAF) and the Four WAIS Dimensions From the Final Saturated Model

	Correlation (IAF-IQ)		Effects on the mean					
	Young cohort	Older cohort	Grand mean	Deviation of older cohort	Regression weight of age in young cohort	Regression weight of age in older cohort	Deviation of males in young cohort	Deviation of males in older cohort
IAF	—	—	10.03	0	0	0	−0.18	−0.03
VC	0.06	−0.04	51.39	+22.5	+0.47	−0.28	+3.86	+5.92
WM	−0.04	0.15*	62.71	0	0	−0.15	+3.21	+8.56
PSPD	0.02	0.04	44.17	+10.26	0	−0.38	−4.69	0
PORG	−0.03	0.08	79.74	+12.07	0	−0.50	+4.03	+3.63

IAF = Individual alpha peak frequency.
VC = Verbal comprehension.
WM = Working memory.
PSPD = Processing speed.
PORG = Perceptual organization.
*Statistically significant at the 0.05 level.

Table II. MZ and DZ Correlations as Estimated by Maximum Likelihood From the Saturated Model in Two Different Age Cohort (see Table I for abbreviations)

		N pairs*	IAF	VC	WM	PSPD	PORG
Young	MZ	54 (47)	*0.73*	0.84	*0.70*	*0.62*	0.69
Cohort	DZ/sib pairs	283 (253)	*0.26*	0.46	*0.16*	*0.24*	0.34
Older	MZ	48 (44)	*0.83*	0.82	0.67	*0.70*	0.69
Cohort	DZ/sib pairs	242 (192)	*0.17*	0.45	0.34	*0.23*	0.25

*Number of pairs for IAF in brackets; sibpairs included all possible sib pairings within a family.
Italic: Variables for which an ADE model instead of an ACE model is fitted.

Variance Decomposition Model Fitting Results and Descriptives

Bivariate variance decomposition models of IAF and each of the four WAIS dimensions were fitted in order to determine the nature of the possible covariance between IAF and IQ. The statistical significance of the estimates in the full bivariate variance decomposition models was established by fitting nested models and comparing the fit statistic to the preceding model using the likelihood ratio χ^2 test. Results are presented in Table III. Equality of variances due to A, D, C, or E across cohorts was also tested and showed no differences in A, D or C, and E estimates for Verbal Comprehension, Processing Speed, and Perceptual Organization across cohorts.

Estimates from the full bivariate variance decomposition models are given in Table IV. Estimates in the most parsimonious variance decomposition models are given in Table V.

The observed phenotypic variance in IAF is mainly due to genetic variance. The genetic variance is decomposed into additive genetic variance (39%) and variance due to nonadditive generic influences (32%) in the young cohort. In the older cohort, only a very small part of the variance is ascribed to additive genetic variance (<1%) and the main genetic variance is due to nonadditive genetic variance (83%). Because models including nonadditive generic influences but excluding additive genetic influences are biologically implausible (Falconer and Mackay, 1996), the additive variance component is always retained in the model.

For the WAIS dimensions, except for Working Memory in the young cohort and Processing Speed in the both cohorts, a model which included an additive genetic component and a nonshared environmental component best fit the data. For Working Memory in the young cohort and Processing Speed in the both cohorts, however, the nonadditive genetic component could not be dropped from the model without significantly worsening the fit. The variance due to nonadditive genetic influences in Working Memory in the young cohort was large (71%). Estimates of the genetic and nonshared environmental variance components of Processing Speed were homogeneous across cohorts. Thirty-two percent of the total variance was due to additive genetic influences, 34% to nonadditive genetic influences, and 34% to nonshared environmental influences. Also, for Perceptual Organization and Verbal Comprehension, no difference in variance components estimates was found between cohorts; 68% and 83%, respectively, was due to additive genetic influences, and 32% and 17%, respectively, to nonshared environmental influences.

All common pathways from the bivariate variance decomposition models could be dropped, except the common nonshared environmental factor between IAF and Verbal Comprehension in the young cohort and the common nonshared environmental factor between IAF and Working Memory in both cohorts. The corresponding nonshared environmental correlation was 0.31 and the corresponding phenotypic correlation 0.07 for IAF and Verbal Comprehension. For IAF and Working Memory, the nonshared environmental correlation was 0.17 and the corresponding phenotypic correlation was 0.05 in the young cohort and 0.04 in the older cohort.

In conclusion, although a high heritability for IAF and all four WAIS dimensions was found, no genetic correlation between IAF and any of the four measures emerged.

Post-hoc Investigation

Because it is known that head size and alpha peak frequency tend to correlate negatively (Nunez *et al.,* 1978), whereas head size and IQ correlate positively (e.g., Jensen, 1994), we conducted *ad hoc* tests in SPSS to determine whether mediating effects of head size

Table III. Fit Statistics of Nested Bivariate Variance Decomposition Models of Individual Alpha Peak Frequency (IAF) and Each of the Four WAIS Dimensions

		χ^{2}*	*df**	$\Delta\chi^{2}$**	Δ*df***
IAF	*Verbal Comprehension*				
ADE young, ADE older	ACE young ACE older with A-/E-correlation young and older	8.70#	2	—	—
ADE young, ADE older	ACE young = ACE older with A-/E-correlation young and older	8.83	5	0.13	3
ADE young, ADE older	ACE young = AE older with A-/E-correlation young and older	9.69	6	0.86	1
ADE young, ADE older	AE young = AE older	19.20#	10	9.51#	4
ADE young, ADE older	**AE young = AE older, with E-correlation young**	**12.20**	**9**	**2.51**	**3**
IAF	*Working Memory*				
ADE young, ADE older	ADE young, ACE older with A-/D-/E-correlations young, A-/E-correlations older	1.62	1		
ADE young, ADE older	AE young, ACE older with A-/D-/E-correlations young, A-/E-correlations older	9.17#	3	7.55	2#
ADE young, ADE older	ADE young, AE older with A-/D-/E-correlations young, A-/E-correlations older	1.62	2	0.00	1
ADE young, ADE older	ADE young, AE older	25.40#	7	23.78	6#
ADE young, ADE older	ADE young, AE older with A-/D-/E-correlations young, A-/E-correlations older and E-correlation young = E-correlation older	1.687	3	0.07	1
ADE young, ADE older	ADE young, AE older with A-/D-correlations young, A-correlation older	11.36	4	9.67#	1
ADE young, ADE older	ADE young, AE older with A-correlation young, A-correlation older with E-correlation young = E-correlation older	2.25	4	0.56	1
ADE young, ADE older	ADE young, AE older with A-correlation older with E-correlation young = E-correlation older	4.17	5	1.92	1
ADE young, ADE older	**ADE young, AE older with E-correlation young = E-correlation older**	**7.05**	**6**	**2.89**	**1**
IAF	*Processing Speed*				
ADE young, ADE older	ADE young, ADE older with A-/D-/E-correlations	0.48	0		
ADE young, ADE older	ADE (young = older) with A-/D-/E-correlations	1.18	3	0.70	3
ADE young, ADE older	**ADE young = ADE older, no correlation**	**6.55**	**9**	**5.38**	**6**
IAF	*Perceptual Organization*				
ADE young, ADE older	ACE young, ADE older with A-/E-correlations young, A-/D-/E-correlations older	0.81	1		
ADE young, ADE older	AE young, AE older with A-/E-correlations young, A-/E-correlations older	3.03	4	2.22	3
ADE young, ADE older	AE young = older with A-/E-correlations young=older	3.09	6	0.06	2
ADE young, ADE older	**AE young = AE older, no correlation**	**4.66**	**10**	**1.57**	**4**

*Against saturated model.
**Against less restrictive model.
Bold indicates most parsimonious model; # fits significantly worse than saturated/less restrictive model at $\alpha < .05$.
Note: In this table results are shown for dropping more than one effect at a time. The significance of each effect, however, was also tested by itself. This means, for example, that when dropping 5 effects at a time resulted in a χ^2 of 8.83, it was tested whether dropping each effect of these 5 by itself exceeded the critical χ^2-value of 3.84 for a 1 *df*-test.

Table IVa. Variance Decomposition Estimates (95% CI) in the Full Bivariate Models

		%A	%D	%C	%E
Young	IAF	40 (0−74)	31 (1−78)	—	28 (18−42)
	VC	74 (56−87)	—	9 (0−26)	17 (12−23)
	WM	11 (0−57)	59 (11−78)	—	30 (21−45)
	PSPD	33 (2−65)	33 (1−66)	—	35 (26−46)
	PORG	69 (34−79)	—	0 (0−26)	31 (21−46)
Older	IAF	1 (0−54)	82 (28−89)	—	17 (11−28)
	VC	74 (56−87)	—	9 (0−26)	17 (12−23)
	WM	67 (28−78)	—	0 (0−27)	33 (22−51)
	PSPD	33 (2−65)	33 (1−66)	—	35 (26−46)
	PORG	37 (0−76)	32 (0−77)	—	31 (20−50)

may have blurred a positive relation between IAF and IQ. Head circumference was measured with a measuring tape. In the complete sample, the correlation between IAF and head size was −0.12 ($p = 0.003$). This negative correlation was mainly due to a negative correlation between IAF and head size in females from the young cohort (−0.17, $p = 0.016$).

The correlations in the complete sample between head size and each of the four WAIS dimensions were all significant ($p < 0.001$), except for Processing Speed. For Perceptual Organization, Verbal Comprehension, and Working Memory the correlations were 0.15, 0.20, and 0.23, respectively.

When, in the complete sample, the correlation between IAF and each of the four WAIS dimensions was corrected for the effects of head size (in addition for correcting for the effects of age), still no correlation between IAF and any of the WAIS-dimensions was found. Also, when the dataset was divided into the four groups of young females, young males, older females, and older males, no correlation between IAF and any of the WAIS dimensions was observed after correction for head size, although for the older males the correlations of IAF and verbal comprehension and IAF with working memory were almost significant (0.17 with $p = 0.063$ and 0.16 with $p = 0.081$, respectively). In conclusion, mediating effects of head size did not explain the absence of a relation between IAF and IQ.

DISCUSSION

The present study, which includes a large representative sample of healthy Dutch adults, is the first large study to report heritability estimates of alpha peak frequency. It is also the first study to investigate the genetic and/or environmental correlation between alpha peak frequency and IQ. No significant correlation between alpha peak frequency and IQ at either the genetic, environmental, or phenotypic level was found, with the exception of a small correlation of peak frequency with Working Memory and Verbal Comprehension in the older cohort. These correlations were

Table IVb. Estimates (95% CI) of Genetic and Environmental Correlations in the Full Bivariate Models

		A correlation with IAF	D correlation with IAF	E correlation with IAF
Young	IAF	—	—	—
	VC	0.05 (−1.00–1.00)	—	0.30 (0.04–0.51)
	WM	−1.00 (−1.00–1.00)	0.25 (−0.95–1.00)	0.18 (−0.10–0.43)
	PSPD	−0.98 (−1.00–0.24)	0.83 (−0.28–1.00)	0.09 (−0.16–0.33)
	PORG	−0.08 (−1.00–1.00)	—	0.03 (−0.21–0.27)
Older	IAF	—	—	—
	VC	0.05 (−1.00–1.00)	—	0.08 (−0.20–0.35)
	WM	1.00 (0.22–1.00)	—	0.12 (−0.18–0.40)
	PSPD	1.00 (−0.84–1.00)	−0.29 (−1.00–0.61)	0.10 (−0.93–0.39)
	PORG	1.00 (−1.00–1.00)	−0.33 (−1.00–1.00)	0.06 (−0.23–0.35)

Table V. Estimates (95% CI) in the Most Parsimonious Bivariate Variance Decomposition Models

		%A	%D	%E	E correlation with IAF
Young	IAF	39 (0–74)	32 (1–80)	29 (18–44)	—
	VC	83 (78–87)	—	17 (12–22)	0.31 (0.04–0.50)
	WM	0 (0–53)	71 (16–80)	29 (20–43)	0.17 (0.01–0.33)
	PSPD	32 (1–69)	34 (1–72)	34 (26–46)	—
	PORG	68 (57–76)	—	32 (24–43)	—
Older	IAF	0 (0–62)	83 (28–89)	17 (11–28)	—
	VC	83 (78–87)	—	17 (12–22)	—
	WM	67 (50–78)	—	33 (22–50)	0.17 (0.01–0.33)
	PSPD	32 (1–69)	34 (1–72)	34 (26–46)	—
	PORG	68 (57–76)	—	32 (24–43)	—

Note: In Tables IVa and IVb, estimates for IAF are taken from the bivariate model IAF with VC, which was representative of all four bivariate models. This causes slightly different parameter estimates for IAF in Table IVb compared with Table IVa.

completely mediated by a common nonshared environmental factor. Because they did not survive the Bonferroni correction for multiple testing, however, these correlations should be regarded with caution.

The absence of a genetic correlation between alpha peak frequency and any of the four WAIS dimensions in this study suggests that genetic differences among individuals in the speed with which the thalamo-cortical feedback loops within the brain oscillate do not contribute to differences among individuals in IQ. This result is at odds with findings in previous studies. Studies in subjects with mental retardation (see Ellingson, 1966; Vogel and Broverman, 1964 for a review of the early studies) or Alzheimer's disease (Lehtovirta *et al.,* 1996; Klimesch, 1997) most clearly show that when the brain is not functioning optimally, both alpha peak and IQ are depressed. In addition, however, a significant link between alpha peak frequency and IQ has been found in populations with a normal IQ range (e.g., Klimesch *et al.,* 1996; Klimesch, 1999; Köpruner *et al.,* 1984; Lebedev, 1994; Osaka *et al.,* 1999; Anokhin and Vogel, 1996). Compared with these previous studies, our study differs mainly in the operationalization of IQ; in the present study, the correlation between alpha peak frequency and WAIS dimensions was investigated. Most previous studies did not use the WAIS to measure IQ (e.g., Klimesch *et al.,* 1996; Klimesch, 1997, 1999; Lehtovirta *et al.,* 1996; Köpruner *et al.,* 1984; Lebedev, 1994; Osaka *et al.,* 1999). Anokhin and Vogel (1996) did use a measure similar to the WAIS to tap general IQ, spatial IQ, and arithmetic abilities and found no significant correlation with any of these and alpha peak frequency. However, in the same study, a significant correlation of 0.35 between alpha peak and verbal abilities, as measured by the Amthauer's Intelligence Structure Test and Horn's Leistungsprüfsystem test, was reported, suggesting that alpha peak frequency may correlate with very specific mental abilities and may not be related to general IQ. Klimesch (1997), who repeatedly linked high alpha peak frequency to good memory performance, used several tests other than the WAIS to tap both working memory and memory: a Sternberg test, a verbal recognition test, an experimental learning test, and an incidental learning test.

A possible explanation for the absence of a correlation between alpha peak frequency and scores on the WAIS-dimensions could be that neural speed *per se* does not play a prominent role in general IQ. Rather, the degree of connectivity between areas or the total gray and white matter (brain volume) may be of greater importance. In other words, efficient interconnectivity of the brain could result in high processing speed without the need of fast oscillating thalamo-cortical feedback loops. Studies relating coherence (a measure of connectivity of the brain) to IQ have indeed reported a relation between efficient connectivity and measures of intelligence (e.g., Jausovec and Jausovec, 2000; Anokhin *et al.,* 1999).

Alpha peak frequency was shown to be highly heritable: In the young adult cohort, 71% of the total variance could be ascribed to genetic variance; in the older cohort this was 83%. These estimates of heritability are among the largest heritabilities reported for a quantitative trait (Plomin and DeFries, 1990). A large part of the genetic variance was estimated to be caused by nonadditive genetic variance; 32% in the young cohort and

83% in the older cohort. Nonadditive genetic variation can be either dominance variation or epistatic variation or both. Dominance variation of a trait refers to the variation due to the interaction effect of the two alleles that define the genotype at a locus. Dominance is distinct from the interaction that may occur between genotypes at separate loci (i.e., epistasis). However, these sources of variance are confounded in the classical twin study (i.e., including only MZ twin pairs and DZ twin pairs/sib pairs) as in most non-experimental genetic studies.

A large estimate of nonadditive influences and a near-zero estimate of additive genetic influences was found for alpha peak frequency in the older cohort. However, the confidence intervals around the estimates of nonadditive genetic variance and additive genetic variance are very broad and highly overlapping, indicating the difficulty in the separate detection of these two influences. The real additive variation and real dominance variation could be anywhere between 1% and 74%, or 1% and 80%, respectively. In the classic twin design, estimates of nonadditive genetic influences and additive genetic influences are highly negatively correlated (−0.9), resulting in stable broad heritability estimates, but large fluctuations in the estimates of these two influences (Eaves, 1972). Including subjects of many different genetic relationships (e.g., MZ twin pairs. DZ twin pairs, half siblings, parent-offspring) will increase the reliability to separate additive from nonadditive genetic influences.

An alternative explanation for the large estimate of nonadditive genetic influences may be that the observed DZ correlation was slightly lower than the true DZ correlation. This bias may occur when twins are sampled from a truncated distribution, which may lead to a slightly misrepresented sample. Martin and Wilson (1982) showed that this selection reduces the correlation between twin pairs and has a proportionally larger effect on lower correlations as compared to higher correlations. This, in turn, may easily result in the estimation of huge nonadditive genetic effects and zero additive genetic effects. For example, when the true MZ correlation is 0.8 and the true DZ correlation is 0.3, the corresponding true percentages of the total variation explained by additive and nonadditive genetic influences are 40% and 40%, respectively. However, if the observed correlations are 0.8 for MZs and 0.2 for DZs, the percentage of observed variation explained by additive genetic influences is estimated to be zero and the percentage of variation explained by nonadditive influences is estimated to be 80% (the percentage of variation explained by nonadditive genetic influences can quickly be obtained by calculating (2 × MZ-correlation minus 4 × DZ-correlation) × 100).

Although the magnitude of the nonadditive genetic influences on alpha peak frequency is likely to be overestimated, other studies support our results in suggesting the presence of nonadditive genetic effects in at least some loci. Lykken *et al.* (1982) reported an MZ correlation of 0.81 and a DZ correlation of −0.15. Although Lykken *et al.* (1982) did not test the departure from the additive model nor estimate the proportion of nonadditive influences, their results were explained in terms of dominance, epistasis, and gene-environment interactions. Christian *et al.* (1996) did estimate the influence of both dominance variance and variance due to epistasis. They found no evidence of additive genetic variance on alpha peak frequency, but the dominance variance and the epistatic variance were estimated at 21% and 18%, respectively.

An alternative explanation for MZ correlations to be more than two-fold the DZ correlation is a specific MZ environment (Wyatt, 1993). Because the twin correlations on EEG parameters for MZs reared together and MZs reared apart are similar (van Beijsterveldt and Boomsma, 1994), this specific MZ environment can only reasonably be sought in a more similar prenatal environment for MZs compared with DZs. It is known that a dysfunctional prenatal environment may result in dysfunctional neuropsychological functioning, as measured by EEG (Scher, 1997a, 1997b). When, for example, MZs are exposed to a specific prenatal environment that causes them to have more similar alpha peak frequencies later in life, the MZ correlation will be inflated compared with the DZ correlation and will falsely result in an estimation of nonadditive genetic influences (Christian *et al.,* 1975). However, such an effect will also be present in different mean alpha peak frequencies. In the present study, no mean differences were found between MZs, DZs and sibs. In addition, when MZs are under the influence of an additional source of variance (i.e., their specific prenatal environment) the result will be a greater total variance for MZ twin pairs compared with DZ twin pairs and sibs. Again, we found no evidence for a difference in variance as a function of zygosity. The nonadditive genetic influences in alpha peak frequency thus appear to be genuine nonadditive genetic influences.

While addressing its primary question, this study uncovered a number of noteworthy findings on the genetic architecture of the IQ dimensions. As expected, differences among individuals in the four WAIS dimensions could be attributed to genetic factors and non-

shared environmental factors, but not to shared environmental factors. The absence of shared environmental influences on specific cognitive abilities measures in adults is consistent with reports from other studies (Plomin *et al.,* 1994a, 1994b). On average 70% of the total interindividual variance was accounted for by additive genetic factors for Verbal Comprehension in both cohorts, Perceptual Organization in both cohorts, and Working memory in the older cohort. For Perceptual Organization in the older cohort, the full variance decomposition model estimated a moderate amount of nonadditive genetic variance that did not reach significance. However, for Processing Speed in both cohorts and Working Memory in the young cohort, the nonadditive genetic variance was significantly different from zero and explained 34% and 71% of the total variance, respectively.

The presence of nonadditive genetic variance in specific cognitive abilities or IQ in general is not often explicitly tested for, presumably because only very large samples have enough statistical power to detect it (Martin *et al.,* 1978). The large amount of variance due to nonadditive genetic sources in combination with the use of an extended twin design (Posthuma and Boomsma, 2000) gave enough power to detect nonadditive genetic variance in the present study, although the detection was not very accurate as indexed by the broad confidence intervals. As discussed earlier, very large sample sizes and information from many different genetic relationships between subjects are needed to separate additive genetic influences from nonadditive genetic influences reliably.

The presence of nonadditive genetic influences on cognitive abilities in the present study complies with the early work of Jinks and Fulker (1970), who reanalyzed the IQ data of Burt and Howard (1956) and concluded that ". . . dominant gene action for IQ almost certainly exists." Reported MZ and DZ correlations from some recent IQ studies also suggest the influence of nonadditive genetic variance. For example, Plomin *et al.* (1994b) reported an MZ correlation of 0.60 and a DZ correlation of 0.08 for the WAIS digit span subtest in the SATSA sample of 67-year-old subjects. In addition, for several other subtests the reported MZ and DZ correlations were suggestive of nonadditive genetic variance. However, nonadditive genetic variance was not included in the analyses.

Fulker and Eysenck (1979) noted that for ". . . many genes influencing IQ there is a marked degree of dominance." Evidence from human inbreeding studies, they argued, clearly indicate the presence of recessive alleles for low IQ and dominant alleles for high IQ. Normally, offspring show a regression to the population mean; i.e., the children of parents who are of lower-than-average IQ tend to be of average IQ as well as the children of parents of higher-than-average IQ. If the IQ of children of blood-related parents tends to be lower than that of children from unrelated parents, then there is evidence of recessive alleles influencing low IQ. This is exactly what was observed in an Israeli study by Bashi (1977); controlling for socioeconomic status, children born to biologically related parents were of lower IQ than children born to unrelated parents. In fact, children born to double first cousins showed a larger adverse effect than children born to first cousins. Another study by Seemanova (1971) found that the IQ of 161 children born from incestuous relationships was severely depressed. In contrast, the IQ of 95 children born to the same mothers but from a different relationship was completely normal. These findings clearly suggest the existence of recessive alleles decreasing IQ and, more generally, of nonadditive genetic variation in IQ.

In conclusion, this study, which included 688 healthy Dutch adult family members, showed that both alpha peak frequency and specific cognitive abilities, as measured with the WAIS, were highly heritable. Possibly as a consequence of the large sample size and the power added by the extended twin design, significant evidence was obtained for nonadditive genetic influences on IQ and on alpha peak frequency. No association between alpha peak frequency and WAIS-IQ at either the genetic, environmental, or phenotypic level was found.

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Chapter 9

Perceptual speed and intelligence[1]

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Perceptual Speed and IQ Are Associated Through Common Genetic Factors

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Individual differences in inspection time explain about 20% of IQ test variance. To determine whether the association between inspection time and IQ is mediated by common genes or by a common environmental factor, inspection time and IQ were assessed in an extended twin design. Data from 688 participants from 271 families were collected as part of a large ongoing project on the genetics of adult brain function and cognition. The sample consisted of a young adult cohort (mean age 26.2 years) and an older adult cohort (mean age 50.4 years). IQ was assessed with the Dutch version of the WAIS-3R. Inspection time was measured in the so-called Π-paradigm, in which a subject is asked to decide which leg of the Π-figure is longest at varying display times of the Π-figure. The number of correct inspections per second (i.e., the reciprocal of inspection time) was used to index perceptual speed. For Verbal IQ and Performance IQ, heritabilities were 85% and 69%, respectively. For perceptual speed, 46% of the total variance was explained by genetic variance. No differences in heritability estimates across age cohorts or sexes were found. Across the whole sample, a significant phenotypic correlation was found between perceptual speed and Verbal IQ (0.19) and between perceptual speed and Performance IQ (0.27). These correlations were entirely due to a common genetic factor that accounted for 10% of the genetic variance in verbal IQ and for 22% of the genetic variance in performance IQ. This factor is hypothesized to reflect the influence of genetic factors that determine axonal myelination in the central nervous system.

KEY WORDS: Neural speed; information processing; intelligence; extended twin design; inspection time.

INTRODUCTION

In 1996, Deary and Stough stated that "inspection time is, to date, the only single information processing index that accounts for approximately 20% of intelligence-test variance." Inspection time is defined as the minimum display time a subject needs to make an accurate perceptual discrimination on an obvious stimulus, and is often thought to reflect speed of apprehension or perceptual speed (Kranzler and Jensen, 1989). Visual inspection time is usually measured in the so-called Π-paradigm in which subjects are asked to decide which leg of the Π-figure is longest. There is no need to make this decision quickly; all that is required is an accurate response. Display time of the Π-figure is varied in order to determine the display time at which a predefined percentage (e.g., 80%) of the subjects' answers is correct. The manipulation of display time (also called SOA; stimulus onset asynchrony) is usually implemented by using a backward masking method, i.e., covering the stimulus with a Π-figure of which both legs are equally long. This reduces after-image of the stimulus on the computer screen, which otherwise would have allowed subjects to gain time beyond the actual display time of the stimulus. The use of different masking methods or no mask at all may blur inspection time-IQ correlations, because smarter people may benefit more from after-image artifacts. A prerequisite for obtaining a reliable inspection time-IQ association is the use of a good mask (Knibb, 1992).

A meta-analysis conducted by Kranzler and Jensen (1989) indicated that inspection time and IQ correlate

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around −0.50: The less time a person needs to make an accurate decision on an obvious stimulus, the higher his or her IQ. Inspection time correlates somewhat higher with performance IQ (−0.54) than with verbal IQ (−0.40) and correlations seem constant over age (Kranzler and Jensen, 1989). It is attractive to hypothesize that inspection time indexes the speed of perceptual processing, or even central nervous (CNS) system processing in general, hence explaining its association with IQ. In fact, the primary idea behind studies investigating the correlation between inspection time and IQ has been that a faster brain should result in a smarter brain. If this idea holds true, then unravelling the determinants of interindividual variance in inspection time in adult humans may also cast light on factors that determine interindividual differences in IQ.

In the present paper, we investigate which factors (genetic or environmental) contribute most to interindividual variability in inspection time and which factors mediate the observed correlation between inspection time and IQ. An extended twin design (i.e., including families consisting of twins and additional siblings) is used to maximize statistical power to detect genetic and environmental influences (Posthuma and Boomsma, 2000).

METHOD

Subjects

Subjects were recruited from The Netherlands Twin Registry (Boomsma, 1998) and participated in a large and as yet ongoing project on the genetics of cognition and adult brain function.

Analyses are based on the 688 family members from a total of 271 extended twin families that had entered the study by December 2000. Fig. 1 depicts the age distribution of the complete sample showing it actually consisted of two cohorts: a young adult cohort with a mean of 26.2 (*SD* 4.19) years of age and an older adult cohort with a mean of 50.4 (*SD* 7.51) years of age. We did not want to rule out possible differential age effects on IQ or inspection time for the two age cohorts. For example, in the young cohort age may not have any effect at all on IQ, whereas in the older cohort a gradual decrease in IQ with age seems reasonable to expect. It was decided, therefore, to include cohort-status in the analyses. Allocation of a family member to one of the two cohorts (young cohort under 36 years of age, older cohort above 36 years of age) was based on the age of the twins. There was a slight overlap in age of the non-twin siblings between the two cohorts.

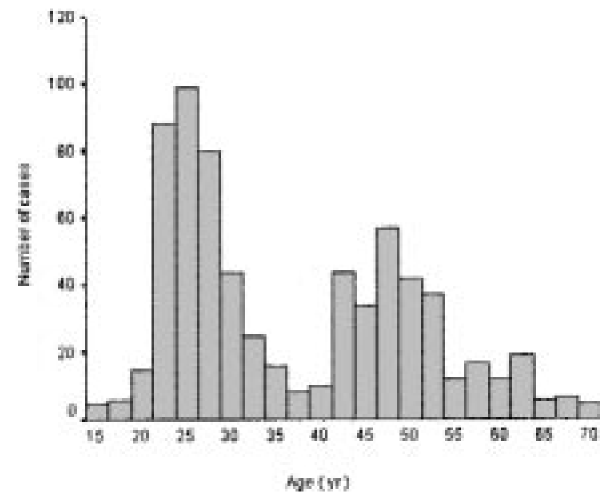

Fig. 1. Age distribution ($N = 688$) showing two cohorts.

Table I lists the complete sample configuration. For example, in the young cohort, 20 MZ families consisting of a complete MZ pair and one additional sibling participated. Participating family members ranged from 1 to 8, with an average of 2.5 subjects per family. In the young cohort, 171 males and 210 females participated, in the older cohort 135 and 172, respectively.

Table II lists the specific distribution of sex, age, educational level, and zygosity groups within the two cohorts.

The Dutch classification system for education level (Standaard Onderwijs Indeling [SOI], 1998) follows the International Standard Classification of Education (ISCED, 1997). The Dutch standard has seven categories, ranging from primary education (category 1) through tertiary education (category 7). The average SOI educational level was 4.21 (*SD* 1.05), meaning that on average subjects received schooling until 16 years of age, which is compatible with the general Dutch population (CBS, 2000). The subjects in the young cohort had a significantly higher average education category (mean 4.4, *SD* 1.03) than subjects in the older cohort (mean 4.0, *SD* 1.04). The same was true for males (mean 4.3, *SD* 1.04) and females (mean 4.1, *SD* 1.03). This pattern was also compatible with males and females of different ages in the general Dutch population (CBS, 2000).

Task and Variables

Inspection Time

A Parameter Estimation by Sequential Testing (PEST) procedure (Findlay, 1978; Pentland, 1980) was

Table I. Family Configuration in the Sample According to Zygosity, Cohort, and Number of Additional Non-Twin Siblings

	Additional siblings												
	0		**1**		**2**		**3**		**4**		**6**		
	fams	*ss*	fams	*ss*	fams	*ss*	fams	*ss*	fams	*ss*	fams	*ss*	
Young cohort													
MZ twin pair	31	*62*	20	*60*	2	*8*	1	*5*	—	—	—	—	Total MZ pairs: 54
single twin	1	*1*	3	*6*	—	—	—	—	—	—	—	—	
DZ twin pair	16	*32*	24	*72*	7	*28*	—	—	—	—	—	—	Total DZ pairs: 47
single twin	1	*1*	4	*8*	1	*3*	—	—	—	—	—	—	
DOS twin pair	11	*22*	12	*36*	2	*8*	1	*5*	—	—	—	—	Total DOS pairs: 26
single twin	2	*2*	4	*8*	1	*3*	—	—	1	*5*	—	—	
no twins	—		2	*2*	2	*4*	—	—	—	—	—	—	
Total Young	62	*120*	69	*192*	15	*54*	2	*10*	1	*5*	—	—	Total additional siblings: 109
Older cohort													
MZ twin pair	26	*52*	16	*48*	4	*16*	1	*5*	—	—	1	*8*	Total MZ pairs: 48
single twin	2	*2*	3	*6*	—	—	—	—	1	*5*	—	—	
DZ twin pair	20	*40*	15	*45*	1	*4*	—	—	—	—	—	—	Total DZ pairs: 36
single twin	3	*3*	1	*2*	2	6	—	—	—	—	—	—	
DOS twin pair	11	*22*	8	*24*	2	*8*	—	—	1	*6*	—	—	Total DOS pairs: 22
single twin	2	*2*	1	*2*	—	—	—	—	—	—	—	—	
no twins	—	—	1	*1*	—	—	—	—	—	—	—	—	
Total Older	64	*121*	45	*128*	9	*34*	1	*5*	2	*11*	1	*8*	Total additional siblings: 80
Total	126	*241*	114	*320*	24	*88*	3	*15*	3	*16*	1	*8*	

Note: Fams = number of families, ss = number of subjects, MZ = monozygotic twins, DZ = dizygotic same sex times, DOS = dizygotic opposite sex twins. Example: In the young cohort, 24 families consisting of a full DZ pair and one additional sibling participated (72 subjects). In the complete sample, 114 families consisting of one additional sibling and either a complete or an incomplete twin pair participated.

Table II. Descriptives of the Two Cohorts by Zygosity and Sex

	ss	Age range (yrs)	Mean age (SD) (yrs)	Education (SOI* categories)
Young cohort				
MZM	50	22.4–33.9	26.0 (3.07)	4.6 (1.14)
MZF	62	22.5–33.9	25.5 (3.42)	4.1 (0.93)
DZM	38	21.8–30.0	26.0 (2.13)	4.5 (0.76)
DZF	62	22.5–33.4	25.8 (2.72)	4.7 (0.92)
DOS	60	18.8–31.8	25.4 (2.87)	4.4 (0.85)
Add. siblings-males	54	13.9–42.6	27.3 (6.67)	4.0 (1.02)
Add. siblings-females	55	16.7–39.3	27.3 (5.85)	4.5 (1.03)
Total	*381*	*13.9–42.6*	*26.2 (4.19)*	*4.4 (0.95)*
Older cohort				
MZM	48	36.0–69.1	49.1 (6.92)	4.3 (1.09)
MZF	53	42.2–67.4	52.5 (7.8)	3.8 (0.96)
DZM	26	42.7–64.1	52.4 (5.07)	4.3 (1.37)
DZF	52	42.1–62.7	50.5 (6.21)	3.7 (1.09)
DOS	47	41.6–71.0	49.8 (7.98)	4.2 (1.09)
Add. siblings-males	37	37.0–68.4	50.8 (8.48)	4.3 (1.09)
Add. siblings-females	44	29.1–70.9	48.3 (8.50)	3.6 (0.97)
Total	*307*	*29.1–71.0*	*50.4 (7.51)*	*4.0 (1.11)*

*SOI = Dutch standard classification system; ss = number of subjects.

incorporated into a Π-paradigm following the description in Luciano *et al.* (2001). Briefly, the PEST procedure uses a staircase method in which stimulus duration is altered based on the subjects' response. If a correct answer is given, stimulus duration time of the next trial is decreased; if an incorrect answer is given, stimulus duration of the next trial is increased. The amount of increase or decrease is dependent on the number of previous reversals of increase/decrease. Thus, after many reversals, increases and decreases on subsequent trials become smaller and the PEST procedure converges on the subjects' inspection time. The task ends when the PEST estimate has become sufficiently stable or as soon as the maximum number of trials is presented.

For each subject, a cumulative normal function (mean = 0) was fitted *post hoc* to the stimulus duration times. The *SD* of this curve is the SOA at which 84% accuracy (corrected for guessing) is achieved (as described in detail in Luciano *et al.* 2001). The reciprocal of the $SD \times 1000$ can be interpreted as the number of inspections per second resulting in a correct judgement (Smith, 2000). This measure was used throughout this paper and will be referred to as *perceptual speed.* In contrast to inspection time itself, the number of correct inspections per second or perceptual speed is expected to correlate positively with IQ, i.e., a high value on perceptual speed means that more correct perceptions per time unit are made and refers to a fast inspection time.

To ensure accurate SOAs, a dynamic backward mask (Evans and Nettelbeck, 1993) was used (Fig. 2). All instructions were given on a computer screen and the importance of accuracy over reaction time was stressed in the instruction.

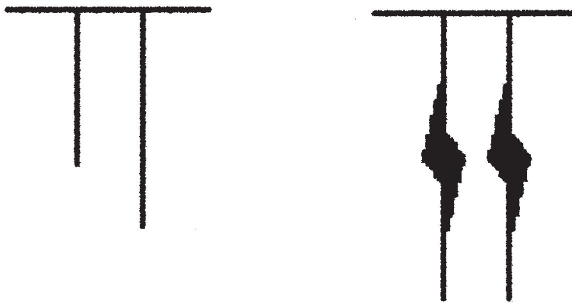

Fig. 2. Π-paradigm with backward masking; the Π is briefly presented and covered with the mask. The amount of increase/decrease of stimulus duration in each trial is dependent on whether or not the subject answered correctly or incorrectly in the previous trials (see text also).

Intelligence Testing

IQ was measured with the Dutch adaptation of the WAIS-3R (WAIS-III, 1997). Standardization norms for this version are currently being determined and at this point it is not possible to report standard IQ scores. Performance IQ was calculated as the mean of three subtests (picture completion, block design, matrix reasoning) and verbal IQ was based on the mean score on four subtests (information, similarities, vocabulary, arithmetic).

Statistical Analysis

Because the sample consisted of unbalanced pedigrees and had some missing data, models were fitted to the raw data rather than covariance matrices. This was accomplished by using the rectangular data file option in Mx (Neale, 1997). Saturated models were fitted in order to determine the fit of the variance components models. The saturated models included modeling a linear regression effect of age within each cohort and a deviation for males within each cohort. The significance of these effects of the means were estimated in the saturated models and the following assumptions of the (extended) twin method were tested: (1) heterogeneity of variances across MZ twins, DZ twins, and siblings, across males and females, and across cohorts; (2) heterogeneity of correlations across MZM twins and MZF twins, and across DZM twins, DZF twins, DOS twins, and sib-sib male/female pairings; (3) heterogeneity of DZ correlations and sib-sib correlations; (4) differences in means between MZ twins, DZ twins, and siblings; and (5) differences in means between cohorts. The resulting most parsimonious saturated model is the model against which the variance components models are tested.

In the variance components models, the observed variance was decomposed in three of four possible latent sources of variance: additive genetic (A), non-additive genetic (D), shared environment (C), and non-shared environment (E) following Neale and Cardon (1992). For DZ twins (and sib pairs if the saturated models indicated no difference in correlation between DZ pairs and sib pairs) similarity in shared environmental influences was fixed at 100%, similarity of additive genetic influences at 50%, similarity of non-additive genetic influences at 25%, and no similarity in non-shared environmental influences. For MZ twins similarities of additive genetic, non-additive genetic and shared environmental influences were fixed at 100% and no similarity in non-shared environmental influences.

Results

Twenty seven subjects of the total 688 subjects took an IQ test at home and did not have data on the computerized inspection time task. Ten subjects who came to the laboratory were unable to perform the inspection time task due to a lack of time or computer problems. Inspection time results from another 10 subjects were discarded from the analyses because they had an unusual long inspection time (2000 ms), which raised the suspicion that they did not perform the task as intended. This left 688 subjects with IQ data of whom 641 also had data on perceptual speed.

Saturated Model Fitting Results and Descriptives

The saturated model fitting procedures indicated that for perceptual speed, Verbal IQ and Performance IQ (1) the variances were homogenous across sexes and across zygosity; (2) the MZF and MZM correlations were homogenous; (3) the DZM, DZF, and DOS correlations were homogenous, and the DZ correlations and sibpair correlations were homogenous; (4) no differences in means were found between MZs, DZs and sibs; and (5) the variances and twin correlations across cohorts were homogenous.

Table III shows the significant effects on the means in the most parsimonious trivariate saturated model. Males performed better on all three measures in both the young and the old cohort, except for perceptual speed, where males and females scored equally well. The difference in means between males and females was larger in the older cohort compared with the young cohort. In the young cohort, there was no effect of age on perceptual speed and Performance IQ. On Verbal IQ every year would raise the score with 0.22 points, i.e., being 25 years of age adds $0.22 * 25 = 5.5$ to the grand mean. In the old cohort, for all three measures a higher age decreases the score.

The grand means were equal for both cohorts except on Verbal IQ. Although the sign of the deviation of the old cohort is positive, this does not correspond to a higher mean in the older cohort compared with the young cohort. This can easily be demonstrated by calculating the expected scores for a female aged 26.2 years (i.e., the average age in the young cohort) and a female aged 50.4 years (the average age in the old cohort). For the 26.2-year-old female, the expected verbal IQ score is $22.70 + (0.22 * 26.2) = 28.46$, whereas for the 50.4-year-old female, the expected verbal IQ score is $22.70 + 6.55 - (0.07 * 50.4) = 25.72$.

The phenotypic correlations between the three measures were homogeneous over cohorts, sex, and zygosity. The correlation between perceptual speed and Verbal IQ was 0.19, between perceptual speed and Performance IQ was 0.27, and the correlation between Verbal IQ and Performance IQ was 0.49. These were all statistically significant at the 0.01 level.

Twin and sibling correlations were also homogeneous over cohorts and sexes, and there was no difference between DZ correlations and sib-correlations. The MZ and DZ correlations (and 95% CI) for perceptual speed were 0.48 (0.31 − 0.60) and 0.20 (0.10 − 0.31), respectively, for Verbal IQ 0.84 (0.79 − 0.88) and 0.47 (0.37 − 0.55), respectively, and for Performance IQ 0.69 (0.58 − 0.77) and 0.32 (0.22 − 0.42), respectively. The overall pattern of correlations indicates additive genetic influences and perhaps some common environmental influences.

Variance Components Modelling

The minus two log likelihoods (-2LLs) of the nested trivariate variance components models were compared to the -2LLs of the final saturated model by way of likelihood ratio test. In this way, a measure of goodness of fit of the variance components models was obtained.

The full trivariate ACE model fitted reasonably well with a chi-square of 0.78 compared with the sat-

Table III. Grand means, Standard Deviations (SD), and Effects on the Means of Perceptual Speed, Verbal IQ, and Performance IQ (as estimated with ML in the final saturated trivariate model)

	Grand mean	*SD*	Deviation of older cohort	Regression weight of age in young cohort	Regression weight of age in older cohort	Deviation of males in young cohort	Deviation of males in older cohort
Perceptual Speed	14.16	4.67	—	—	−0.05	—	1.70
Verbal IQ	22.70	5.41	6.55	0.22	−0.07	1.00	2.89
Performance IQ	23.63	3.63	—	—	−0.09	1.10	1.46

urated model and the same amount of degrees of freedom. However, shared environmental influences could be dropped from the model without significantly worsening the fit of the model ($\chi^2_6 = 2.82$). For the same reason, all common non-shared environmental factors could be dropped from the model ($\chi^2_3 = 3.22$). In contrast, the common genetic factors could not be dropped from the model without significantly worsening the fit. Dropping the common genetic factor for perceptual speed and Verbal IQ resulted in a χ^2 of 11.06 with one degree of freedom, for the common genetic factor between perceptual speed and Performance IQ the χ^2 with one degree of freedom was 18.18, and for the common genetic factor between verbal IQ time and performance IQ the χ^2 with one degree of freedom was 31.62.

Thus, a trivariate model that included additive genetic influences and non-shared environmental influences, and that allowed all phenotypic correlation between the three measures to be explained by common additive genetic factors fitted the data best. Fig. 3 illustrates this model and the standardized path coefficients.

As can also be calculated from Fig. 3 (see Neale and Cardon, 1992), the percentage of variation explained by additive genetic factors for perceptual speed, Verbal IQ, and Performance IQ was 46% (95% CI 33–58), 85% (80–89) and 69% (60–77), respectively. The remaining variation explained by non-shared unique environmental influences was 54% (42–67), 15% (11–20), and 31% (23–40) for perceptual speed, verbal IQ, and performance IQ.

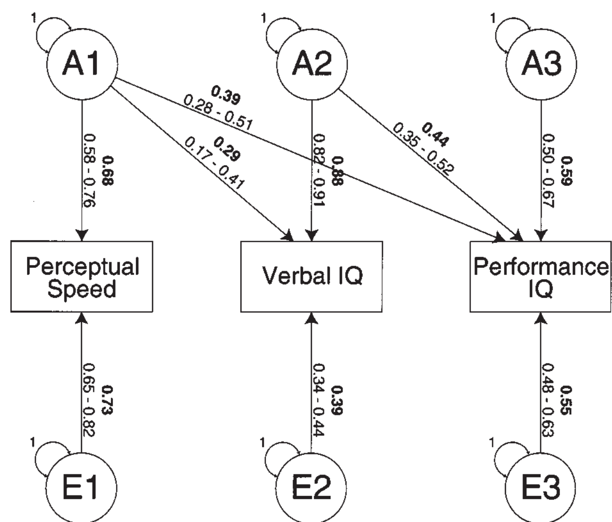

Fig. 3. Standardized estimates (95% CI) in best-fitting trivariate model. Reported values are estimated simultaneously with effects of age and sex on the observed scores.

The genetic correlation between perceptual speed and Verbal IQ was 0.31 (0.18–0.44). Or in other words, 10% of the genetic variance in Verbal IQ is explained by genetic factors that are shared with perceptual speed. The genetic correlation between perceptual speed and Performance IQ was 0.47 (0.33–0.61), indicating that 22% of the genetic variance in performance IQ was explained by genetic factors shared with perceptual speed.

Not surprisingly, a high genetic correlation was also observed between Verbal IQ and Performance IQ; 0.65 (0.56–0.72), corresponding to 28% of the genetic variance in Performance IQ that is shared with genetic factors important to Verbal IQ. This also means that 50% of the genetic variance in Performance IQ is unique to Performance IQ, and thus unshared with either perceptual speed or Verbal IQ.

DISCUSSION

In a large sample of 688 individuals, the phenotypic correlation between the number of correct inspections per second and Verbal IQ was 0.19 and between the number of correct inspections per second and Performance IQ was 0.27. The magnitudes of these correlations are lower than the current consensus (e.g., Nettelbeck, 1987; Kranzler and Jensen, 1989) that sets the correlation between inspection time and IQ around −0.50 (the difference in sign simply reflects the reverse scaling of the number of correct inspections per second in comparison to inspection time). A possible source of difference is the use of different strategies by our subjects that may blur inspection time-IQ correlations (Knibb, 1992). This is not likely because a backward masking procedure was used to prevent the use of strategy. Furthermore, it has been shown that if strategies are used, the inspection time–IQ relation tends to be lower rather than higher than when no strategies are used (Deary and Stough, 1996).

It remains unclear why the inspection time–IQ relation in our sample is below the estimate derived from the meta-analysis (Kranzler and Jensen, 1989). It should be pointed out that the uncorrected correlations in this meta-analysis were very comparable to ours (around −0.30). Only when an attempt was made to correct for artifact effects inherent in pooling over studies for conducting a meta-analysis the corrected inspection time–IQ correlations came into the −0.50 range. Two sources of evidence suggest that the lower estimates for phenotypic inspection time–IQ correlation may be more

correct. First, the number of subjects in this study (688) is larger than the total number of subjects used in the meta-analysis ($n = 88$ for PIQ, $n = 218$ for VIQ, and $n = 633$ for total IQ). Second, our results are consistent with findings from another recent large study, which included 390 twin pairs aged 16 years (Luciano *et al.,* 2001). In this study, a phenotypic correlation between inspection time and IQ of -0.36 was found.

Variance components analysis suggested moderate genetic influences on perceptual speed as indexed by inspection time; 46% of the interindividual variance was explained by genetic variance and 54% was explained by non-shared environmental sources of variance including measurement error. Shared environmental sources of variance did not significantly contribute to the interindividual variance. This pattern was uniform over two age cohorts and over both sexes. The influence of genetic variation on interindividual variation in IQ was much higher; 85% and 69% for Performance IQ and Verbal IQ, respectively, with the estimates uniform over cohorts and sexes. The observed correlation between our measure of perceptual speed and the two IQ measures was mediated completely by the sharing of underlying genetic factors; 10% of the genetic variance in Verbal IQ was explained by genetic factors shared with perceptual speed. Twenty-two percent of the genetic variance in performance IQ was explained by genetic factors shared with perceptual speed. These results are similar to the results obtained by Luciano *et al.* (2001) in a sample of 16-year-old twins.

Although no structural biological theories exist which specifically address inspection time, the existing biological model for visual processing based on the monkey brain holds strong clues to the possible source of genetic influences on perceptual speed/inspection time. Fig. 4 briefly explains this model.

A recent meta-analysis on the latencies of responses evoked by visual stimuli in the monkey, mostly obtained by intracranial electrophysiological recordings, showed that earliest responses in the lateral geniculate nucleus of the thalamus occurred at 28 to 31 msec, earliest responses in the primary and extrastriate visual cortices at 35 (V1), 54 (V2), and 61 (V4) msec, and earliest responses in the posterior part of the inferior temporal cortex (TE1) at 57 msec (Lamme and Roelfsema, 2000). Presumably, activation of V2 and inferior temporal cortex is minimally required when discriminating a simple two-dimensional object such as the Π figure. These latencies of the early visual pathways in monkeys compare quite reasonably to the earliest visual evoked potentials over the occipital cortex that occur around 60 msec (Celesia, 1993).

The interest in inspection time in intelligence research is driven mainly by the notion that it indexes a basic process in brain function, like perceptual, or even general information processing speed (Jensen, 1993; Eysenck, 1995), although this notion has been debated (Stankov and Roberts, 1997). If differences in inspection time reflect perceptual speed, then Fig. 4 reveals that a major source for genetic influences are the conduction velocity in the optic nerve to the thalamic LGN and the projection of LGN neurons to the primary cortex, and on to extrastriate areas. Optic nerve conduction velocity and conduction velocity from LGN neurons to higher areas depend on the fibre diameter of the axons, the number and form of ion channels in the axon membrane, and the quality (thickness and stability) of the myelin sheath generated by the oligodendrocytes (Kandel *et al.,* 1991). We hypothesize that part of the common genetic factors underlying IQ and inspection time are factors that determine myelination of axons by oligodendrocytes. Results from aging studies have indicated that, with aging, white matter (which is mainly composed of myelinated axons) density tends to decrease, whereas gray matter (cell bodies) density remains stable (Courchesne *et al.,* 2000). This suggests that aging does not result in neuronal apoptosis but instead goes along with a reduction in myelin, either by thinning of myelin sheaths or axonal degeneration. This will influence axonal conduction velocity and may explain the reduction in inspection time in the older cohort compared with the young cohort in this study.

Several genes that influence CNS axonal myelination have been implicated from animal models, some of which are known to cause dysmyelination in humans as well. The *Plp* gene (Xq22.3), for example, codes for two membrane proteins important for myelination. Disruption of expression of the *Plp* gene in mice causes a disruption in the assembly of the myeline sheath, which leads to a profound reduction in conduction velocity of CNS axons (Boison and Stoffel, 1994; Griffiths *et al.,* 1995, Ikenaka and Kagawa, 1995; Lemke, 1993). The influence of the *Plp* gene is specific to CNS axonal myelination because it does not affect peripheral conduction velocity nor give rise to gross behavioral anomalies (Boison and Stoffel, 1994). Although the exact role of the *Plp* gene in the CNS remains poorly defined (Knapp, 1996; Griffiths *et al.,* 1998), mutations in the same gene in humans are known to result in Pelizaeus-Merzbacher disease (PMD) (e.g., Anderson *et al.,* 1999; Griffths *et al.,* 1995; Woodward and Malcolm, 1999).

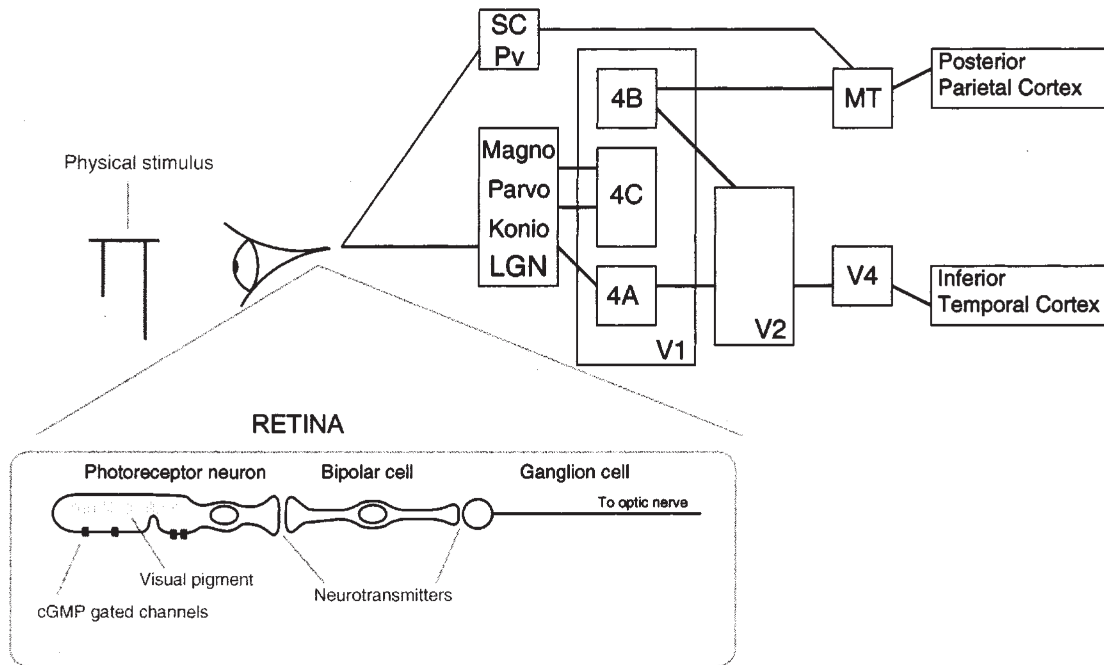

Fig. 4. The visual pathway in monkeys. Visual information processing-starts with the absorption of light by the visual pigments in the photoreceptors of the retina. This stimulates cGMP (cyclic nucleotide 3′–5′ cyclic gunosine monophosphate) phosphodiesterase, which reduces the amount of cytoplasmic cGMP and closes the cGMP gated channels, changing the ionic current across the membrane. This, in turn, leads to a hyperpolarization of the photoreceptor membrane and results in the reduction of glutamate in the synaptic cleft between photoreceptor and interneuron. The interneuron then transduces the electrical signal by way of graded potentials, eventually triggering an action potential in the ganglion cell. The axons of the ganglions cells leave the retina at the optic disc, where they become myelinated by oligodendrocytes and form the optic nerve (Tessier-Lavigne, 1991). That oligodendrocytes are a source of optic nerve myelination contrasts with other peripheral nerves where myelin is always generated by Schwann cells; this makes the optic nerve a good model for central nervous system conduction velocity.

Most detailed anatomical information exists on the monkey brain (Kandel *et al.,* 1991; Salin and Bullier, 1995). Information from both eyes is conducted through neurons in the optic tract to the lateral geniculate nucleus (LGN) of the thalamus. Retinal information also travels to the pretectal area of the midbrain for the control of pupillary constriction, to the superior colliculus (SC), the pulvinar (Pv) for the control of (saccadic) eye movements, and to the cerebellum to control movement in response to visual input. The lateral geniculate nucleus projects to layer 4 of the primary visual cortex (V1) that projects on to V2 and higher visual association cortices (V4, MT), eventually leading to visual awareness. From the retina to the LGN and from the LGN to the area V1, parallel pathways (magno-, parvo-, and koniocellular) transfer different kinds of information that are recombined in areas V1 and V2. After recombination, two pathways emerge: a dorsal, magno-dominated pathway to the posterior parietal cortex involved with space and movement, and a ventral, parvo-dominated pathway concerned with object identification and perception to the inferior temporal cortex.

PMD is a hypomyelination disease which, in its mildest form, may lead to optic atrophy and dementia. Other genes implicated to be important for myelination in knock out mouse studies are the *cgt gene* (Stoffel and Bosio, 1997), the *MAG* gene (Fujita *et al.,* 1998, Sheikh *et al.,* 1999; Bartsch, 1996, for a review), and the *tn-r* gene (Weber *et al.,* 1999).

Obviously, as is apparent from Fig. 4, aspects of visual processing other than conduction velocity determine inspection time as well. Speed of receptor potential generation in the photoreceptors and its transduction to ganglion cells depends on the availability of cGMP, the number of cGMP gated channels, and the availability of glutamate—factors that may well be under genetic control. Most important, the efficiency of synaptic neurotransmission in the LGN and striate neurons is a major determinant of visual processing speed. Given the staggering amount of protein interactions involved in neurotransmission, it is easy to envision how synaptic transmission could introduce genetic variance in inspection time (and IQ). In fact, a sodium channel isoform was recently identified that influenced both axonal conduction velocity as well as synaptic responses (Caldwell *et al.,* 2000). Finally, although inspection time seems to depend largely on the "fast feedforward sweep of visual information processing," we

cannot rule out effects of horizontal connections within the visual layers, e.g., within V1, or of recurrent processing from hierarchically higher visual areas (Lamme and Roelfsema, 2000), which brings in a number of possible additional genetic factors.

In summary, we found that the correlations between perceptual speed and Verbal IQ and between perceptual speed and Performance IQ were entirely due to a common genetic factor that accounted for 10% of the genetic variance in Verbal IQ and for 22% of the genetic variance in Performance IQ. We conclude that perceptual speed as indexed by inspection time can be used as an intermediate phenotype in linkage and association studies aimed at detecting genetic loci that determine interindividual variance in intelligence. Genes related to CNS axonal conduction velocity constitute good candidate genes for intelligence.

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Chapter 10

Speed of response selection, inhibition and intelligence[1]

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Genetic analysis of IQ, processing speed and stimulus-response incongruency effects

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Abstract

Psychometric IQ (WAIS-III), onset and peak latency of the lateralized readiness potential (LRP), decision time, and accuracy were assessed during an Eriksen Flanker task in a young (149 families) and in an older (122 families) cohort of twins and their siblings. Stimulus-response incongruency effects were found on all measures of processing speed and accuracy. The effects on the percentages of wrong button presses and too slow (> 1000 ms) responses were larger in the older than in the younger age cohort. Significant heritability was found for processing speed (33–48%), accuracy (41%), and stimulus-response incongruency effects (3–32%). Verbal and performance IQ correlated significantly with stimulus-response incongruency effects on accuracy (−0.22 to −0.39), and this correlation was completely mediated by an underlying set of common genes. It is concluded that measures of the ability to perform well under conditions of stimulus-response incongruency are viable endophenotypes of cognitive ability. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Intelligence; Endophenotype; Lateralized readiness potential (LRP); Heritability; Genetic correlation

1. Introduction

The presence of genetic influences on cognitive ability is well established (e.g. Bouchard and McGue, 1981; Plomin et al., 2001). Little is known, however, about the pathways that lie between genes and cognitive ability. Two strategies may be

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employed to identify these pathways. The first, bottom-up strategy starts with the sequence of known genes, identifies the gene product, establishes the function of the gene product at the cellular level, its possible role in neuronal networks and ultimately its effects on cognition. A second, top-down strategy focuses on individual variability in cognitive ability. It consecutively traces individual differences in cognitive ability back to differences in brain function, to the neurophysiological substrates determining brain function, to the cellular pathways underlying this neurophysiology, to the proteins involved in these cellular pathways, and finally to the genes coding for these proteins.

In the top-down strategy, cognitive psychophysiological experimentation plays a crucial role by indexing the first important element in this approach; individual differences in brain function. Measures of brain function that correlate with cognitive ability through shared genetic factors are called "endophenotypes" (de Geus and Boomsma, 2002). A rapidly increasing number of potential endophenotypes have already been tested for crucial properties of heritability and (genetic) covariation with cognitive ability (for a review see Posthuma et al., 2002). A specific class of these endophenotypes came from the theoretical framework of the neural speed theory of intelligence (Eysenck, 1986; Vernon, 1987, 1993). Within this framework, many studies have looked at the heritability of reaction times and their correlation with measures of intelligence (e.g. Baker et al., 1991; Ho et al., 1988; Finkel and Pedersen, 2000; Luciano et al., 2001; Neubauer and Knorr, 1997; Rijsdijk et al., 1998). Reaction times are moderately to highly heritable (40–80%) and correlate around −0.20 to −0.40 with measures of intelligence. This association is largely (70–100%) explained by common underlying genetic factors that influence both reaction times and intelligence.

Reaction time in a typical choice reaction time task reflects the final outcome of a multi-stage process of stimulus detection, stimulus evaluation, response selection, response activation, and response initiation. Processing speed of each of these stages can be indexed separately, and tested for heritable individual differences and their relevance to intelligence. For instance, we previously showed that 46% of the individual differences in the speed of early stimulus detection (as measured by inspection time), can be ascribed to genetic differences among subjects (Posthuma et al., 2001b). Moreover, the correlation between this early step and IQ was shown to be completely mediated through a common genetic pathway (Posthuma et al., 2001b). Besides early stimulus detection and reaction times, a number of studies have looked at P3 latency as a measure of the speed of stimulus evaluation. van Beijsterveldt and van Baal (2002) reported a "meta"-heritability across these studies of 51%. Also, a number of studies have reported correlations of P3 latencies with IQ, although not systematically (for a review see Wright et al., 2002). To date there have been virtually no investigations of individual differences in the speed of other stages of information processing.

A potential measure of another aspect of processing speed is the lateralized readiness potential (LRP). The LRP is mathematically derived from the Bereitschaftspotential or Readiness Potential (RP; Kornhuber and Deecke 1965). The onset of the LRP is considered to reflect the output of the response selection stage (Coles,

1989; Eimer, 1998) and its peak latency is thought to additionally reflect central motor processes that take place after response selection has taken place, i.e. response activation (Falkenstein et al., 1994). Actual response initiation can be measured by EMG onset or alternatively as the release of a home button (decision time). In this paper, we examined the heritability of the latency of (pre-) motor selective response activation using the onset and peak latency of the LRP. The heritability of the speed of response initiation was examined using decision time. Since large individual differences in speed–accuracy trade off may exist, even under standardized instructions, we also assess the heritability of accuracy. To test their viability as endophenotypes of cognitive ability, we examined the phenotypic and genetic correlation of processing speed and accuracy with psychometric IQ.

The LRP was obtained during an Eriksen flanker task (Eriksen and Eriksen, 1974). We tested processing speed during the performance of the *congruent* trials because these are comparable to the two-choice reaction time tasks used in many studies testing the neural speed of intelligence hypothesis. In addition, the Eriksen flanker task can be used to specifically test the effects of stimulus-response incongruency. Stimulus-response incongruency in this task generally induces slowing and loss of accuracy (Botvinick et al., 1999; Cohen et al., 1992; Kramer et al., 1994). This may reflect impairment of selective attentional control over the local inhibitory circuits in the perceptual or premotor cortices (Cohen et al., 1992; Servan-Schreiber, 1990; Spencer and Coles, 1999) or of top-down inhibitory control of frontal executive areas (e.g. Kramer et al., 1994; West, 1996). The concepts of selective attention as well as inhibitory control are included in almost all theories of higher cognitive function (Anderson and Spellman, 1995; Baddeley, 1986; Dempster, 1991, 1992; Fuster, 1997 West, 1996). Therefore, we examined the heritability of stimulus-response incongruency effects and explored their phenotypic and genetic correlation to IQ.

All assessments were made in a large sample of twin pairs and their singleton siblings. Twin families had been recruited from two separate age cohorts: 149 families with a mean age of 26 (SD 4.2) and 122 families with a mean age of 50 (SD 7.5). A randomly drawn sample of one subject per family was used to explore the effects of age and sex on stimulus-response incongruency effects on the onset and peak latency of the LRP, decision time and the number of too slow and incorrect responses. Structural equation modelling on the complete sample of genetically related subjects (twins and additional siblings) was used to examine whether individual differences in processing speed during trials with congruent stimulus-response mapping are influenced by genetic factors. Following this, the heritability was tested for stimulus-response incongruency effects using the contrast between stimulus-response congruent and stimulus-response incongruent trials. For all Eriksen flanker task derived measures we then computed the phenotypic correlation with psychometric IQ. In the main multivariate analyses, these phenotypic correlations were decomposed into a genetic and environmental part to test (1) whether common underlying genetic or environmental factors influence processing speed, accuracy and intelligence and (2) whether common underlying genetic or

environmental factors influence stimulus-response incongruency effects and intelligence.

2. Methodology

2.1. Subjects

Subjects were recruited from the Netherlands Twin Register (Boomsma, 1998) as part of a large ongoing project on the genetics of cognition and adult brain function (Posthuma et al., 2001a,b; Wright et al., 2001). Adult twins and their non-twin siblings were asked to participate in a testing protocol lasting 4.5 hrs. In one half of the protocol, psychometric intelligence, inspection time and decision time were assessed, in the other half electroencephalographic activity (EEG) was measured. The EEG registration consisted of a resting EEG measurement (Posthuma et al., 2001a), an oddball task (van Beijsterveldt et al., 2001), a spatiovisual working memory task (Hansell et al., 2001) and the Flanker task (Eriksen and Eriksen, 1974). The order of these tasks within the EEG session was fixed. The order of the two halves of the protocol was randomized across family members. In the present paper only data from the IQ test and from the Eriksen Flanker Task are reported.

Six hundred and eighty-eight family members from 271 extended twin families had participated in the study by December 2000. Participating families consisted of one to eight siblings (including twins). On average 2.5 subjects per family participated. In a young adult cohort 171 males and 210 females participated, in an older cohort this was 135 males and 172 females. The young cohort included 54 MZ pairs, 73 DZ pairs, 18 single twins and 109 additional siblings. The older cohort included 48 MZ pairs, 58 DZ pairs, 15 single twins, and 80 additional siblings.

2.2. Intelligence testing

IQ was measured with the Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III, 1997). Standardization norms for this version are currently being determined and at this point we can report unstandardized raw IQ scores only. All analyses, however, will explicitly model effects of sex and age on the raw IQ scores. Performance IQ was calculated as the mean score of three subtests (picture completion, block design, matrix reasoning) and verbal IQ was based on the mean score on four subtests (information, similarities, vocabulary, arithmetic).

2.3. Flanker task procedure

Subjects were in a supine position facing a monitor at 80 cm distance, in a dimly lit sound attenuated, and electrically shielded chamber. Two boxes with an upper and a lower button were placed left and right in front of the subject. A single randomized sequence of 120 trials was generated and used for all subjects. A trial was started when the subject simultaneously pressed the left and right lower buttons. Subjects

always used the index fingers. The trials started with a tone (1 KHz, 100 ms) and a simultaneously presented fixation dot in the centre of the monitor. After 1000 ms, the stimulus array was presented for 100 ms (see Fig. 1). Stimuli consisted of a horizontal stimulus array comprising five arrowheads. Left and right arrowheads occurred with equal probability. Likewise, the flanking arrowheads were as often congruent as incongruent with the target arrow. This resulted in four conditions each containing 30 trials: left congruent ($<<<<<$), right congruent ($>>>>>$), left incongruent ($>><>>$), and right incongruent ($<<><<$).

Subjects were instructed to respond with the left hand if the central arrowhead pointed to the left, and with the right hand if the central arrowhead pointed to the right. Responding meant releasing the lower "home" button and pushing the upper "response" buttons. They were asked to respond as fast and accurately as possible and to ignore the flanking arrowheads. Visual feedback ("right", "wrong" or "too slow", and total current points) was presented 1000 ms after the onset of the stimulus array, and lasted 1500 ms. They gained 1 point for each correct response and lost 5 points for wrong button presses or too-slow responses. Wrong button presses incorporated all premature responses, wrong home button releases, and wrong response button presses. Responses were too slow when they exceeded the maximum response time of 1000 ms. Trials were separated by an inter trial interval of 1500 ms after which the next trial started as soon as both home buttons had been pressed.

Home button release time and time of response button pressing were stored for all trials as well as the number of too-slow responses (>1000 ms) and wrong button presses. Performance measures were decision time, the number of incorrect and the number of too-slow responses. These measures were all averaged over left and right hand trials. Decision time was computed as the time interval between stimulus onset and home button release. Too-slow responses and wrong button presses were counted and converted to a percentage, because in a small number of subjects, timing information on a few of the 120 trials was lost. Before recording, all subjects received 30 practice trials.

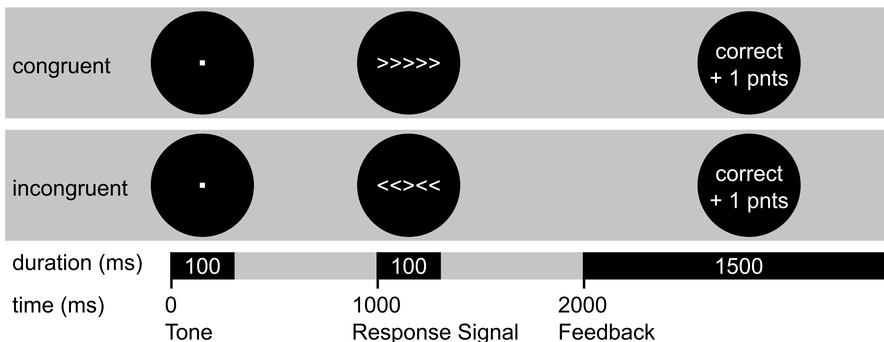

Fig. 1. Temporal structure of the LRP task.

2.4. EEG recording and LRP computation

The EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (Enschede, The Netherlands). Signals were continuously represented online on a Nec multisync 17” computer screen using POLY 5.0 software (POLY, 1999) and stored for offline processing. Standard 10–20 positions were F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, and O2 (Jasper, 1958). Additionally F1 and F2 were placed halfway between F3 and Fz, and between Fz and F4, respectively. Positions C3 and C4 are located above the right and left motor cortices, respectively, and are used in this analysis.

Software-linked earlobes (A1 and A2) served as reference. The vertical electrooculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed 1 cm below the right eye and 1 cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed 1 cm left from the left eye and 1 cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified (0.05–30 Hz), digitized at 250 Hz and stored for offline processing.

LRPs were computed for correct trials only. Per trial, the epoch used for data analysis started 250 ms preceding stimulus array onset, and ended 1000 ms after onset of the stimulus array. The mean amplitude in the 250 ms preceding the stimulus array was defined as the baseline. Epochs were discarded from further analyses if values exceeded 200 μV on the vertical or horizontal EOG channels, or values exceeded 80 μV on the EEG channels. A three step subtraction method was performed to calculate the LRP waveforms. First, we subtracted the time series recorded from C4 from those recorded over C3 on each trial for the right hand responses. Second, we subtracted the time series recorded from C4 from those recorded over C3 on each trial for left hand responses. Third, the two difference waves for left and right hand responses were subtracted, which resulted in the LRP waveform. This method is also known as the double subtraction method:

$$\text{LRP} = (\text{C3} - \text{C4})_{\text{righthand}} - (\text{C3} - \text{C4})_{\text{lefthand}}$$

Peak latency of the LRP was determined by searching the most negative value in the 350–900 ms post stimulus window. Onset of the LRP was calculated by a single-subject based regression procedure with one degree of freedom (Mordkoff and Gianaros, 2000). This method fits a linear regression to the LRP slope using the individually fixed LRP peak negativity. The intercept with the x-axis denotes LRP onset.

2.5. Statistical procedure

2.5.1. Effects of SEX and AGE COHORT on stimulus-response incongruency effects

As the total sample existed of genetically related subjects, a subset of unrelated subjects was obtained by randomly drawing one subject from each family. On this subset of genetically *un*related subjects, effects of sex and age cohort and their interactions with condition were tested using a repeated measurements MANOVA (GLM, SPSSwin v10.0, 1999). The within subjects factor was CONDITION (congruent, incongruent), and between subjects factors were SEX (female, male) and AGE COHORT (younger, older). Stimulus-response incongruency effects and modulation by age and sex are reflected in the CONDITION main effects, and the AGE COHORT $\times$ CONDITION and SEX $\times$ CONDITION interaction effects, respectively.

2.5.2. Phenotypic correlation of IQ with processing speed, accuracy and stimulus-response incongruency effects

In the subset of genetically *un*related subjects, Pearson correlations of verbal IQ and performance IQ with the onset and peak latency of the LRP, decision time, percentage too-slow responses or wrong button presses were calculated using SPSS 10.0. As the percentages of too-slow responses and wrong button presses were highly skewed both a log-transformation and a transformation to an ordinal scale were used. The transformation to the ordinal scale was done by regrouping the data into four categories: 0–5% slow, 5–10%, 10–15%, and more than 15%. Polyserial correlations with IQ were calculated using the software package PRELIS (version 2.12a; Jöreskog and Sörbom, 1996). The log-transformed and the ordinally transformed variables gave highly similar results. Only correlations obtained using the log-transform of the percentages too-slow responses and wrong button presses will be presented.

2.5.3. Estimating heritability of processing speed, accuracy, and stimulus-response incongruency effects

To estimate heritability of the processing speed we used onset and peak latency of the LRP and decision time in the *congruent condition*. To estimate heritability of accuracy we used the percentages too-slow responses or wrong button presses in the *congruent condition*. However, the data from the congruent condition were analyzed in a single analysis with the data from the incongruent condition to allow us to simultaneously estimate the heritability of the stimulus-response incongruency effects on speed and accuracy by using a linear combination of the two scores ($+1 \times \text{incongruent} + -1 \times \text{congruent}$) in the path model. The percentages of too-slow responses or wrong button presses had to be log transformed to obtain normality, so here a linear combination of the log-transformed variables in the congruent and incongruent condition would not work. Stimulus-response incongruency effects on accuracy, therefore, were obtained from a separate analysis on the log transform of the contrast between the two conditions.

Heritability was derived from structural equation modelling that estimates sources of (co-) variance in the observed measures due to additive genetic variation (A) or due to shared (C) and non-shared environmental (E) variation (Neale and Cardon, 1992). MZ twins share 100% of their genes, while DZ twins share on average 50% of their genes, as do singleton sibling pairs. Shared environment is per definition 100% shared by the twins of both MZ and DZ pairs, and will consist mainly of the family environment. Thus, the expectation for the covariance between two members of an MZ twin pair is A+C. The expectation for the covariance between two members of a DZ twin pair or between singleton sibling pairs is 1/2A+C. Non-shared environmental factors incorporate those factors in the environment that are not shared by siblings. The expectation for the variance is A+C+E.

Our extended twin design (i.e. consisting of twins and additional siblings) provides data characterized by families of variable size. Such ‘incomplete’ data can be analyzed in Mx (Neale, 1997) via full information maximum likelihood, which uses the observed data, and provides parameter estimates that make the observed data most likely. In order to obtain a measure of how well the specified model for means and covariances fits the observed values, the raw data option in Mx calculates the negative Log-Likelihood (−LL) of the raw data for each pedigree (Lange et al., 1976), as:

$$-\mathrm{LL} = -k\,\log(2\pi) + \log|\Sigma| + (y_i - \mu)'\,\Sigma^{-1}(y_i - \mu),$$

where k ($k = 1, \ldots, p$; p is the number of family members times the number of phenotypes) denotes the number of observed variables within a family (and can vary over families), Σ is the expected covariance matrix of family members with dimension p by p, y_i (for $i = 1, \ldots p$) is the vector of observed scores, μ is the column vector of the mean expected values of the variables for that pedigree, and $|\Sigma|$ and $\Sigma - 1$ are the determinant and inverse of matrix Σ, respectively.

Since the families are independent, their joint likelihood is the product of their individual likelihoods and the log of the joint likelihood is the sum of the log likelihoods per family. Thus, summing the negative likelihoods (−LLs) of all families gives the −LL of the model. In Mx the −LL of the model is doubled because twice the difference between two models (2{−LL full model−(−LL nested model)}) is—under certain regularity conditions—asymptotically distributed as χ^2. Thus, two nested models (a nested model includes fewer parameters and does not introduce new parameters compared to the model under which it is nested) which provide −2LLs, may be subtracted to provide a Δ(−2LL) which has a χ^2 distribution. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model.

When the model is written in terms of matrix algebra, generalization from the univariate case to a multivariate case becomes straightforward. Let matrices **A**, **C** and **E** be of dimensions $n \times n$, where n denotes the number of variables measured on each subject. Matrix **A** denotes the genetic component, matrix **C** denotes the shared environmental component, while matrix **E** denotes the non-shared environmental component. The diagonal elements of matrix **A** denote the genetic variances of the three variables. For example, element a_{11} is the genetic variation in the first variable.

The off-diagonal elements of matrix **A** represent the genetic covariance between variables. Analogously, the diagonal elements of matrices **C** and **E** denote the shared and non-shared environmental variances of the three variables, and the off-diagonal elements denote the covariances due to shared and non-shared environmental influences.

As matrices **A**, **C**, and **E** are covariance matrices, they are restricted to be positive definite. This is accomplished by calculating matrices **A**, **C**, and **E** as the product of a triangular matrix and its transpose. Thus, matrix **A** is calculated as $\mathbf{X}\times\mathbf{X}'$, where **X** is triangular and of dimensions 3×3 (for three variables). Analogously, matrix **C** is $\mathbf{Y}\times\mathbf{Y}'$, and matrix **E** is $\mathbf{Z}\times\mathbf{Z}'$. This is also known as a Cholesky factorization of matrices **A**, **C** and **E**.

2.5.4. Decomposition of phenotypic correlations with IQ into environmental and genetic correlation

A multivariate decomposition of covariances into genetic and environmental components was used for each measure that showed a significant phenotypic correlation with verbal or performance IQ. The decomposition of covariances into genetic and environmental components necessitates the use of a genetically informative design, such as the twin design. The variance is formally represented as

$$\mathbf{A} + \mathbf{C} + \mathbf{E} = \mathbf{X} \times \mathbf{X}' + \mathbf{Y} \times \mathbf{Y}' + \mathbf{Z} \times \mathbf{Z}'.$$

The covariance is formally represented as

$$\mathbf{A} + \mathbf{C} = \mathbf{X} \times \mathbf{X}' + \mathbf{Y} \times \mathbf{Y}' \text{ for MZ twins,}$$

$$0.5 \times \mathbf{A} + \mathbf{C} = 0.5 \times \mathbf{X} \times \mathbf{X}' + \mathbf{Y} \times \mathbf{Y}' \text{ for DZtwins.}$$

The *genetic correlation* between variables i and j (r_{gij}) is derived as the genetic covariance (a_{ij}) between variables i and j divided by the square root of the product of the genetic variances of variables i (a_{ii}) and j (a_{jj});

$$r_{gij} = \frac{a_{ij}}{\sqrt{a_{ii} \times a_{jj}}}.$$

Analogously, the *shared* (r_{cij}) *and non-shared* (r_{eij}) *environmental correlation* between variables i and j are derived as the respective environmental covariances between variables i and j divided by the square root of the product of the respective environmental variances of variables i and j. The phenotypic correlation (r) is the sum of the product of the genetic correlation and the square roots of the genetic variances of the two phenotypes and the product of the environmental correlation and the square roots of the environmental variances of the two phenotypes.

$$r = r_{gij} \times \sqrt{\frac{a_{ii}}{(a_{ii} + c_{ii} + e_{ii})}} \sqrt{\frac{a_{jj}}{(a_{ii} + c_{ii} + e_{ii})}} + r_{cij} \times \sqrt{\frac{c_{ii}}{(a_{ii} + c_{ii} + e_{ii})}} \sqrt{\frac{c_{jj}}{(a_{ii} + c_{ii} + e_{ii})}} + r_{eij} \times \sqrt{\frac{e_{ii}}{(a_{ii} + c_{ii} + e_{ii})}} \sqrt{\frac{e_{jj}}{(a_{ii} + c_{ii} + e_{ii})}}.$$

$$r = \text{genetic contribution} + \text{shared environmental contribution} + \text{non-shared environmental contribution.}$$

3. Results

3.1. Effects of SEX and AGE COHORT on stimulus-response incongruency effects

Psychometric IQ scores were available for 688 subjects (271 families). Table 1 shows age and IQ for the random selection of unrelated individuals, one from each of these families. Analyses of sex and age cohort effects on verbal and performance IQ for this sample have been described elsewhere (Posthuma et al., 2001b). Briefly, it was found that males generally had higher IQ scores than females and younger subjects had higher IQ scores than older subjects.

Seventy eight subjects did not perform the Eriksen flanker task. For the remaining 610 subjects (250 families) data on the average decision time over correct trials and the percentage of trials with too-slow responses or wrong button presses are shown

Table 1
Age and IQ in the randomly selected group of unrelated subjects

		Age	Verbal IQ	Performance IQ
Young females	*N*	74	74	74
	Mean	26.02	28.24	23.64
	Sd	3.78	5.03	3.51
Young males	*N*	75	75	75
	Mean	25.96	29.23	24.34
	Sd	4.41	4.83	3.14
Older females	*N*	63	63	63
	Mean	51.11	26.04	19.40
	Sd	7.46	6.22	3.89
Older males	*N*	59	59	59
	Mean	50.59	29.33	20.62
	Sd	7.36	5.07	4.05

Table 2
Decision time, percentage wrong button presses and percentage responses 'too slow' in the randomly selected group of unrelated subjects

		Decision time (ms)		Percentage wrong button presses		Percentage 'too slow'	
		Congruent	Incongruent	Congruent	Incongruent	Congruent	Incongruent
Young females	*N*	68	68	68	68	68	68
	Mean	456.99	552.66	0.20	3.31	2.52	8.52
	Sd	39.47	41.89	0.68	8.83	3.20	8.89
Young males	*N*	69	69	69	69	69	69
	Mean	467.34	562.91	0.23	2.05	3.07	9.00
	Sd	36.27	40.27	1.01	5.75	4.81	9.47
Older females	*N*	59	59	59	59	59	59
	Mean	499.66	586.08	2.38	6.78	8.50	22.12
	Sd	44.93	51.22	5.47	11.46	8.10	18.68
Older males	*N*	54	54	54	54	54	54
	Mean	497.60	589.26	0.74	5.94	7.26	17.71
	Sd	46.92	53.38	2.44	11.50	8.60	16.50

in Table 2. The expected effects of CONDITION were found for the percentage too-slow ($F(1, 246) = 188.98$, $P < 0.0001$), percentage wrong button presses ($F(1, 246) = 44.27$, $P < 0.0001$), and decision time ($F(1, 246) = 1872.92$, $P < 0.0001$): stimulus-response incongruency resulted in a prolonged decision time (+92.33 ms), more wrong button presses (3.63%) and more too-slow (9.00%) responses. No main or interaction effects were found involving SEX.

Significant effects of AGE COHORT were found for the percentage too-slow ($F(1, 246) = 46.67$, $P < 0.0001$), percentage wrong button presses ($F(1, 246) = 12.68$, $P < 0.0001$), and decision time ($F(1, 246) = 40.84$, $P < 0.0001$). Older subjects made more responses that were 'too-slow' (+8.12%), made more wrong button presses (+2.51), and had prolonged decision times (+33.17 ms) as compared to younger subjects. In addition, AGE COHORT significantly interacted with CONDITION for the percentage too slow ($F(1, 246) = 21.49$, $P < 0.0001$) responses and wrong button presses ($F(1, 246) = 4.56$, $P < 0.05$). Stimulus-response incongruency led to a larger percentage wrong button presses responses in the older cohort (4.80%) than in the younger cohort (2.47%). Likewise, it affected the percentage too-slow responses more in the older cohort (12.03%) than in the younger cohort (5.96%). In contrast, the AGE COHORT × CONDITION interaction failed to reach significance for decision time ($F(1, 246) = 2.38$, $P = 0.12$). Because the number of too-slow responses was higher in the older cohort, particularly during the incongruent condition, the lack of an interaction effect on decision time may have reflected the exclusion of the correct but slow trials. To explore this, we plot histograms of the reaction time (decision time + movement time) from all correct trials in the congruent and incongruent conditions for the two age cohorts in Fig. 2. In the incongruent condition of the older cohort it is evident that a number of correct trials are missing from the distribution because we classified reaction times above 1000 ms as too-slow. However, extrapolating from the normal curve this missing tail accounts for only about 3–5% of the responses. In reality, 20% of the trials were coded too slow. This means that 15–17% of the too-slow responses were not simply "correct but slow", but must have been drawn from another distribution.

Two further measures of processing speed were derived from the LRP: onset and peak latency. We found that a number of subjects did not show a waveform resembling a readiness potential, which made computation of the LRP problematic. We then decided to select only subjects with a minimum of 30 correct trials (for the congruent as well as the incongruent condition) who had unambiguous LRP traces, even if this meant compromising statistical power of the genetic analyses in terms of lowered sample sizes.

A reliable onset of the LRP was available for 376 subjects in the congruent condition and 361 subjects in the incongruent condition. Peak latency of the LRP was available for 407 subjects in the congruent condition and 376 subjects in the incongruent condition. Fig. 3 shows the grand averages of the LRP waveforms in the congruent and incongruent conditions for the remaining participants in both age cohorts. The figure nicely demonstrates the stimulus-response incongruency effects on the onset and peak latency of the LRP. The positive dip before the onset of the negative shift in the incongruent condition reflects activation of the wrong response.

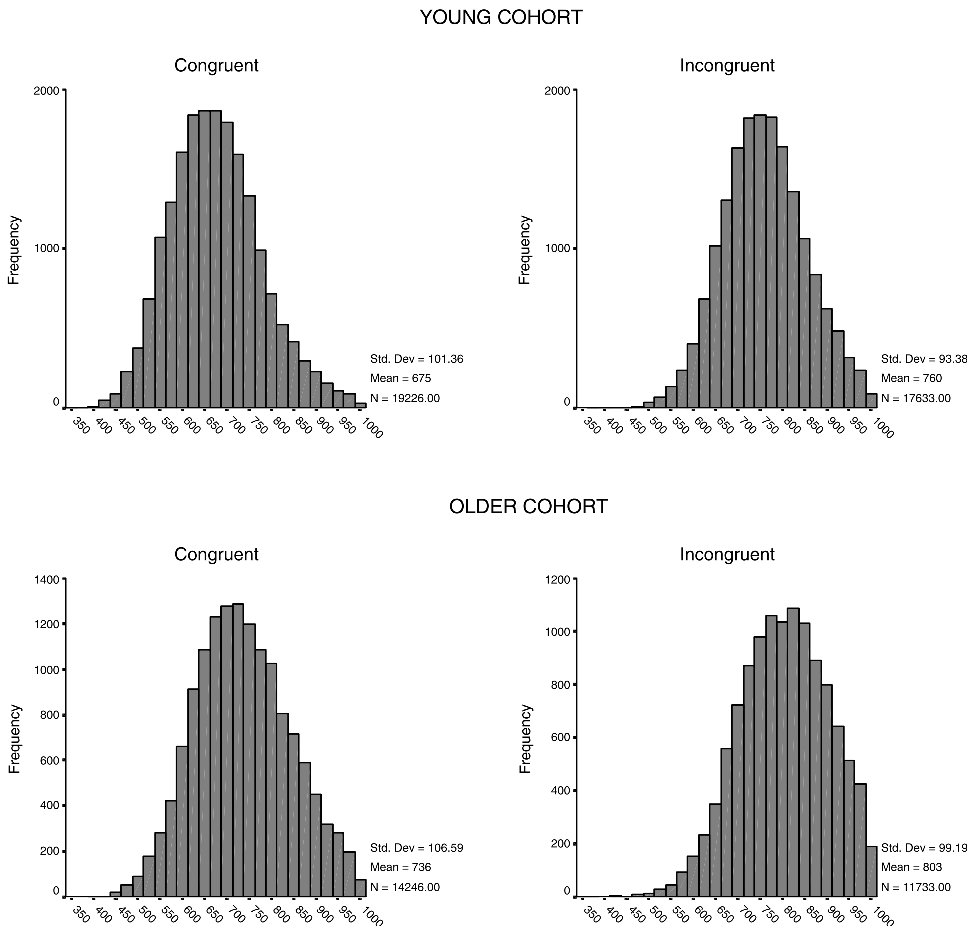

Fig. 2. Distribution of the single trial reaction times (decision time+movement time). Reaction time was recorded in correct trials only; trials with reaction times over 1000 ms were coded too slow.

The analyses of the effects of SEX and AGE COHORT were again performed on the subset of genetically unrelated subjects. LRP latencies of these subjects are shown in Table 3. For the onset ($F(1, 175) = 666.16$, $P < 0.0001$) and peak latency of the LRP ($F(1, 184) = 450.32$, $P < 0.0001$) significant effects of CONDITION were found. The presence of incompatible flankers resulted in a prolonged onset (+115.86 ms) and prolonged peak latency (+96.96 ms). The main effect of AGE COHORT was significant for the onset ($F(1, 175) = 6.07$, $P < 0.05$) and peak latency of the LRP ($F(1, 184) = 16.77$, $P < 0.0001$) and indicated that the onset (+13.55 ms) and the peak latency of the LRP (+32.60 ms) were slower in the older compared to the young cohort. There were no main effects of SEX, and no interactions of SEX with either AGE COHORT or CONDITION.

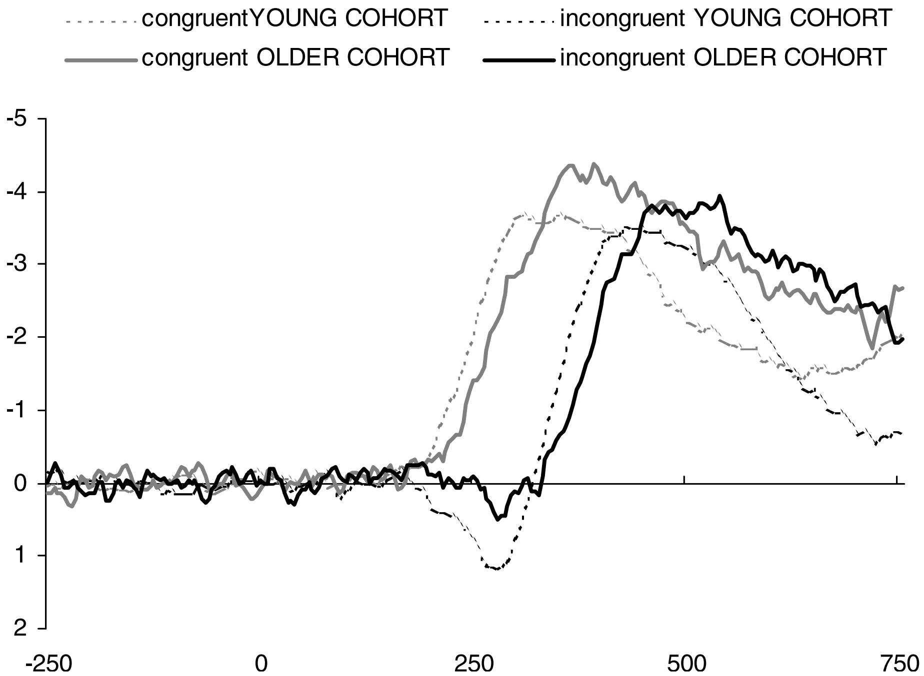

Fig. 3. Grand averages of the LRP.

3.2. *Genetic analyses of processing speed and accuracy*

The distribution of percentage wrong button presses and percentage too-slow responses was highly skewed. In view of the comparable effects of stimulus-response

Table 3
Onset and peak latency of the LRP in the randomly selected group of unrelated subjects

		Onset (ms)		Peak latency (ms)	
		Congruent	Incongruent	Congruent	Incongruent
Young females	*N*	50	50	55	55
	Mean	186.96	301.04	347.75	441.93
	Sd	49.61	52.39	62.04	56.01
Young males	*N*	57	57	60	60
	Mean	194.63	322.84	359.00	452.20
	Sd	39.55	45.94	65.83	53.47
Older females	*N*	35	35	36	36
	Mean	214.46	312.43	386.33	487.00
	Sd	51.93	49.48	41.73	56.18
Older males	*N*	37	37	37	37
	Mean	204.81	318.97	379.08	478.86
	Sd	42.38	39.84	82.28	69.02

incongruency effects and aging on both type of incorrect responses, we collapsed them into a single percentage for the genetic analyses. This percentage was still highly skewed, and analyses were run using both a threshold model and a log transform. The ordinal transformation and log-transformation gave highly similar results (data not shown) and below we report only on the log-transform of the percentage incorrect response. Maximum likelihood estimates of the twin correlations are given in Table 4. Virtually all MZ correlations are higher than DZ twin correlations. This suggests the presence of genetic influences on the variance in onset and peak latency of the LRP, decision time, percentage incorrect responses, and verbal and performance IQ.

Decomposing the variance in IQ measures by structural equation modelling into genetic, shared and non-shared environmental components confirmed our previous finding (see Posthuma et al., 2001b) that verbal and performance IQ are highly heritable (85 and 69%, respectively). No evidence was found for shared environmental influences. Although the final sample size for the LRP measures is significantly larger than any previous study on the LRP, and more than sufficient to estimate age and sex effects on the mean, it is still critically small for the separate detection of genetic influences and shared environmental influences (see e.g. Posthuma and Boomsma, 2000). We choose, therefore, to decompose the variance in genetic variance (A) and non-shared environmental variance (E; including measurement error) and not to include shared environmental variance in the model. Thus, although the factor A is modelled as additive genetic influences, it should be kept in mind that this factor may also contain shared environmental influences. Table 5 shows the fit statistics of the full AE model and the best reduced variance decomposition models in which different models were allowed for the young and older cohort in each of the two conditions.

The congruent condition was used to assess heritability of processing speed and accuracy. Under the most parsimonious models, genetic influences explained 43% of interindividual differences in the onset of the LRP in the young cohort and 46% of interindividual differences in the peak latency of the LRP in the older cohort (see Table 6). Genetic influences explained 33% of the variance in decision time in the young and 48% of the variance in the older cohort. In the older cohort, 41% of the variance in accuracy derived from genetic influences. No genetic influences on the percentage incorrect responses in the young cohort could be detected. This may not be surprising as in the young cohort very few incorrect responses were given in the congruent condition, keeping the interindividual variance very low.

3.3. *Genetic analyses of the effects of stimulus-response incongruency*

The contrast between the congruent and incongruent conditions was used to assess heritability of the effects of stimulus-response incongruency on processing speed and accuracy. Table 7 shows that individual differences in the effects of stimulus-response incongruency on onset and peak latency of the LRP were not due to genetic differences, with the exception of the onset of the LRP in the young cohort. However, individual differences in the effects of stimulus-response incongruency on

Table 4
Twin correlations

	Onset		Peak latency		Decision time		Percentage incorrect		IQ	
	Congruent	Incongruent	Congruent	Incongruent	Congruent	Incongruent	Congruent	Incongruent	VIQ	PIQ
Young cohort										
MZ	0.69 (16)	0.15 (18)	0.04 (23)	0.73 (20)	0.56 (46)	0.49 (46)	−0.24 (46)	0.30 (46)	0.84 (54)	0.70 (54)
DZ	0.24 (117)	−0.02 (119)	0.21 (136)	0.03 (129)	0.06 (241)	0.35 (239)	0.07 (241)	0.26 (241)	0.47 (283)	0.31 (283)
Older cohort										
MZ	0.35 (16)	0.44 (13)	0.41 (18)	0.16 (14)	0.50 (45)	0.33 (45)	0.39 (45)	0.38 (45)	0.84 (48)	0.70 (48)
DZ	−0.32 (68)	0.09 (54)	0.29 (81)	0.48 (47)	0.24 (185)	0.22 (183)	0.23 (185)	0.30 (185)	0.47 (242)	0.31 (242)

MZ = monozygotic twins; DZ = dizygotic twin and twin-sibling pairs. VIQ, PIQ = verbal and performance IQ. Between brackets: number of pairs.

Table 5
Fit statistics of the full AE and the best (reduced) variance decomposition models (**bold**)

	−2LL	df	χ^2	Δdf	*P*
ONSET					
Full AE-model	7852.73	721			
E-model, AE-model for congruents in young cohort	**7857.34**	**726**	**4.62**	**5**	**0.47**
PEAK LATENCY					
Full AE-model	8577.71	767			
E-model in young cohort, AE-model in older cohort	**8579.36**	**770**	**1.65**	**3**	**0.65**
DECISION TIME					
Full AE-model	**12 393.12**	**1200**			
% INCORRECT					
Full AE-model	12 322.22	1201			
AE-model, E-model for congruents in young cohort	**12 323.94**	**1202**	**1.72**	**1**	**0.19**

All models are bivariate models that include the congruent and incongruent conditions and a linear combination of these two conditions to derive estimates for the stimulus-response incongruency effects.

Table 6
Percentage of the variance in processing speed and accuracy explained by additive genetic variation (A) and non-shared environmental variation (E)

	YOUNG COHORT		OLDER COHORT	
	A	E	A	E
ONSET				
Full AE-model	48 (7–76)	52 (24–93)	3 (0–28)	97 (72–100)
E-model, AE-model for congruents in young cohort	**43 (3–73)**	**57 (27–97)**	**–**	**100**
PEAK LATENCY				
Full AE-model	2 (0–23)	98 (77–100)	46 (20–66)	54 (34–80)
E-model in young cohort, AE-model in older cohort	**–**	**100**	**46 (20–66)**	**54 (34–80)**
DECISION TIME				
Full AE-model	**33 (10–54)**	**67 (46–90)**	**48 (25–66)**	**52 (34–75)**
% INCORRECT				
Full AE-model	3 (0–23)	97 (77–100)	41 (20–58)	59 (42–80)
AE-model, E-model for congruents in young cohort	**–**	**100**	**41 (20–58)**	**59 (42–80)**

decision time and on the percentage incorrect responses (including too slow) were under significant genetic control. Under the most parsimonious models, genetic influences explained 25% of interindividual differences in decision time in the young cohort and 32% in the older cohort. Genetic influences explained 23% of the variance in percentage incorrect in the young and 29% in the older cohort.

Table 7
Percentage of the variance in *stimulus-response incongruency effects* on processing speed and accuracy explained by additive genetic (A) and non-shared environmental variation (E)

	YOUNG COHORT		OLDER COHORT	
	A	E	A	E
ONSET				
Full AE-model	15 (0–49)	85 (51–100)	10 (0–45)	90 (55–100)
E-model, AE-model for congruents in young cohort	**26 (2–47)**	**74 (53–98)**	**–**	**100**
PEAK LATENCY				
Full AE-model	6 (0–28)	94 (72–100)	3 (0–35)	97 (65–100)
E-model in young cohort, AE-model in older cohort	**–**	**100**	**3 (0–35)**	**97 (65–100)**
DECISION TIME				
Full AE-model	**25 (6–44)**	**75 (56–94)**	**32 (3–69)**	**68 (31–97)**
% INCORRECT				
Full AE-model	**23 (6–40)**	**77 (60–94)**	**29 (5–12)**	**71 (48–95)**

3.4. Phenotypic correlations with verbal IQ and performance IQ

The phenotypic correlations (by age cohort) of onset and peak latency of the LRP and decision time with verbal and performance IQ are shown in Table 8. These correlations do not show a meaningful pattern for the young cohort, but suggest a significant relation between processing speed and IQ in the older cohort. However, in contrast to our expectation, this IQ/processing speed correlation was not reflected in the onset and peak latency of the LRP.

Table 9 shows the pattern of correlations of stimulus-response incongruency effects with verbal and performance IQ. Significant correlation was found with IQ for the effects on accuracy. Incongruency effects on the number of too slow and the number of wrong button presses were significantly larger in the subjects with lower IQ scores.

3.5. Decomposition of the phenotypic correlations into genetic and environmental correlations

Only the significant phenotypic correlations in Tables 8 and 9 were selected for decomposition into genetic and environmental components. The results of this decomposition are depicted in Table 10. The correlation of verbal and performance IQ with decision time in the older cohort was completely explained by an underlying set of genes. Dropping the environmental contributions to verbal IQ/decision time and performance IQ/decision time correlations did not cause a significant worsening of the fit of the model (VIQ $\chi^2_1 = 0.02$, $P = 0.88$; PIQ $\chi^2_1 = 0.001$, $P = 0.98$). The correlation of verbal and performance IQ with percentage incorrect in the congruent condition in the older cohort was also completely explained by an underlying set of

Table 8
Phenotypic correlation of verbal (VIQ) and performance (PIQ) with processing speed and accuracy

		Onset	Peak latency	Decision time	Wrong button presses	'Too slow'	Total incorrect
Young	VIQ	0.01	0.10	0.06	0.11	−0.04	−0.01
Cohort	PIQ	−0.02	0.04	0.09	0.00	−0.14	−0.13
Older	VIQ	0.13	0.06	−0.21*	−0.07	−0.25**	−0.23*
Cohort	PIQ	0.03	−0.16	−0.25**	−0.07	−0.24**	−0.23*

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Table 9
Phenotypic correlation of verbal (VIQ) and performance IQ (PIQ) with stimulus-response incongruency effects

		Onset	Peak latency	Decision time	Wrong button presses	'Too slow'	Total incorrect
Young	VIQ	−0.07	−0.24**	0.01	−0.11	−0.24**	−0.22**
Cohort	PIQ	0.08	−0.10	−0.18*	−0.33**	−0.29**	−0.39**
Older	VIQ	0.01	−0.13	0.14	−0.28**	−0.29**	−0.36**
Cohort	PIQ	−0.06	−0.04	0.11	−0.29**	−0.32**	−0.39**

* Significant at the 0.05 level.
** Significant at the 0.01 level.

Table 10
Genetic correlation and genetic contribution to the significant phenotypic correlations with verbal (VIQ) and performance IQ (PIQ)

	VIQ		PIQ	
	Genetic correlation	Genetic contribution[a] (%)	Genetic correlation	Genetic contribution[a] (%)
Decision time (older)	−0.20 (−0.41 to −0.001)	100	−0.34 (−0.56 to −0.12)	100
Percentage incorrect (older)	−0.51 (−0.70 to −0.31)	100	−0.52 (−0.73 to −0.30)	100
Incongruency effects on the percentage incorrect (young)	−0.44 (−0.79 to −0.20)	100	−0.68 (−1.00 to −0.43)	100
Incongruency effects on the percentage incorrect (older)	−0.37 (−0.89 to −0.12)	100	−0.48 (−0.93 to −0.21)	100

[a] Genetic contribution = percentage of the phenotypic correlation explained by a genetic correlation.

genes. Dropping the environmental contributions from the model did not cause a significant worsening of the fit (VIQ $\chi^2_1 = 0.37$, $P = 0.54$; PIQ $\chi^2_1 = 0.01$, $P = 0.94$).

The correlation of verbal and performance IQ with stimulus-response incongruency effects on the percentage incorrect responses in both cohorts was completely explained by an underlying set of genes. Dropping the environmental contributions to these correlations for both cohorts did not cause a significant worsening of the fit of the model (VIQ;$\chi^2_2 = 1.20$, $P = 0.55$; PIQ:$\chi^2_2 = 2.80$, $P = 0.25$).

4. Discussion

This study examined the genetic contribution to interindividual variance in the speed of selective response activation, decision time and accuracy in the congruent condition of the Eriksen Flanker task. It also examined the genetic contribution to slowing and loss of accuracy induced by stimulus-response incongruency. It was specifically tested whether processing speed, accuracy and stimulus-response incongruency effects were genetically correlated with IQ. These analyses required a large sample of genetically related subjects, in our case twins and their singleton siblings. This large sample provided us with the opportunity to evaluate effects of age and sex on these measures for which most previous samples using the Flanker task had only low statistical power. Below, we review these age and sex effects and follow this with a discussion of phenotypic and genetic correlations with IQ.

As expected, the presence of incongruent flankers led to a significant increase in the onset (115.86 ms) and peak latency of the LRP (96.96 ms) and in decision time (92.33 ms), which is in line with previous findings on this task (e.g. Eriksen and

Eriksen, 1974; Botvinick et al., 1999; Casey, et al., 2000; Gratton et al., 1988; Kopp et al., 1996; Kramer et al., 1994). No evidence of sex differences was found on the performance of the Eriksen flanker task throughout. For age, the expected effects were found on all measures of processing speed. Subjects from the older age cohort (mean age 50) had an onset of the LRP that was on average 13.55 ms delayed compared to subjects in the younger age cohort (mean age 26). The peak latency of the LRP was delayed by an average of 32.60 ms in the older age cohort, and decision times were prolonged by 33.17 ms. At first sight, this cognitive slowing did not seem amplified by stimulus-response incongruency, since no evidence was found for an interaction of age-cohort and condition on decision time. This is consistent with findings from a previous study that looked at decision time during an Eriksen flanker task and compared means across a cohort of 32 young (mean age 20.6) subjects and a cohort of 30 older subjects (mean age 67.8) subjects (Kramer et al., 1994). They found significant differences in mean decision time between the two cohorts (i.e. the older subjects had a longer decision time) and significant prolongation of decision time in the incongruent condition compared to the congruent condition in both cohorts, but no interaction effects.

It should be noted, however, that our measures of processing speed were all computed over trials in which a correct response had to be given within 1000 ms. Slower trials were coded as ‘too slow’ and no mean decision time was recorded for these trials; instead the ‘too slow’ feedback was given instantaneously. This stern criterion was chosen to make sure that the subjects would remain motivated to respond as fast as possible. Fig. 2 suggests that at least part of the potentially correct trials in the incongruent condition in the older cohort fell in the ‘too slow’ category, which meant they were not used to compute average decision time, onset and peak latency of the LRP. We found a significantly larger stimulus-response incongruency effect on the percentage responses too slow in the older cohort: the presence of incongruent flanking stimuli induced 12.03% more too-slow responses than the congruent condition. This figure was only half (5.96%) in the young cohort. These findings do allow for possible amplification of cognitive slowing by stimulus-response incongruency in the older cohort. The failure of the age cohort by condition interaction on decision time to reach significance may have been due to removing these “correct but slow responses just after 1000 ms”. However, three observations suggest that a substantial part of the too-slow responses were qualitatively different from such correct but slow responses. First, unless the distribution in Fig. 2 is extremely skewed to the right, only a few percent of the correct trials are missing—far less than the actual percentage of too-slow responses found. Secondly, in 74% of the too-slow responses the home button was never released. This means that even the decision time was larger than 1000 ms, almost double of what it is in the correct trials. In these trials subjects literally ‘did not lift a finger’. Thirdly, the number of wrong button presses also showed evidence of stronger stimulus-response incongruency effects in the older than in the younger cohort. Stimulus-response incongruency, therefore, seems to do more harm than response slowing alone. A fair summary of our findings is that older subjects experience more interference by

incongruent flankers than younger subjects when they have to respond correctly within a fixed time frame.

The source of individual differences in the interference induced by stimulus-response incongruency is still unresolved. Larger interference may derive from impairments in local inhibitory connections in the motor or perceptual system (Cohen et al., 1992; Servan-Schreiber, 1990; Spencer and Coles, 1999) or from impairments in top-down inhibitory control signals generated by a supervisory attentional system (Kramer et al., 1994; West, 1996) or a conflict monitoring system (Botvinick et al., 2001).

Localisation of these impairments in cognitive control in the brain is still unresolved although the frontal cortex seems to play an important role (Botvinick et al., 1999; Dempster, 1991; Fuster, 1997; Hazeltine et al., 2000; MacDonald et al., 2000; Smith and Jonides, 1999; Ullsperger and von Cramon, 2001). For our purposes it suffices that processes of inhibitory control and attentional selection are highly plausible source of individual differences in cognitive ability. Although cognitive ability (or IQ) in itself is highly heritable, it is likely to be influenced by a number of genes of small effect. These genes are more easily uncovered by focussing on elementary aspects of cognition, such as processing speed or resistance to interference. The main goal of our study was to test Flanker task derived behavioural and electrophysiological measures of processing speed and resistance to interference as viable “endophenotypes” of cognitive ability. This endophenotype approach requires that the Flanker-task derived measures must be heritable and show evidence of genetic correlation to intelligence (de Geus and Boomsma, 2002).

Using the complete dataset of genetically related subjects, it was found that genetic effects accounted for over 40% of the variance in LRP-onset (young cohort) and LRP-peak amplitude (older cohort) in the congruent condition. Neither parameters, however, were systematically associated with verbal and performance IQ, and no genetic correlation could be found. This contrasted with our expectation that the more intelligent subjects would be fastest in their selective response activation. This expectation derived from the theoretical framework of the neural speed theory of intelligence (Eysenck, 1986; Vernon, 1987, 1993). Within this framework, previous studies have systematically found reaction time to be a heritable trait that is both genetically and phenotypically correlated with measures of intelligence (e.g. Baker et al., 1991; Finkel and Pedersen, 2000; Ho et al., 1988; Neubauer and Knorr, 1997; Rijsdijk et al., 1998; Luciano et al., 2001). In an earlier report on these same subjects we found that the speed of early stimulus detection (as measured by inspection time) was significantly correlated with IQ through a common genetic pathway (Posthuma et al., 2001b). We now extend these findings by showing a similar pattern for decision time in the older cohort, where a significant genetic correlation was found of decision time with verbal (−0.20) and performance IQ (−0.34).

It is unclear why the onset or peak latency of the LRP did not show the expected (genetic) correlation with IQ that we did find in these same subjects with the other processing speed measures (inspection time and decision time), and that others found with total reaction time (Finkel and Pedersen, 2000; Luciano et al., 2001). A first explanation is that the largest source of individual differences relevant to IQ may

simply be in the early perceptual stage of a response, in the stage between selective response activation and the actual response execution, or even in movement execution itself. A second more humble explanation may be the difference in the reliability of the methodologies to assess the various parameters. Reaction times can be recorded with a high level of fidelity, whereas the ERPs, almost by their nature, are highly noisy. Error variance is further increased by the use of a difference score, i.e. the subtraction of left and right EEG signals. Although LRP data are highly useful to compare groups, they may be less suitable to a pure individual differences design. Interestingly, the latency of another ERP, the P3 latency, also showed no evidence of a genetic correlation with IQ in a group of adolescent twins in who IQ and reaction time did derive from common genetic factors (Wright et al., 2002). Aware of the potential problems in the reliability of the LRP, we rigidly selected only those traces in which a clear readiness potential was visible, and used only subjects in which we could average 30 of such traces. As a consequence of this selection of highly reliable LRP traces, a substantial number of subjects were lost, eroding the power to detect low but reliable correlation with IQ.

In addition to processing speed, we also examined the effects of stimulus-response incongruency as a possible genetic correlate of IQ. Effects of stimulus-response incongruency on the LRP-derived measures did not classify as useful endophenotypes of verbal or performance IQ, and neither did the effects on decision time. In contrast, the effects of incongruent flankers on the percentage of incorrect responses were heritable in both age cohorts and correlated at a genetic level with psychometric IQ. In other words, the genetic factor underlying these stimulus-response incongruency effects also explained part of the variance in verbal and performance IQ. We conclude that the ability to perform correctly on a speeded choice reaction time task under conditions of response conflict is a viable endophenotype of cognitive ability.

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Chapter 11

Combined linkage and association tests in Mx[1]

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Combined Linkage and Association Tests in Mx

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Statistical methods aimed at the detection of genes for quantitative traits suffer from two problems: (i) when a linkage approach is employed, relatively large sample sizes are usually required; and (ii) when an association approach is employed, effects of population stratification may blur genuine locus–trait associations. The variance components method proposed by Fulker *et al.* (1999) addressed both these problems; it is statistically powerful because it involves a combined analysis of linkage and association and can include information from multiplex families, which reduces the overall amount of necessary individual genotypes. In addition, it includes an explicit test for the presence of spurious association. After a brief illustration of the various ways in which population stratification may affect locus–trait associations, the implementation in Mx (Neale, 1997) of the method as proposed by Fulker *et al.* (1999) is discussed and illustrated. In addition, an extension to this method is proposed that allows the use of (variable) sibship sizes greater than two, the estimation of additive and dominance association effects, and the use of multiple alleles. These extensions can be implemented when parental genotypes are available or unavailable.

KEY WORDS: QTL; population stratification; structural equation modeling; variance components modeling; quantitative trait.

INTRODUCTION

Statistical methods aimed at the detection of quantitative trait loci (QTLs) have primarily focused on detecting linkage between a QTL (or a marker in linkage disequilibrium with the QTL) and a trait (e.g., Almasy and Blangero, 1998; Amos, 1994; Boomsma and Dolan, 1998; Eaves *et al.,* 1996; Fulker and Cardon, 1994; Fulker and Cherny, 1996; Goldgar, 1990; Haseman and Elston, 1972; Schork, 1993). Recently, however, attention has shifted toward methods designed to detect *associations* between QTLs and traits (e.g., Abecasis *et al.,* 2000; Fulker *et al.,* 1999; Lesch *et al.,* 1996; Plomin *et al.,* 2001). Under certain conditions, testing for association can be more powerful than testing for linkage (Risch, 2000; Risch and Merikangas, 1996; Sham *et al.,* 2000), even without assuming that one of the typed markers is the actual trait locus (Long and Langley, 1999).

A widely used design to test for an association between a locus and a trait is the case-control design. This design, however, is sensitive to the effects of population stratification that may confound genuine locus—trait associations (Hamer and Sirota, 2000). Spurious associations may arise in a population that is a mix of two or more genetically distinct subpopulations. Any trait that is more frequent in one of the subpopulations compared to the other subpopulation(s) (e.g., because of cultural differences or assortative mating) will show a statistical association with any allele that has a different frequency across those two populations (e.g., as a result of different ancestors or genetic drift). This association is called spurious because within each population the allele is unrelated to variation in the trait. In practice, more than two populations may have combined and it will not be obvious from the combined populations whether or not the sample is stratified and in what way.

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Population stratification is often considered the culprit for nonreplication of previously found associations (Cardon and Bell, 2001; Ioannidis *et al.,* 2001; Plomin and Caspi, 1999; Risch, 2000; Sullivan *et al.,* 2001). However, what is frequently overlooked is that population stratification is as likely to obscure genuine associations as it is to falsely introduce them. The first aim of this paper is to illustrate these opposing impacts of population stratification on association under various admixtures of subpopulations with different trait means and different allele frequencies.

To control for the confounding effects of population stratification, family-based tests have been developed in which locus–trait associations are compared across genetically related individuals. Because these individuals stem from the same stratum, locus–trait associations observed within genetically related individuals are genuine. Most available family-based tests for association have been developed for binary traits, such as the Haplotype Relative Risk test (HRR, Falk and Rubinstein, 1987; Terwilliger and Ott, 1992) and the Transmission Disequilibrium Test (TDT, Spielman *et al.,* 1993). Under the assumption of random ascertainment, a clinical binary diagnosis such as "depressed" or "not depressed" or "hypertensive" vs. "normotensive," however, is less powerful for gene finding than a continuous trait such as the score on a depression scale or blood pressure (Boomsma *et al.,* 2000; Van den Oord, 1999). For this reason the TDT has recently been extended to the analysis of quantitative traits (q-TDT; Allison, 1997; Rabinowitz, 1997). The TDT is based on the comparison of transmitted alleles from the parents to affected offspring with nontransmitted alleles. In its original form the TDT has some drawbacks: (i) it requires parental genotypes that complicates its application to late-onset diseases; (ii) two homozygous parents are noninformative, resulting in a decrease of the available sample size; and (iii) no more than one affected child per family can be included because siblings are not genetically independent. Recently, extensions of the TDT have been developed that deal with some of its original drawbacks (reviewed in Zhao, 2000).

Fulker *et al.* (1999) proposed a variance components sib-pair analysis for mapping QTL. This method is based on the modeling of allelic effects on the trait values as a test for association and simultaneous modeling of the sibship covariance structure as a test for linkage (Fulker *et al.,* 1999). By partitioning the association effects into a *between family* component and a *within family* component, spurious associations can be separated from genuine associations. The *between family* effects reflect both the genuine and the possible spurious association between locus alleles and a trait (or allelic association between locus alleles and trait locus alleles). The *within family* effects reflect only the genuine association.

When simultaneously modeling linkage (using identity by descent (IBD) information at positions across the genome) and association (using the alleles from candidate genes/markers) lying within the region that shows linkage), evidence for linkage in a genomic region is expected to decrease; by modeling the allelic effects on the trait values, the residual variance will show less evidence for linkage. If the evidence for linkage does not completely decrease in the presence of a significant genuine association effect of a marker within the linkage region, this could imply that the linkage derives from some other gene within that genomic region, that not all relevant alleles of that locus have been genotyped, or that (part of) the observed linkage may have been artefactual (i.e., because of marker genotype errors) (Abecasis *et al.,* 2000, 2001; Cardon and Abecasis, 2000; McKenzie *et al.,* 2001).

The second aim of this paper is to present an implementation of the combined linkage and association test, including the test for the presence of spurious associations. Although we will present this implementation in the context of using Mx software (Neale, 1997), the general algebraic formulas can also be implemented in other genetic software, such as MERLIN (Abecasis *et al.,* 2002) or SOLAR (Almasy and Blangero, 1998). Mx (Neale, 1997) is a matrix algebra interpreter that uses numerical optimization to obtain parameter estimates by maximum likelihood. Its flexibility allows the relative simple implementation of extensions to multiple (marker) alleles, dominance as well as additive association effects, and variable sibship sizes. In addition, either parental genotypes or sibling genotypes can be used to derive the coefficients used for the decomposition of the association into spurious and genuine effects. These extensions will also be discussed in algebraic terms and implemented in an example Mx script.

Effects of Population Stratification on Statistical Association

We start with a brief definition of some terms used in this paper and will mostly adhere to the definitions given by Terwilliger and Göring (2000). *Linkage between a marker and a trait locus* refers to the nonindependent segregation of the marker and the trait locus, implying that the recombination fraction between them is less than 0.5. *Linkage between a locus and a*

trait is related to this and denotes that pairs of genetically related individuals that share two locus alleles IBD are phenotypically more alike than pairs of genetically related individuals that share none of their alleles on the locus IBD. The locus may either be the trait locus itself or be a marker linked to the trait locus (i.e., a recombination fraction between the marker and the trait locus of less than 0.5); it is in *linkage disequilibrium* (LD), but not necessarily in *disequilibrium* with the trait locus, *LD* or *allelic association* refers to the situation in which certain alleles of a marker are preferentially cosegregated with certain alleles of a trait locus. LD may occur because two loci are in tight linkage but can also occur as a result of population stratification or when certain allele combinations at different loci confer enhanced reproductive fitness. In the latter two cases we speak of disequilibrium. *Association between a locus and a trait* refers to the apparent allelic effects of a locus on trait values. This locus may either be the trait locus itself (i.e., the actual gene) or be a marker in LD with the trait locus.

When several populations have combined, spurious association between a locus and a trait may arise. The size and direction of this association depend on the combination of allele frequencies and trait means in the subpopulations. Different trait means for the same genotypic category across subpopulations will generally result in a difference of the overall means across subpopulations, which is why a difference in overall trait means across subpopulations is generally given as a prerequisite for spurious association to occur. Yet, it should be kept in mind that the crucial events leading to spurious associations between alleles at a locus and a trait are a difference in allele frequencies at that locus and a difference in the trait means for a given *genotype* across subpopulations.

Consider two subpopulations A and B that combine to form the mixed population M. Let subpopulation A have a trait mean μ_A of 105 and subpopulation B a trait mean μ_B of 100. Consider a diallelic locus with alleles E and e and frequencies p and q, respectively, where $q = 1 - p$. Let p in subpopulation A (p_A) be 0.9 and p in subpopulation B (p_B) be 0.5. This locus contributes neither to μ_A nor to μ_B; in other words, within each subpopulation there is no association between the locus and the trait. Let μ_m and p_m denote the trait mean and the frequency of allele E, respectively, in the mixed population (M). Let P, H, and Q denote the genotypic frequencies of the three possible genotypes EE, Ee, and ee, respectively. As subpopulations A and B are in Hardy-Weinberg equilibrium (HWE), P, H, and Q may be calculated from the allele frequencies of each subpopulation

$$P_A = p_A^2,\ H_A = 2p_A q_A,\ Q_A = q_A^2$$

and

$$P_B = p_B^2,\ H_B = 2p_B q_B, \text{ and } Q_B = q_B^2$$

(see also Table I).

As the locus is not related to the phenotypic trait values, the three genotypic categories have equal means within subpopulations. Across subpopulations, however, the trait means are different for individuals that have similar genotypes. Assuming equal population sizes for subpopulations A and B, mixing the subpopulations creates population M, where the genotypic frequencies P_M, H_M, and Q_M are derived from the genotypic frequencies of the two subpopulations A and B

Table I. Formulas and Hypothetical Situation Illustrating the Effects of Population Stratification in the Absence of a Genuine Association

	Population mean	Allele frequencies		Genotypic frequencies			Trait means (μ_g) for given genotype		
	μ	p(E)	q(e)	P(EE)	H(Ee)	Q(ee)	EE	Ee	ee
A	105.00	0.9	0.1	0.81	0.18	0.01	105.00	105.00	105.00
B	100.00	0.5	0.5	0.25	0.50	0.25	100.00	100.00	100.00
M	102.50	0.7	0.3	0.53	0.34	0.13	103.82	101.32	100.19

Note: Following Falconer and Mackay (1996) p denotes the frequency of allele E, $q = 1 - p$ and denotes the frequency of allele e. P, H, and Q denote the genotypic frequencies of genotypes EE, Ee, and ee, respectively. P, H, Q, p, and q in the mixed population are derived from the genotypic frequencies in the subpopulations. P_M is derived as $\sum_{t=1}^{T} P_t \times n_t / \sum_{t=1}^{T} n_t$, where n is the total sample size of subpopulation t, and $t = 1, \ldots, T$. Analogously, H_M is derived as $\sum_{t=1}^{T} H_t \times n_t / \sum_{t=1}^{T} n_t$, and Q_M is derived as $\sum_{t=1}^{T} Q_t \times n_t / \sum_{t=1}^{T} n_t$. The allele frequencies p and q in the combined population M are derived as $p_M = P_M + \frac{1}{2}H_M$ and $q_M = Q_M + \frac{1}{2}H_M$ respectively.

Two subpopulations A and B of equal size, differ both in trait means (per genotype) and in allele frequencies of a diallelic locus. Within each population no locus-trait association exists, whereas in the mixed population M a spurious locus-trait association is clearly evident.

(Table I). As is shown in Table I, P_M, H_M, and Q_M are 0.53, 0.34, and 0.13, respectively. The allele frequencies are calculated following the rules of the biometrical model (Falconer and Mackay, 1996): $p_M = P_M + \frac{1}{2}H_M$ and $q_M = Q_M + \frac{1}{2}H_M$. Note that population M is no longer in HWE.

The trait means for each genotype in population M are a function of the trait means and frequencies of each genotype in subpopulations A and B. Assuming equal population sizes, the trait mean of individuals with genotype g in population M is calculated as follows ($\mu_{g,M}$):

$$\mu_{g,M} = \frac{G_{g,A} \times \mu_{g,A} + G_{g,B} \times \mu_{g,B}}{G_{g,A} + G_{g,B}} \tag{1}$$

where $G_{g,A}$ refers to the frequency of genotype g in population A, $G_{g,B}$ refers to the frequency of genotype g in population B, $\mu_{g,A}$ refers to the trait mean for genotype g in population A, and $\mu_{g,B}$ refers to the trait mean for genotype g in population B.

For the example given in Table I, this results in different trait means for each of the three genotypic categories in population M, reflecting a spurious statistical association between the locus and the trait. Figure 1 presents this effect graphically.

In the biometrical model, which is drawn in Figure 2, a denotes the (additive) effect of genotype EE on the trait, $-a$ denotes the (additive) effect of genotype ee on the trait, and d denotes the dominance deviation for the heterozygous genotype Ee. In association analysis we aim to quantify a and d. In the situation described in Table I and Figure 1, both a and d are 0 for subpopulations A and B. From the values given in the

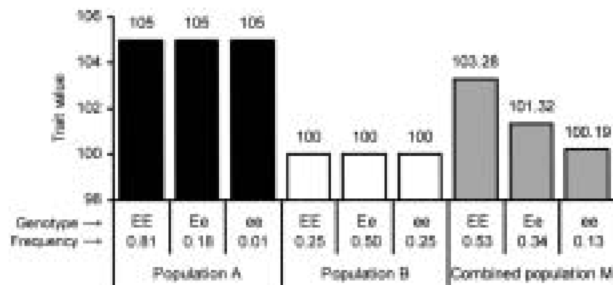

Fig. 1. Graphical representation of the effects of population stratification. Two populations A and B differ both in overall trait means (and trait means per genotype) and in allele frequencies of a diallelic locus. Within each population no locus–trait association exists, whereas in the mixed population a spurious locus–trait association is clearly evident. Specifics concerning this situation are given in Table I. Genotypes and their frequencies are given on the x-axis, whereas the trait means per genotype are scaled on the y-axis.

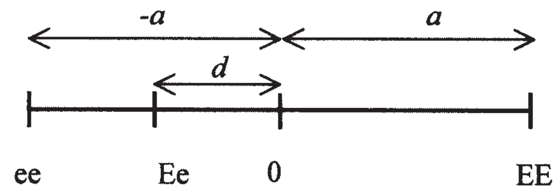

Fig. 2. Biometric model for a diallelic trait with alleles E and e. Let a be the effect of genotype EE on the trait mean, $-a$ the effect of ee, and d the dominance deviation of the heterozygous genotype Ee.

last three cells from Table I, however, the estimated a and d in the mixed population M would be obtained as $(103.82 - 100.19)/2 = 1.82$ and $101.32 - (103.82 + 100.19)/2 = -0.69$, respectively.

For the example given in Table I and represented in Figure 1 we used extreme allele frequency differences ($\Delta p = p_A - p_B = 0.4$) between the two subpopulations and a mean difference of 5 scale points. Figure 3a plots the effects of varying allele frequency differences between populations A and B for four $\Delta\mu$s ($\mu_A - \mu_B = 10, 5, -5,$ or -10) on the estimated value of a in the mixed population, in the absence of a genuine association (i.e., $a = 0$ in subpopulations A and B). In Figure 3b the effect on the calculated value of d in the mixed population is plotted for the same situations and a d of 0 in subpopulations A and B. The allele frequency p_B is constant at 0.5, whereas the allele frequency p_A is varied in steps of 0.01 from 0.99 to 0.01. The mean μ_B is constant at 100, whereas μ_A is 110, 105, 95, or 90.

As becomes evident from Figures 3a and b, population stratification will result in spurious associations between a locus and a trait. As the genuine a and d values were 0, the estimated a and d values in the mixed population are always biased (except when $\Delta p = 0$), and may result both in positive effects of a and d, as well as in negative values of a and d. The bias in estimation of d becomes relatively small when the difference in allele frequency between subpopulations A and B is small to moderate (between -0.3 and 0.3).

Using the same situations as described above, yet assuming a value of $+2$ for a in subpopulations A and B, shows that in the presence of a genuine association the estimated value of a in the mixed population may be overestimated, underestimated, or of reversed sign.

As the genuine dominance deviation was fixed at 0, the calculated dominance deviation from the mixed population is always biased (except when $\Delta p = 0$ or when the genotypic means are equal across populations) and is similar to the effects seen in Figure 3b. Our purpose is to clarify the different ways in which

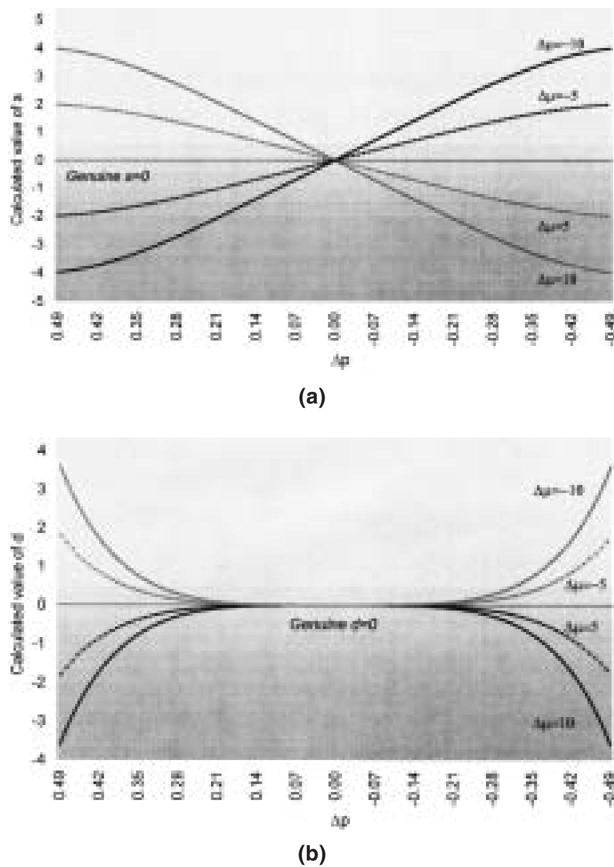

Fig. 3. a, Effect of population stratification on the calculated value of a in the absence of a genuine locus–trait association ($a = 0$; $d = 0$) for varying levels of allele frequency differences. The mixed population exists of populations A and B with constant μ_B (100) whereas μ_A is varied from 110, 105, 95, and 90. Allele frequency p_B is constant at 0.5. Allele frequency p_A is varied with steps of 0.01 from 0.99 to 0.01. b, Effect of population stratification on the calculated value of d in the absence of a genuine locus–trait association ($a = 0$; $d = 0$) for varying levels of allele frequency differences. The mixed population exists of populations A and B with constant $\mu_B(100)$, whereas μ_A is varied from 110, 105, 95, and 90. Allele frequency p_B is constant at 0.5. Allele frequency p_A is varied with steps of 0.01 from 0.99 to 0.01.

population stratification may affect genetic effects in general; thus we chose not to discuss situations in which a genuine dominance deviation is present.

Implementing the Test for Combined Linkage and Association in Mx

Modeling Spurious and Genuine Association

When allelic effects are estimated from genetically related subjects, effects of population stratification can be controlled for. The method proposed by Fulker *et al.,* 1999 uses the *within family genetic effects* on the trait value as an estimate of the genuine association. The *between family genetic effects* on the trait value include both the genuine and the possible spurious association. When the *between family genetic effects* and the *within family genetic effects* are unequal, a spurious association is said to exist, which may either be in the same direction (*between genetic effects* > *within genetic effects*) or in the opposite direction (*between genetic effects* < *within genetic effects*) compared to the genuine association. Thus, equating the *between effects* and the *within effects* serves as a test of the presence (and direction) of spurious associations between a locus and a trait in the data set. This test can be conducted on DNA markers as well as candidate genes.

Estimation of the *between genetic effects* is based on defining the contribution of each family or sibship to the population mean in terms of genetic effects. Thus, for each sibship the genetic mean needs to be calculated. Estimation of the *within genetic effects* is based on defining each individual's genetic deviation from the genetic mean of his sibship. The genetic family/sibship mean can be calculated using the sibling genotypes (if parental genotypes are unavailable) or using the parental genotypes (if available). In this section the implementation in Mx (Neale, 1997) of the combined linkage and association method for these two situations (parental genotypes unavailable and parental genotypes available) as can be applied to real data, is discussed.

Parental Genotypes Unavailable

In Table II the coefficients for the *within* (genuine) and *between* (possibly spurious and genuine) additive and dominance effects are derived for a diallelic locus using sibpairs. The general expression for the means, following Fulker *et al.* (1999) yet including both additive effects and dominance, for the observed score in sib j from the ith family (y_{ij}) is:

$$y_{ij} = \mu + a_b A_{bi} + a_w A_{wij} + d_b D_{bi} + d_w D_{wij} + e_{ij} \qquad (2)$$

where μ denotes the overall trait mean (equal for all individuals), A_{bi} is the derived coefficient (e.g., $\frac{1}{2}$, or $-\frac{1}{2}$, 1, etc.) for the *between families* additive genetic effect for the ith family, as calculated in the fifth column of Table II. A_{wij} denotes the coefficient by which the *within families* additive genetic effects need to be multiplied for sib j from the ith family as derived in the last two columns of Table II. D_{bi} is the coefficient by which the *between families* dominant genetic effect needs to be multiplied for the ith family, as calculated in the fifth column of Table II. D_{wij} denotes the coefficient as derived for the *within families* dominant genetic effects for sib j from the ith family (see last two columns of Table II). Parameters a_b and a_w are the estimated

additive *between* and *within* effects; parameters d_b and d_w are the estimated dominance *between* and *within* effects, and e_{ij} denotes that part of the grand mean that is not explained by the genotypic effects.

For a diallelic locus, derivation of the additive *between* and *within* coefficients and the dominance *between* and *within* coefficients is straightforward and can be taken from Table II (e.g., $\frac{1}{2}$, or $-\frac{1}{2}$, 1, etc.). For a locus with more than two alleles, however, this becomes a daunting task. We therefore chose to have the necessary coefficients calculated by the program instead of specifying them in a matrix (e.g., Neale, 2000; Neale *et al.,* 1999).

Let matrices **A** and **C** be vectors of dimensions $1 \times n$, where $n = 2, \ldots, n$ for the number of alleles at the locus. Let matrices **D** and **F** be subdiagonal matrices of dimensions $n \times n$. Matrix **A** contains the estimated combined spurious and genuine (i.e., *between*) additive allelic effects. Matrix **C** contains the estimated genuine additive (i.e., *within*) allelic effects. Matrix **D** contains the estimated spurious and genuine (i.e., *between*) dominance deviations for the heterozygous genotypes, and matrix **F** contains the estimated genuine (i.e., *within*) dominance deviations. Let matrix **I** be a vector containing one's of dimension $1 \times n$. In the Mx script language this is written (see also Appendices I and II for full Mx script example; anything after ! on the same line is not read by the Mx program and can be used for additional remarks):

```
#define n 5                  !number of alleles = 5 ; the letter n will be substituted
                             !by the number 5, except when n occurs as part of a word
Begin matrices;              !start declaration of matrices
  A Full 1 n  free           !will contain additive allelic effects WITHIN
  C Full 1 n  free           !will contain additive allelic effects BETWEEN
  D Sdiag n n free           !will contain dominance deviations within
  F Sdiag n n free           !will contain dominance deviations between
  I Unit 1 n                 !unit vector to multiply allelic effects [1 1 1 1 1]
End matrices;                !end declaration of matrices
```

With these matrices, two symmetric matrices of dimensions $n \times n$, one for the *between* (i.e., the sum of the spurious and genuine effects) and one for the *within* (i.e., the genuine effects) estimates, are calculated that contain the genotypic effects of the homozygous genotypes on the diagonal and the genotypic effects of the heterozygous genotypes on the off-diagonals.

```
Begin algebra;
  K = (A'@I) + (A@I') ;      !calculates linear combinations of the allelic effects
  L = D + D' ;               !dominance deviations below and above diagonal
  W = K + L ;                !creates one full n x n matrix containing the WITHIN
                             !genotypic effects
  M = (C'@I) + (C@I') ;      !calculates linear combinations of the allelic effects
  N = F + F' ;               !dominance deviations below and above diagonal
  B = M + N ;                !creates one full n x n matrix containing the BETWEEN
                             !genotypic effects
End algebra;
```

The symbol @ denotes the Kronecker product ($\otimes$) in Mx and results in the multiplication of each element of the first matrix by the second matrix. For a locus with five alleles, matrix **W** is a symmetric matrix of dimension $n \times n$ containing the following estimated effects for a locus with five alleles ($n = 5$):

$$\mathbf{W}\left\{\begin{array}{lllll} a_{w,1}+a_{w,1} & & & & \\ a_{w,1}+a_{w,2}+d_{w,12} & a_{w,2}+a_{w,2} & & & \\ a_{w,1}+a_{w,3}+d_{w,13} & a_{w,2}+a_{w,3}+d_{w,23} & a_{w,3}+a_{w,3} & & \\ a_{w,1}+a_{w,4}+d_{w,14} & a_{w,2}+a_{w,4}+d_{w,24} & a_{w,3}+a_{w,4}+d_{w,34} & a_{w,4}+a_{w,4} & \\ a_{w,1}+a_{w,5}+d_{w,15} & a_{w,2}+a_{w,5}+d_{w,25} & a_{w,3}+a_{w,5}+d_{w,35} & a_{w,4}+a_{w,5}+d_{w,45} & a_{w,5}+a_{w,5} \end{array}\right.$$

where $a_{w,1\ldots n}$ refers to the genuine additive allelic effects of the alleles labeled $1 \ldots n$, and $d_{w,12\ldots nn}$ refers to the genuine dominance deviation of the heterozygous genotypes labeled $12 \ldots nn$. Note that with this notation $a_{w,1\ldots}a_{w,n}$ refers to *allelic* effects, whereas a_w refers to *genotypic* effects. Similarly, matrix **B** will be symmetric, of dimension $n \times n$ and will contain the analogous estimated genuine and spurious additive and dominance genotypic effects (subscripted b).

We now proceed to the calculation of the sibship genetic means and each individual's deviation from the sibship's genetic mean. For sibships of size two, each individual's deviation from the sibship genetic mean can easily be deducted by precalculating half the difference between the genetic effects of each sib (as is done in Table II). For sibship sizes larger than two, the *within* component is no longer simply "half the difference," but instead is mathematically represented by the deviation of sib j from the ith sibship mean. The individual genotypes should be in the datafile (which is the "raw" datafile and not a variance/covariance matrix). These are selected from the list of input variables to be *used* and will be specified in a matrix. They need to be treated differently from variables that are to be *analyzed* (the phenotype). The `definition variable` function in Mx can be used to separate variables that are used as covariates (such as sex, age, and allelic effects) from the dependent variables.

```
G2: datagroup
Select pheno1 pheno2 pheno3 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3   !Select all variables to
                                                            !be used or analysed
..
Definition_variables a1s1 a2s1 a1s2 a2s2 a1s3 a2s3          !define which variables
                                                            !need to be treated as a
                                                            !covariate
Begin matrices ;                 !begin declaration of matrices for group 2
..
  K Full 1 4 Fixed               !Will contain first and second allele of sib1
  L Full 1 4 Fixed               !Will contain first and second allele of sib2
  M Full 1 4 Fixed               !Will contain first and second allele of sib3
..
End matrices ;                   !end declaration of matrices for group 2
Specify K a1s1 a2s1 a1s1 a2s1    !put alleles of sib 1 into vector
Specify L a1s2 a2s2 a1s2 a2s2    !put alleles of sib 2 into vector
Specify M a1s3 a2s3 a1s3 a2s3    !put alleles of sib 3 into vector
```

For each individual, two alleles need to be present in the data file. The alleles should be coded as 1, 2, 3, . . . , n. For each sibship, different elements need to be taken from matrices **B** and **W** to calculate the family genetic mean and each individual's deviation from that mean. The definition variables that have now been put into matrices (**K, L,** and **M**) that contain numbers that correspond to the specific alleles from the respective individual. For example, if the first sib has genotype 11, the second sib has genotype 34, and the third sib has genotype 13 at a marker locus, matrix **K** contains [1 1 1 1], matrix **L** contains [3 4 3 4], and matrix **M** contains [1 3 1 3].

Matrices **K, L,** and **M** can now be used to select the relevant cells from matrices **B** and **W:**

```
!For sibships of size 3 for a univariate trait
  Begin matrices
    B Computed n n  = B1               !spurious and genuine genotypic effects,
                                       !precalculated in previous Mx group
    W Computed n n  = W1               !genuine genotypic effects
    S Full 1 1 Fixed                   !to contain sibshipsize (3)
    G Full 1 1 Free                    !grand mean, to be estimated
                                       !dimensions 1 x number of variables
  End matrices
   Matrix S 3                          !sibship size = 3
  Begin Algebra;
      V = (\part(B,K) + \part(B,L) + \part(B,M) ) % S ;
     !sib genetic mean: between effects (spurious and genuine)
      D = (\part(W,K) + \part(W,L) + \part(W,M) ) % S ;
     !used for individual's deviation from sib mean: within effects (genuine)
  End Algebra;
  Means G + V + (\part(W,K)-D) | G + V + (\part(W,L)-D) | G + V + (\part(W,M)-D) ;
     !means model: grand mean + sib genetic mean effects + individual's deviation
     !from sib genetic mean, for three sibs
```

The `\part` statement in Mx allows one to select a rectangular submatrix from a larger matrix. For example, \part(**B,K**) tells Mx to select from matrix **B** the part specified in matrix **K**. Matrix **K** should always be of dimension 1×4 (start row, start column, end row, end column) and specifies the elements of matrix **B** where the

Table II. Partitioning of Additive and Dominance Genotypic Effects into Between and Within Components for a Diallelic Locus with Alleles E and e in Sib-pairs

Genotype		Genotypic effect		Additive		Dominance		Partitioned genotypic effects	
Sib 1	*Sib 2*	*Sib 1*	*Sib 2*	*Mean*	*Difference/2*	*Mean*	*Difference/2*	*Sib 1*	*Sib 2*
EE	EE	a	a	a_b	0	0	0	a_b	a_b
EE	Ee	a	d	$\frac{1}{2}a_b$	$\frac{1}{2}a_w$	$\frac{1}{2}d_b$	$-\frac{1}{2}d_w$	$(\frac{1}{2}a_b + \frac{1}{2}a_w) + (\frac{1}{2}d_b - \frac{1}{2}d_w)$	$(\frac{1}{2}a_b - \frac{1}{2}a_w) + (\frac{1}{2}d_b + \frac{1}{2}d_w)$
EE	ee	a	−a	0	a_w	0	0	a_w	$-a_w$
Ee	EE	d	a	$\frac{1}{2}a_b$	$-\frac{1}{2}a_w$	$\frac{1}{2}d_b$	$\frac{1}{2}d_w$	$(\frac{1}{2}a_b - \frac{1}{2}a_w) + (\frac{1}{2}d_b + \frac{1}{2}d_w)$	$(\frac{1}{2}a_b + \frac{1}{2}a_w) + (\frac{1}{2}d_b - \frac{1}{2}d_w)$
Ee	Ee	d	d	0	0	d_b	0	d_b	d_b
Ee	ee	d	−a	$-\frac{1}{2}a_b$	$\frac{1}{2}a_w$	$\frac{1}{2}d_b$	$\frac{1}{2}d_w$	$(-\frac{1}{2}a_b + \frac{1}{2}a_w) + (\frac{1}{2}d_b + \frac{1}{2}d_w)$	$(-\frac{1}{2}a_b - \frac{1}{2}a_w) + (\frac{1}{2}d_b - \frac{1}{2}d_w)$
ee	EE	−a	a	0	$-a_w$	0	0	$-a_w$	a_w
ee	Ee	−a	d	$-\frac{1}{2}a_b$	$-\frac{1}{2}a_w$	$\frac{1}{2}d_b$	$-\frac{1}{2}d_w$	$(-\frac{1}{2}a_b - \frac{1}{2}a_w) + (\frac{1}{2}d_b - \frac{1}{2}d_w)$	$(-\frac{1}{2}a_b + \frac{1}{2}a_w) + (\frac{1}{2}d_b + \frac{1}{2}d_w)$
ee	ee	−a	−a	$-a_b$	0	0	0	$-a_b$	$-a_b$

relevant submatrix (which can also be a single element) starts and ends. Because matrix **K** contains the alleles of an individual, the submatrix is selected conditional on that individual's genotype.

In our example, in which the first sib is of genotype 11, the second sib has genotype 34, and the third sib has genotype 13, the mean of the estimates in cells (denoted by row and column) 11, 34, and 13 from matrix **B** is calculated as the sibship genetic mean (representing the *between family* effects of that sibship, in matrix **V**). Similarly, for the first sib the *within family* effect is calculated by subtracting the estimate in cell 11 from matrix **W** from the mean of the parameters in cells 11, 34, and 13 from matrix **W** (i.e., `(\part (W,K)-D)`).

Because of linear dependency between the allelic effects, two constraint groups (one for the *within* effects and one for the *between* effects) are needed in which the sum of all the allelic effects is constrained to be 0 (see Appendices I and II).

Abecasis *et al.* (2000) showed that calculation of the sibship genetic mean based on both parental genotypes is less error prone than calculation of the sibship genetic mean based on available sibling genotypes. For sibship sizes of four and above the two methods are equally powerful and error rates are closer to nominal significance rates. The above method can be used when genotype information from *both* parents is unavailable.

Parental Genotypes Available

When both parental genotypes are available, the expected mean additive genotypic value of the offspring (a_{bi}) equals the midparental genotypic value

$$a_{bi} = \frac{G_{iF} + G_{iM}}{2}, \qquad (3)$$

where $G_{i,F}$ is the additive genotypic value of the father in family i, and $G_{i,M}$ is the additive genotypic value of the mother in family i.

When dominance effects are also considered, the midparental genotypic value is no longer an estimate of the expected offspring mean, because parents and offspring are uncorrelated in terms of dominance effects. The genotypes of the parents, however, do provide information on the expected dominance effects in the offspring. For example, when one parent is of genotype EE, with a corresponding genotypic value of a, and the other parent is of genotype ee, with a corresponding value of $-a$, the midparental genetic value will be 0. However, all of their offspring will be of genotype Ee, with a corresponding genetic value of d.

For each type of parental mating we therefore need to calculate all possible genotypes in the offspring and their probability, given the parental mating type. The mean value in terms of a and d of the possible genotypes in the offspring weighted by their probability gives the expected offspring (i.e., sibling) genetic mean. In Table III the coefficients for additive and dominance between and within effects are derived, conditional on the parental genotypes.

Extending this to a multiallele locus quickly becomes a large undertaking, and it is more convenient to use a program such as Mx that can calculate the necessary coefficients (A_{bi}, A_{wijk}, D_{bi}, and D_{wijk}) by which the effects (a_b, a_w, d_b, and d_w) need to be multiplied conditional on the parental genotypes. For a given parental mating type, the possible genotypes of offspring and their probabilities may be calculated in Mx by using the parental alleles to select elements from the matrices that contain the *between* and *within* effects (matrices **B** and **W**). Whereas in the previous section both alleles that were used to select from matrices **B** and **W** were from the same person (i.e., one sib), we now pair paternal and maternal alleles to obtain all possible genotypes of the offspring. The maximum number of genotypic categories in the offspring from one mating type is four (i.e., when both parents are heterozygous and have four different alleles). We thus specify in Mx the following matrices:

```
Specify N a1p1 a1p2 a1p1 a1p2    !first allele parent one first allele parent two
Specify O a1p1 a2p2 a1p1 a2p2    !first allele parent one second allele parent two
Specify X a2p1 a1p2 a2p1 a1p2    !second allele parent one first allele parent two
Specify Y a2p1 a2p2 a2p1 a2p2    !second allele parent one second allele parent two
```

These are used to select relevant submatrices from matrix **B** and **W** to calculate the genetic offspring (i.e., sibship) mean and each offspring's individual deviation from that mean (see Appendix II for the full Mx script). The additive and dominance coefficients can be calculated in Mx in this manner for an arbitrary number of alleles and an arbitrary number of offspring.

Modeling Linkage

Implementation of the linkage component in the variance components model is straightforward and can be done by using the "pi-hat" ($\hat{\pi}$) approach, in which the covariance resulting from the marker or trait locus for a sibpair is modeled as a function of the IBD status of that sibpair. Generally, for sibships, the phenotypic variance is decomposed in familial variance (σ_f^2), variance resulting from nonshared environmental influences (σ_e^2), additive variance from the QTL or marker in LD with the QTL (σ_a^2), and dominance variance resulting from the QTL, or a marker in LD with the QTL (σ_d^2). The variance-covariance matrix for the ith family, Ω_{ijk} is then given by

$$\Omega_{ijk} = \begin{cases} \sigma_f^2 + \sigma_a^2 + \sigma_d^2 + \sigma_e^2 & \text{if } j = k \\ \sigma_f^2 + \hat{\pi}_{ijk}\sigma_a^2 + \hat{z}_{ijk}\sigma_d^2 & \text{if } j \neq k \end{cases} \qquad (4)$$

where $\hat{\pi}_{ijk}$ is the estimated proportion of alleles shared IBD between sibs j and k for the ith family, and $\hat{z}_{ijk}$ is the probability of complete IBD sharing between sibs j and k for the ith family. The estimated proportion of alleles shared IBD between sibs j and k ($\hat{\pi}_{ijk}$) is based on the probabilities that sibs j and k share 0, one, or two alleles IBD ($p_{(\mathrm{IBD}=0)}$, $p_{(\mathrm{IBD}=1)}$, $p_{(\mathrm{IBD}=2)}$, respectively) that can be obtained from genetic software such as Genehunter (Kruglyak *et al.,* 1996). The formula to obtain $\hat{\pi}_{ijk}$ for the ith family is given by

$$\hat{\pi}_{ijk} = 0 \times p_{(\mathrm{IBD}=0)_{ijk}} + 0.5 \times p_{(\mathrm{IBD}=1)_{ijk}} + 1 \times p_{(\mathrm{IBD}=2)_{ijk}} \qquad (5)$$

The probability of complete IBD sharing between sibs j and k for the ith family simply equals pIBD2_{ikj}:

$$\hat{z}_{ijk} = p_{(\mathrm{IBD}=2)_{ijk}} \qquad (6)$$

Tests

The test for spurious association consists of the joint test that matrix **A** equals matrix **C** (from the first group in our example script), and that matrix **D** equals matrix **F** (from the first group in our example script). If the parameters in these matrices cannot be constrained to be equal, there is evidence of spurious association. The conservative test for the presence of a genuine association is to test whether matrices **A** and **D** are significantly different from 0.

The test for the presence of dominance effects can be conducted by comparing the minus two loglikelihoods (-2LL's) from the full model and a model without the subdiagonal matrices **D** and **F** from the first group in the example Mx script that contain the deviations of the heterozygous genotypes from the mid value of the two corresponding homozygous genotypes. This can be done conservatively only for the presence of the genuine dominance effects (i.e., dropping matrix **D**) or for the presence of both the genuine and spurious dominance effects (dropping matrices **D** and **F**).

Three models may be evaluated to test whether linkage is present and whether the linkage component

Table III. Partitioning of Additive and Dominance Genotypic Effects into Between and Within Components for a Diallelic Locus with Alleles E and e Conditional on Parental Genotypes

	Offspring						
	EE		Ee		ee		
	a		*d*		$-a$		
Parental mating type ↓	Probability	Deviation from family mean (*Within*)	Probability	Deviation from family mean (*Within*)	Probability	Deviation from family mean (*Within*)	Family mean (*Between*)
EE × EE	1	0	0	n.p.	0	n.p.	a_b
EE × Ee	$\frac{1}{2}$	$\frac{1}{2}a_w - \frac{1}{2}d_w$	$\frac{1}{2}$	$-\frac{1}{2}a_w + \frac{1}{2}d_w$	0	n.p.	$\frac{1}{2}a_b + \frac{1}{2}d_b$
EE × ee	0	n.p.	1	0	0	n.p.	d_b
Ee × Ee	$\frac{1}{4}$	$a_w - \frac{1}{2}d_w$	$\frac{1}{2}$	$\frac{1}{2}d_w$	$\frac{1}{4}$	$-a_w - \frac{1}{2}d_w$	$\frac{1}{2}d_b$
Ee × ee	0	n.p.	$\frac{1}{2}$	$\frac{1}{2}d_w + \frac{1}{2}a_w$	$\frac{1}{2}$	$-\frac{1}{2}d_w - \frac{1}{2}a_w$	$\frac{1}{2}d_b - \frac{1}{2}a_b$
ee × ee	0	n.p.	0	n.p.	1	0	$-a_b$

Note: The expectation for an individual sibling is the sum of the *Between* and *Within* components.
n.p., not possible.

can be partly or completely explained by the association: (i) a model with a linkage component only; (ii) a model with both linkage and association; (iii) a model with the association component only. If the linkage component is reduced in model (ii) as compared to model (i), but still significant, this may indicate that within the linkage region another gene, besides the gene used for the association component, is also influencing the trait, that not all relevant alleles of that locus have been genotyped, or that LD between the marker and the trait locus is incomplete. If the linkage component disappears when modeled simultaneously with association, it indicates that the linkage is completely explained by the association effects of the tested locus or by the effects of another locus that is in complete LD with the tested locus.

Practical Considerations

The implementation in Mx of the analysis as proposed by Fulker *et al.* (1999) is flexible in terms of the number of alleles it can incorporate, variable sibship sizes, the inclusion of both additive and dominance effects and can be used both when parental genotypes are available or unavailable. Theoretically, it may include loci with an unlimited number of alleles. With an increasing number of alleles, however, the chance increases that not all possible genotypes are present in the sample. This should be explored beforehand, and the corresponding elements in matrices **A, C, D,** and **F** containing the allelic effects and dominance deviations should be fixed to prevent nonidentification. For example when alleles labelled 3 and 4 do not exist in a heterozygous genotype, the dominance deviation for genotype "3,4" cannot be estimated. Element 3,4 from matrices **D** and **F** needs to be constrained at 0. If, on the other hand, two alleles only occur in a heterozygote, the additive effects cannot be distinguished from the dominance deviation and either one cannot be estimated. Related to this, it is also possible to group certain alleles as if they were one allele (or different alleles with the same effect) and to contrast the effect of one allele against the effects of all other alleles. This can be implemented in Mx by using constraints on the corresponding matrix elements containing the allelic effects. If alleles that differ in size are used (e.g., variable number tandem repeats [VNTRs], a linear regression of allele size may be incorporated into the model (see for example Zhu *et al.,* 1999).

Sibship size may vary across families. In this case one may use the `variable length` datafile option in Mx and use sibship size (specified in Matrix **S** from

the second Mx group in the example script) as a `definition variable`, which is read from the datafile and varies across families. The simultaneous implementation of an arbitrary number of alleles, for an arbitrary sibship size, using parental genotypes or sibling genotypes, and decomposing both the additive effect and the dominance deviations into genuine and spurious effects is unique to Mx.

CONCLUSION

We have illustrated the effects of population stratification on quantitative traits and have shown that in the absence of a genuine association, population stratification may result in a spurious association between any trait that differs in mean between subpopulations and any locus that differs in allele frequency between subpopulations. This situation is illustrated by the well-known "chopsticks gene" example as described by Hamer and Sirota (2000). As was also mentioned by Witte *et al.* (1999; for binary traits), population stratification may not only result in overestimation of allele effects on quantitative traits, but also in an underestimation. More specifically, in the presence of a genuine association population, stratification may result in: (i) an overestimation of the genuine association effects, (ii) an underestimation of the genuine association effects, or (iii) a reversal or incorrect direction of allelic effects.

Genuine association effects will be overestimated because of the effects of population stratification when within the subpopulations' higher trait values are associated with a higher frequency of the increaser allele and lower trait values are associated with a lower increaser allele frequency. Or, in other words, a positive $\Delta p(p_A - p_B)$ is related to a positive $\Delta\mu(\mu_A - \mu_B)$, and a negative Δp to a negative $\Delta\mu$ (see also Figure 4). In this case we may speak of *concordant pairing* of allele frequency and trait value. In practice, such a situation may exist, for example, as a result of assortative mating within subpopulations that differ in trait means and allele frequencies. Differences in trait means and allele frequencies may exist as a result of historical or cultural differences or as a result of natural selection. For example, when in one population high trait values increase reproductive fitness, the frequency of the increaser allele for that trait and the overall trait mean may increase in that population. In the other population, in which high trait values are irrelevant for reproductive fitness, the increaser allele frequency and the overall trait mean remain the same. Assortative mating within subpopulations ensures that eventually *concordant pairing* between increaser allele and trait value will exist, and

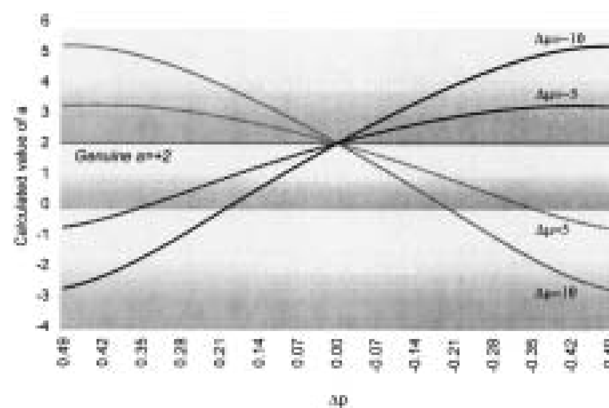

Fig. 4. Effect of population stratification on the calculated value of a in the presence of a genuine locus–trait association ($a = +2$; $d = 0$) for varying levels of allele frequency differences. The mixed population exists of populations A and B with constant μ_B (100), whereas μ_A is varied from 110, 105, 95, and 90. Allele frequency p_B is constant at 0.5. Allele frequency p_A is varied with steps of 0.01 from 0.99 to 0.01.

neglecting the stratified nature of the complete sample will lead to an overestimation of genetic effects.

In the presence of a genuine association, underestimation of the additive genetic effects will occur when, within subpopulations, relatively higher trait values tend to go together with a lower frequency of the increaser allele, or vice versa (either a positive Δp and a negative $\Delta\mu$, or a negative Δp and a positive $\Delta\mu$). In this case we may speak of *discordant pairing* of allele frequency and trait value. This situation may be understood by considering that the overall mean of a subpopulation may also influenced by other (non-) genetic factors. For example, it is well known from mouse model systems, that the same allele at the same locus may cause a major disease in one mouse strain, but no phenotype in a strain with a different genetic background (e.g., Linder, 2001; Liu *et al.,* 2001; Montagutelli *et al.,* 2000). The same has been reported for effects on gene expression in different environmental backgrounds (Cabib *et al.,* 2000; Crabbe *et al.,* 1999). Put differently, in one strain the presence of the particular allele leads to crossing a certain threshold value above which a disease will evolve, whereas in the other strain, because of a different genetic or environmental background, this threshold is not reached. The frequency of the disease-predisposing allele may therefore rise in the population with the genetic or environmental background that prevents the individuals within that population from reaching a threshold. In humans, the presence of different genetic (or environmental) backgrounds that derive from mixed ethnicity may cause the allele frequency of the increaser allele

of a subpopulation with a relatively low trait mean to be higher than the allele frequency of the increaser allele in a population with a higher overall trait mean.

Non-Mendelian traits are likely to be influenced by multiple (risk) factors of which the presence differs across subpopulations; thus *discordant pairing* may realistically hide genuine allele–trait associations when the effects of population stratification are neglected. When the difference in trait means between subpopulations and the difference in increaser allele frequencies becomes extreme in the presence of *discordant pairing,* the genuine allelic effects will appear reversed in sign as a result of population stratification. This suggests that in the mixed population individuals who are homozygous for the increaser alleles (EE) have a lower trait value than individuals who are homozygous for the decreaser allele (ee), whereas in the subpopulations the opposite is true. This statistical effect is known as Simpson's paradox (Simpson, 1951; Yule, 1900) and refers to the reversal of the direction of an association when data from several groups are combined to form a single group. Its importance to gene hunting studies may well have been illustrated by the numerous association studies for schizophrenia, in which the same allele of the same locus has both been associated with increased and decreased risk for schizophrenia (Baron, 2001; Bray and Owen, 2001).

Family-based tests of association explicitly model the consequences of population stratification, by looking at allelic effects within genetically related subjects. In the method proposed by Fulker *et al.* (1999) spurious association is defined as the difference between the allelic effects as estimated from the comparison of unrelated subjects (*between effects*) and the allelic effects as estimated from the comparison of genetically related subjects (*within effects*). This method, which was originally proposed to include sibpairs, diallelic markers, and additive effects, has now explicitly been extended to include variable sibship sizes, multiallele markers, and dominance deviations, using the parental genotypes (if available) or the sibling genotypes.

It is known that the use of multivariate phenotypes may provide more statistical power than univariate phenotypes (e.g., Allison *et al.,* 1998; Boomsma and Dolan, 1998). The method as implemented in Mx can easily be extended to multivariate phenotypes. One can then model the association as an effect on the factor mean of multivariate measurements. In this case it may be assumed that the allelic association effects on the multivariate measurements are all proportionally related. Covariance among the traits resulting from the association will lead to a decrease in the estimated amount of covariance because of the linkage component.

With the rapidly increasing availability of large amounts of genomic data, the detection of linkage and/or association between a marker (and all the linked loci surrounding the marker that are in LD with it) and a trait becomes a realistic tool in the hunt for genes for complex traits. Combining linkage analysis and association analysis has already proved to be a powerful tool in gene finding (e.g., Neale *et al.,* 1999; Trembath *et al.,* 1997; Zhu *et al.,* 1999; see Beekman *et al.,* 2003 for a practical implementation of the method described in the present paper). Particularly when fine mapping is a goal of interest this method is invaluable, because the effect of linkage will be reduced when estimated in the presence of association, thereby providing information on the specific region where the QTL is expected to reside (Cardon and Abecasis, 2000). An explicit test for population stratification is crucial to rule out spurious associations. The Fulker *et al.* (1999) method has all these advantages and, as was shown in the present paper, can easily be conducted in a statistical package such as Mx.

APPENDIX I: PARENTAL GENOTYPES UNAVAILABLE

```
Mx scripts can also be downloaded from the Mx homepage or from the Mx Scripts' Library:
http://www.vcu.edu/mx
http://www.psy.vu.nl/mxbib

!Mx script for the conduction of the combined linkage and association method
!testing for spurious association
!extended to sibships>2, additive and dominance association, multiple alleles
!using sibling genotypes to calculate the mean genotypic value within a sibship

#define n 5          !number of alleles is 5
#define nvar 1       !univariate
#define nsibs 3      !sibshipsize = 3
#ngroups 4           !one precalculation group, one data group, two constraint groups

G1: calculation group between and within effects
```

```
Data Calc
  Begin matrices;          !start declaration of matrices
  A  Full  1  n  free      !will contain additive allelic effects within
  C  Full  1  n  free      !will contain additive allelic effects between
  D  Sdiag  n  n free      !will contain dominance deviations within
  F  Sdiag  n  n free      !will contain dominance deviations between
  I  Unit 1 n              !unit vector to multiply allelic effects [1 1 1 1 1]
 End matrices;             !end declaration of matrices

 Begin algebra;
  K = (A'@I) + (A@I') ;
  L = D + D' ;
  W = K + L ;
  M = (C'@I) + (C@I') ;
  N = D + D' ;
  B = M + N ;
 End algebra ;
st .2 all
end

G2: datagroup: sibship size three
  Data NInput=12
  Missing =-99.00
  Rectangular File=myfile.dat
  Labels ph1 ph2 ph3 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23
  Select ph1 ph2 ph3 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
      !selects 3 phenotypes; one for each sib
      !selects 6 allele variables, a1s1 is allel #1 from sib #1
      !selects pi's and z's
  Definition_variables
      a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
      !declare the allele variables and the pIBD=2 as definition variables
 Begin Matrices;
  F Lower nvar nvar Free               ! familial variance
  Q Lower nvar nvar Free               ! QTL additive variance
  R Lower nvar nvar Free               ! QTL dominance variance
  E Lower nvar nvar Free               ! non-shared environmental variance
  B Computed n n = B1                  ! spurious and genuine genotypic effects
  W Computed n n = W1                  ! genuine genotypic effects
  I Ident nsibs nsibs Fix              !
  P Sym nsibs nsibs Fix                ! To contain pi-hats
  Z Sym nsibs snibs Fix                ! To contain pIBD2's
  T Stand nsibs nsibs Fix
  K Full 1 4 Fix                       ! First and second allele of sib1
  L Full 1 4 Fix                       ! First and second allele of sib2
  M Full 1 4 Fix                       ! First and second allele of sib3
  S Full 1 1 Fix                       ! to contain nsibs
  G Full 1 nvar Free                   ! grand mean
 End Matrices;
 Matrix S 3                            ! sibship size 3
 Matrix K 1 1 1 1
 Matrix L 1 1 1 1
 Matrix M 1 1 1 1
 Matrix P
      0
      1 0
      1 1 0
Matrix  Z
      0
      1 0
      1 1 0
 Specify K a1s1 a2s1 a1s1 a2s1   !genotype sib1 to be used for \part
 Specify L a1s2 a2s2 a1s2 a2s2   !genotype sib2 to be used for \part
 Specify M a1s3 a2s3 a1s3 a2s3   !genotype sib3 to be used for \part
```

```
Specify P    1
             pi12 1
             pi13 pi23 1
Specify Z    1
             z12 1
             z13 z23 1
Specify T    .5                          ! when familial variance is modeled as
             .5 .5                       ! add gen variance

Begin Algebra;
 V = (\part(B,K) + \part(B,L) + \part(B,M) ) % S ; !"B"
 D = (\part(W,K) + \part(W,L) + \part(W,M) ) % S ; !used for deviation: W
End Algebra;

Means G + V + (\part(W,K)-D) | G + V + (\part(W,L)-D) | G + V + (\part(W,M)-D);
Covariance T@(F*F') + P@(Q*Q') + Z@(R*R') + I@(E*E') ;

End

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
  A full 1 n = A1
  O zero 1 1
End Matrices;
Begin algebra;
  B = \sum(A) ;
End Algebra;
Constraint O = B ;
end

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
 C full 1 n = C1
 O zero 1 1
End Matrices;
Begin algebra;
 B = \sum(C) ;
End Algebra;
Constraint O = B ;
option multiple issat
end

save full.mxs

!test for spurious association W=B
Specify 1 A 101 102 103 104 105
Specify 1 C 101 102 103 104 205 !first 4 equal to within; last unequal but because
                                !of second constrain 205 will be equal to 105
Specify 1 D 801 802 803 804 805 806 807 808 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
end

!Drop dominance: non-conservative test (i.e. genuine and spurious)
Specify 1 D 801 802 803 804 805 806 807 808 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
Drop @0 801 802 803 804 805 806 807 808 809 810
end

!Drop all allelic effects: non-conservative test (i.e. genuine and spurious)
```

```
Specify 1 A 101 102 103 104 105
Specify 1 C 101 102 103 104 205
Specify 1 D 801 802 803 804 805 806 807 8O8 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
Drop @0 101 102 103 104 105 801 802 803 804 805 806 807 808 809 810
end

get full mxs

!drop QTL linkage effect while keeping association effects in the model
Drop Q 2 1 1 !QTL additive variance
Drop R 2 1 1 !QTL dominance variance
end
```

APPENDIX II: PARENTAL GENOTYPES AVAILABLE

```
!Mx script for the conduction of the combined linkage and association method
!testing for spurious association
!extended to sibships>2, additive and dominance association, multiple alleles
!using parental genotypes to calculate the mean genotypic value within a sibship

#define n 5          !number of alleles is 5
#define nvar 1       !univariate
#define nsibs 3      !sibshipsize = 3
#ngroups 4           !one precalculation group, one data group, two constraint groups

G1: calculation group between and within effects
Data Calc
   Begin matrices;        !start declaration of matrices
   A Full 1 n free        !will contain additive allelic effects within
   C Full 1 n free        !will contain additive allelic effects between
   D Sdiag n n free       !will contain dominance deviations within
   F Sdiag n n free       !will contain dominance deviations between
   I Unit 1 n             !unit vector to multiply allelic effects [1 1 1 1 1]
  End matrices;           !end declaration of matrices
  Begin algebra;
   K = (A'@I)+(A@I') ;
   L = D + D' ;
   W = K + L ;
   M = (C'@I)+(C@I') ;
   N = F + F' ;
   B = M + N ;
  End algebra ;
st .2 all
end

G2: datagroup: sibship size three
  Data NInput=12
  Missing =-99.00
  Rectangular File=myfile.dat
  Labels ph1 ph2 ph3 a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13
pi23 z12 z13 z23
  Select ph1 ph2 ph3 a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13
pi23 z12 z13 z23;
      !selects 3 phenotypes; one for each sib
      !selects 6 allele variables for sib, a1s1 is allel #1 from sib #1
      !selects 4 allele variables for parents a1p1 is allel #1 parent #1
      !selects pi's and z's
  Definition_variables
      a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
      !declare the allele variables and the pIBD=2 as definition variables
```

```
Begin Matrices;
   F Lower nvar nvar Free            !familial variance
   Q Lower nvar nvar Free            !QTL additive variance
   R Lower nvar nvar Free            !QTL dominance variance
   E Lower nvar nvar Free            !non-shared environmental variance
   B Computed n n = B1               !spurious and genuine genotypic effects
   W Computed n n = W1               !genuine genotypic effects
   I Ident nsibs nsibs Fix           !To multiply E
   P Sym nsibs nsibs Fix             !To contain pi-hats and to multiply Q
   Z Sym nsibs snibs Fix             !To contain pIBD2's and to multiply R
   T Stand nsibs nsibs Fix           !To multiply F
   K Full 1 4 Fix                    !First and second allele of sib1
   L Full 1 4 Fix                    !First and second allele of sib2
   M Full 1 4 Fix                    !First and second allele of sib3
   N Full 1 4 Fix                    !a1p1 a1p2
   O Full 1 4 Fix                    !a1p1 a2p2
   X Full 1 4 Fix                    !a2p1 a1p2
   Y Full 1 4 Fix                    !a2p1 a2p2
   S Full 1 1 Fix                    !to contain 4: maximum of 4 possible
                                     !genetically different offspring

   G Full 1 nvar Free                !grand mean
End Matrices;
Matrix S 4
Matrix K 1 1 1 1
Matrix L 1 1 1 1
Matrix M 1 1 1 1
Matrix N 1 1 1 1
Matrix O 1 1 1 1
Matrix X 1 1 1 1
Matrix Y 1 1 1 1
Matrix P
      0
      1 0
      1 1 0
Matrix  Z
      0
      1 0
      1 1 0
Specify K a1s1 a2s1 a1s1 a2s1   !genotype sib1
Specify L a1s2 a2s2 a1s2 a2s2   !genotype sib2
Specify M a1s3 a2s3 a1s3 a2s3   !genotype sib3
Specify N a1p1 a1p2 a1p1 a1p2   !parental alleles
Specify O a1p1 a2p2 a1p1 a2p2   !parental alleles
Specify X a2p1 a1p2 a2p1 a1p2   !parental alleles
Specify Y a2p1 a2p2 a2p1 a2p2   !parental alleles
Specify P   1
            pi12 1
            pi13 pi23 1
Specify Z   1
            z12 1
            z13 z23 1
Specify T   .5                         ! when familial variance is modeled as
            .5 .5                      ! add gen variance

Begin Algebra;
 V = (\part(B,N) + \part(B,O) + \part(B,X) + \part(B,Y)) % S ; !Between effects
 D = (\part(W,N) + \part(W,O) + \part(W,X) + \part(W,Y)) % S ; !for Within effects
End Algebra;

Means G + V + (\part(W,K)-D) | G + V + (\part(W,L)-D) | G + V + (\part(W,M)-D);
Covariance T@(F*F') + P@(Q*Q') + Z@(R*R') + I@(E*E') ;
End
```

```
Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
  A full 1 n = A1
  O zero 1 1
End Matrices;
Begin algebra;
  B = \sum(A) ;
End Algebra;
Constraint O = B ;
end

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
 C full 1 n = C1
 O zero 1 1
End Matrices;
Begin algebra;
 B = \Sum(C) ;
End Algebra;
Constraint O = B ;
end
```

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Chapter 12

Discussion[1]

[1] This chapter is based on: D Posthuma, EJC de Geus, DI Boomsma. Genetic contributions to anatomical, behavioural, and neurophysiological indices of cognition. To appear in: R. Plomin, J.C. deFries, I.C. Craig, & P. McGuffin (Eds.). *Behavioral genetics in the postgenomic era*. Washington, DC. APA Books. (2002).

This final Chapter summarizes and discusses the results that have been described in Chapters 3 to 11 in the light of existing literature. It is divided in three main sections: the extended twin design, cognitive ability and its endophenotypes, and follow-up research.

The extended twin design

Results from this thesis

This thesis comprises the first large study that employed a twin design with additional non-twin siblings. Effects of adding one or two non-twin siblings to a twin pair on the sample size required to detect additive genetic influences, non-additive genetic influences and shared environmental with a power of 80%, was investigated in Chapter 2. A summary of the results is given in Table 12.1.

Table 12.1
Indication of the effects of adding one or two non-twin siblings to a twin pair on sample size required to detect additive genetic influences (A), non-additive genetic influences (D) or shared environmental influences (C), with a power of 80%.

	Estimating A	*Estimating D*	*Estimating C*
Adding one additional sibling	Slight **decrease** in sample size	Large **decrease** in sample size	Large **decrease** in sample size
Adding two additional siblings	Slight **increase** in sample size	Large **decrease** in sample size	Large **decrease** in sample size

Compared to a design in which only MZ and DZ twins are included, adding one additional sibling leads to an average decrease of 9% (depending on the magnitude of the genetic influences) in sample size needed to detect additive genetic influences (with a power of 80% and alpha level of 0.05). The impact on the detection of shared environmental influences is even larger: on average 50% of the sample size is needed when one sibling is added to the classical twin design. For most complex traits it is found that genetic influences are of greater importance than shared environmental influences (Plomin *et al.*, 2000; Lynch and Walsh, 1996). Small contributions of shared environmental factors may easily go undetected in the classical twin design, which leads to an overestimation of the contribution of genetic factors to the overall variance of a trait. With the same sample size, but with a different design, namely the extended twin design, small contributions of shared environmental factors are less likely to go undetected. Thus, adding an additional sibling to a twin pair enhances the power to decompose familial influences on complex traits into genetic and shared environmental components. In the light of future QTL studies an extended twin design is also desirable as large sibship sizes are known to greatly enhance the power to detect QTL influences on a trait (Dolan, Boomsma and Neale, 1999).

Besides having a positive effect on statistical power, extended twin designs allow the evaluation of certain assumptions made in twin studies. Heritability estimates

derived from twin samples are sometimes criticized for their non-generalizability to the general (non-twin) population. A sub optimal intrauterine environment may have adverse effects later in life (Barker, 1998; Philips *et al.* 2000; Hack *et al.*, 2002). As twins share a womb at the same time, they generally experience a less optimal intrauterine environment than singletons. This is also reflected in lower birth weights in twins and lower birth weights-for-gestational-age in twins as compared to singletons. Studies comparing intellectual abilities of twins and singletons have found that during childhood, twins score significantly less on IQ tests than singletons (e.g. Record *et al.*, 1970). Previous studies, however, compared twins and singletons raised in different families. As was outlined in chapters 3 and 4 this may not be an optimal comparison as twin families and non-twin families may not be perfectly matched. The extended twin design provides an optimal matched singleton to the twins: the non-twin sibling. Non-twin siblings are raised in the same home as the twins; they even shared the same womb, although not at the same time. I found that at adult age, twins and singletons do not differ in mean scores on an IQ test. In Chapters 8 and 9 I also explicitly tested whether the covariance (and correlation) in non-twin sibling pairs differs from the covariance (and correlation) in DZ twins, since this – rather than the mean- is the basis of all heritability estimates. No differences in (co) variance structures of twins and singletons were found. Heritability estimates for IQ derived from twin studies can thus be generalized to the general population.

Using the same optimally matched twin-singleton design no twin-singleton differences in means and variances of intracranial volume, total brain volume, grey matter volume, white matter volume, lateral ventricular volume and third ventricle volume were found, implying that heritability estimates for brain volumes derived from twin studies can be generalized to the general population. We did find that second born twins have a smaller intracranial volume than their first-born co-twins (and siblings), reflecting the less optimal intrauterine environment for second born twins as compared to first born twins. This difference in intracranial volume, however, did not correspond to a difference in intellectual ability later in life.

Chapters 6 to 10 do not explicitly discuss the generalizability of estimates derived from twin samples. However, they all incorporate tests for homogeneity of (co-) variances across twins and their non-twin siblings. A summary of these tests is given in Table 12.2.

Table 12.2
Tested homogeneity in means, variances and covariances across twins and non-twin siblings

		TWIN-SIBLING COMPARISON		
		Mean	Variance	Covariances
COGNITIVE ABILITY	Block design	ok	ok*	ok*
	Letter-number sequencing	ok	ok*	ok*
	Information	ok	ok*	ok*
	Matrix reasoning	ok	ok*	ok*
	Similarities	ok	ok*	ok*
	Picture completion	ok	ok*	ok*
	Arithmetic	twins < non-twin sibling	ok*	ok*
	Vocabulary	ok	ok*	ok*
	Digit symbol coding	ok	ok*	ok*
	Digit symbol free recall	DZ/non-twin siblings < MZ	ok*	ok*
	Digit symbol pairing	ok*	ok	ok
	WAIS-IIIR Full Scale IQ	ok*	ok	ok
	WAIS-IIIR Verbal IQ	ok*	ok	ok
	WAIS-IIIR Performance IQ	ok*	ok	ok
	WAIS-IIIR Verbal Comprehension	ok*	ok	ok
	WAIS-IIIR Working Memory	ok*	ok	ok
	WAIS-IIIR Perceptual Organization	ok*	ok	ok
	WAIS-IIIR Processing Speed	ok*	ok	ok
BRAIN VOLUME	Intracranial volume	2nd born twin < non-twin sibling	ok	ok
	White matter volume	ok	ok	ok
	Grey matter volume	ok	ok	ok
	Lateral ventricular volume	ok	ok	ok
	Third ventricle volume	ok	ok	ok
	Cerebellar volume	ok*	ok	ok
SPEED	Alpha Peak Frequency	ok*	ok	ok
	Perceptual speed	ok*	ok	ok
	Speed of premotor selective response activation	ok*	ok	ok
	Speed of motor selective response activation	ok*	ok	ok
	Decision time	ok*	ok	ok
INHIBITION	Speed of premotor response activation with stimulus-response incongruency	ok*	ok	ok
	Speed of motor response activation & SR incongruency	ok*	ok	ok
	Decision time & SR incongruency	ok*	ok	ok
	Flanker Task Performance & SR incongruency	ok*	ok	ok

* not reported on in this thesis. SR = stimulus-response

Cognitive ability and its endophenotypes

Results from this thesis

Biological, neurophysiological, electrophysiological and behavioural indices of the pathways that connect genes and cognitive ability are called *endophenotypes* of cognitive ability. A summary of the heritability of cognitive ability and its anatomical, electrophysiological and behavioural indices as investigated in this thesis is given in Table 12.3.

Table 12.3
Overview of heritability estimates across two age cohorts of cognitive ability and neurophysiological indices, as investigated in this thesis.

	MEASURE	Young Dutch adults (26 yrs)	Middle aged Dutch adults (50 yrs)
		Heritability	Heritability
COGNITIVE ABILITY	WAIS-IIIR Full Scale IQ	0.86	0.86
	WAIS-IIIR Verbal IQ	0.85	0.85
	WAIS-IIIR Performance IQ	0.69	0.69
	WAIS-IIIR Verbal Comprehension	0.83	0.83
	WAIS-IIIR Working Memory	0.71	0.67
	WAIS-IIIR Perceptual Organization	0.68	0.68
	WAIS-IIIR Processing Speed	0.66	0.66
*BRAIN VOLUME	Intracranial volume	0.89	-
	White matter volume	0.87	-
	Grey matter volume	0.82	-
	Cerebellar volume	0.89	-
SPEED	Alpha Peak Frequency	0.71	0.83
	Perceptual speed	0.46	0.46
	Speed of premotor selective response activation	0.62	0.00
	Speed of motor selective response activation	0.39	0.39
	Decision time	0.43	0.43
INHIBITION	Speed of premotor response activation with stimulus-response incongruency	0.00	0.00
	Speed of motor response activation with stimulus-response incongruency	0.00	0.45
	Decision time with stimulus-response incongruency	0.48	0.48
	Flanker Task Performance (incorrect responses) with stimulus-response incongruency	0.54	0.41

*assessed in the sample (aged 30) of dr. Baaré, which partly overlaps with the young adult age cohort.

The first goal of this thesis was to investigate the heritability of cognitive ability in young and middle aged Dutch adults. Heritability estimates were similar for both age cohorts and were very high. The highest heritability was found for Full scale IQ (86%) and somewhat lower (66-83%) for the dimensions of cognitive ability. These heritability estimates of cognitive ability are among the highest reported for cognitive ability (Bouchard and McGue, 1981).

Combining these results with studies on cognitive ability in Dutch children and adolescents nicely shows the increasing heritability of cognitive ability with increasing age (see Figure 12.1).

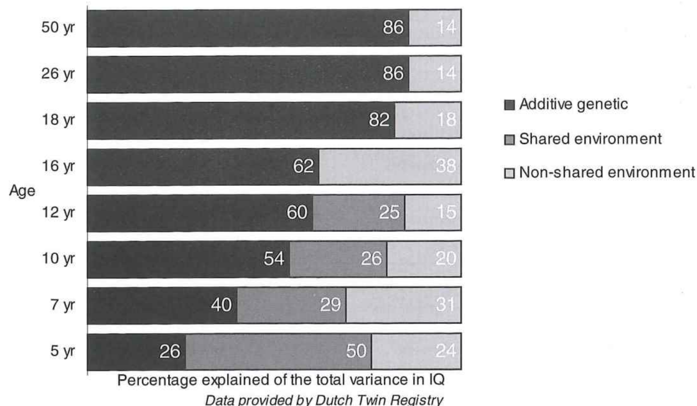


Figure 12.1:
Decomposition of the variance in Full Scale IQ into additive genetic variance, shared environmental variance, and non-shared environmental variance, at different ages in the Dutch population. Significance of additive genetic influences is .03 at age 5; of shared environmental influences it is .06 at age 7, .09 at age 10 and .08 at age 12.

A second goal of this thesis was to gain more insight in the biological pathways connecting genes and cognitive ability. The heritability of several endophenotypes has been investigated (Table 12.3) as well as their relation with cognitive ability (Table 12.4). Investigated endophenotypes were brain volumes, alpha peak frequency, perceptual speed, speed of premotor and motor response selection, decision time, and inhibitory control.

Table 12.4
Phenotypic correlation between cognitive ability and biological indices, and the percentage of the correlation explained by common genetic factors (homogeneous across both age cohorts, unless specified otherwise).

		FSIQ	VIQ	PIQ	VC	WM	PO	PS
BRAIN VOLUMES	White matter volume	0.24 100%	-	-	ns	0.28 100%	ns	0.25 100%
	Grey matter volume	0.25 100%	-	-	ns	0.27 100%	0.20 100%	ns
	Cerebellar volume	-	-	-	ns	0.27 100%	0.18 66%	ns
	Alpha Peak Frequency	-	-	-	ns	ns	ns	ns
SPEED	Perceptual speed	-	0.19 100%	0.27 100%	-	-	-	-
	Speed of premotor response activation	-	ns	ns	-	-	-	-
	Speed of motor response activation	-	ns	- 0.54[1]	-	-	-	-
	Decision time	-	ns	0.14[3]	-	-	-	-
INHIBITION	Speed of premotor response activation & SR incongruency	-	ns	ns	-	-	-	-
	Speed of motor response activation & SR incongruency	-	- 0.35[2]	- 0.41[1]	-	-	-	-
	Decision time & SR incongruency	-	ns	ns	-	-	-	-
	Number of incorrect responses & SR incongruency	-	-0.35 100%	-0.35 100%	-	-	-	-

[1] only for older females
[2] only for young males
[3] only for older males
FSIQ = Full Scale IQ; VIQ = verbal IQ; PIQ = performance IQ; VC = verbal comprehension; WM = working memory; PO = perceptual organization; PS = processing speed; SR = stimulus-response; ns = not significantly different from zero.
Note: Endophenotypes were either analysed in a multivariate design including Full scale IQ, in a multivariate design including verbal and performance IQ, or in a multivariate design including the four WAIS-IIIR dimensions.

Integrating our results with the recent literature[1]

Brain size

An obvious source of individual differences in cognitive abilities is the size of the brain. Since the second half of the 19th century positive relations between head size and intelligence have been observed. Correlations generally range around 0.20 (Jensen, 1994; Posthuma *et al.*, 2001b), but have been reported as high as 0.44 (van Valen, 1974). Head size is usually measured with a measuring tape as circumference of the head. A more accurate measure of the size of the brain can be obtained through Magnetic Resonance Imaging (MRI).

Willerman *et al.* (1991) correlated brain size as measured through MRI with IQ (measured with the revised Wechsler Adult Intelligence Scale, WAIS-R) in a sample of 40 unrelated subjects. They found a correlation of 0.51, which was higher in men (0.65) than in women (0.35). In a follow-up study, Willerman *et al.* (1992) suggested that, in men, a relatively larger left hemisphere better predicted verbal IQ than it predicted performance IQ, whereas in women the opposite was true. Since then, several studies have provided confirmative evidence that brain volume and IQ correlate around 0.40 (e.g. Egan at al. 1994; Andreasen *et al.*, 1993; Raz *et al.*, 1993; Storfer, 1999; Wickett *et al.*, 2000)

In a large MRI study including 112 twin pairs and 34 additional siblings the heritability of volumes of several brain structures was investigated (Baaré *et al.*, 2001). Heritability estimates for intracranial volume, total brain volume, grey matter volume, and white matter volume were all between 80-90%. Genetic intercorrelations between these measures were all very high indicating that a largely overlapping set of genes is responsible for individual differences in each of these measures.

Two early multivariate genetic studies have been conducted to investigate whether the relation between IQ and brain volumes is mediated through a common genetic pathway or through a common environmental pathway. The first study, often cited although only published as an abstract so far (Wickett *et al.*, 1997), was based on MRI and IQ data from 68 adult males from 34 sibships, and compared within-family correlations with between-family correlations of brain volume and IQ. A within-family correlation of 0.24 and a between-family correlation of 0.50 were reported, suggesting that some, but not all, of the phenotypic correlation between brain volume and IQ is due to a common underlying set of genes.

The second study (Pennington *et al.*, 2000), specifically addressed the relation of reading disorder with brain volume, but also included measures of IQ (WISC and WAIS3-R scores). In this study both a reading disorder sample (25 MZ, 23 DZ) and a healthy sample (9 MZ, 9 DZ) were included. The MZ and DZ correlations in the

[1] See Appendix IX for a summary of the multivariate genetic studies investigating the association between putative endophenotypes and cognitive ability discussed in this chapter.

reading disorder sample and the healthy sample were comparable and suggested high heritability of brain volume (90%), which is in line with the larger study on heritability of brain volumes of Baaré *et al.* (2001). Phenotypic correlations between cerebral brain volume and IQ were 0.31 in the healthy sample and 0.42 in the reading disorder sample. The genetic correlation, as calculated from the cross-twin correlations, was 0.48 in the combined sample. This indicates that about half of the genetic influence on either cerebral brain volume or IQ is due to genetic factors influencing both. Put differently, 80% of the phenotypic correlation is explained by genetic mediation.

In a recent study by Thompson *et al.* (2002), voxel based MRI techniques were used on a dataset of 10 MZ twins and 10 DZ twins. They reported high heritability of grey matter volume in several cortical regions (80-90%), specifically for the frontal, parieto-occipital and linguistic regions. In addition they reported a correlation between the size of these regions and cognition, and suggested that, although their study did not have the statistical power to estimate the relative contributions of genetic and environmental correlations, this correlation was likely to be mediated by an underlying set of genes.

In Chapter 7a of this thesis (Posthuma *et al.*, 2002) it was determined that the observed correlation between brain volume and intelligence is completely mediated by an underlying set of common genes. In a data set of 24 MZ pairs, 31 DZ pairs, and 25 additional siblings a correlation of 0.25 (0.24) between cerebral grey (white) matter volume and Full Scale IQ was found and a correlation of 0.29 between the Working Memory dimension of the WAISIII-R and grey or white matter volume. Results from multivariate genetic modelling showed that these correlations were completely determined by a genetic correlation between the genes that influence brain volume and the genes that influence IQ. In Chapter 7b we included cerebellar volume in the analyses as well as all four dimensions of the WAISIIR. It was found that cerebellar volume was related to Working Memory (0.27) and Perceptual Organization (0.18). One hundred percent of the correlation between cerebellar volume and Working Memory was explained by common genetic factors, while 66% of the correlation between cerebellar volume and Perceptual Organization was explained by common genetic factors.

Alpha peak frequency

Electroencephalographic (EEG) recording is a non-invasive technique to measure electrical activity of the brain. EEG activity can be analysed according to the frequency spectrum that is obtained when a Fourier transformation is performed on an EEG time-series. Generally five frequencies are distinguished in the EEG power spectrum: delta (0.5-4 cycles per second), theta (4-8 cycles per second), alpha (8-13 cycles per second), beta (13-30 cycles per second), and gamma (> 30 cycles per second). In the past decade the underlying biological mechanisms of the different

frequencies, especially the alpha and beta rhythms, are well understood and have been described in the literature (Steriade *et al.*, 1990; Lopes da Silva, 1991).

The dominant frequency in an adult human EEG spectrum lies in the alpha range around 10 cycles per second. The alpha peak frequency has been related to cognitive abilities in general and to (working) memory in particular. Lebedev (1990, 1994) proposed a functional role for the human alpha peak frequency in stating that 'cyclical oscillations in an alpha rhythm determine the capacity and speed of working memory. The higher the frequency the greater the capacity and the speed of memory'. In addition, Klimesch (1997) argued that thalamo-cortical feedback loops oscillating within the alpha frequency range allow searching and identification of encoded information. He speculated that faster oscillating feedback loops would correspond to faster access to encoded information. These theories are supported by the results of some recent studies; Klimesch (1997) found that the alpha peak frequency of good working memory performers lies about 1 Hz. higher than that of bad working memory performers. Anokhin and Vogel (1996) reported a correlation of 0.35 between alpha peak frequency and verbal abilities. In addition it is found that within the same subject alpha peak frequencies increase with increasing cognitive load of the task in which they are measured (Klimesch, 1999).

Results from a few small twin studies have suggested that alpha peak frequency is influenced by genetic factors (Christian *et al.*,1996), and it has also been speculated that its relation with IQ is due to a genetic basis (e.g. Vogel, 2000, page 117). In only one multivariate genetic study, however, the nature of the relation between alpha peak frequency and cognitive abilities is formally investigated. Including 102 MZ pairs and 525 DZ/sib pairs from two age cohorts (mean ages 26 and 50 years).

Chapter 8 of this thesis reports on a study from which it was concluded that that alpha peak frequency is highly heritable (Posthuma *et al.* (2001b). In young adults (mean age 26 years) heritability was estimated at 71%, and in older adults (aged 50 years) heritability was somewhat higher at 83%. Heritabilities for the WAIS-3R dimensions ranged from 66 to 83%. Surprisingly, no correlation was found between alpha peak frequency and IQ (WAIS-IIIR), thereby dismissing alpha peak frequency as a valuable electrophysiological substrate of cognitive ability as measured with the WAIS-IIIR.

Inspection Time

Inspection time is a measure of central nervous system processing and is defined as the minimum display time a subject needs for making an accurate perceptual discrimination on an obvious stimulus. It is distinct from reaction time since there is no need to make the discrimination quickly all that is required is an accurate response. Visual inspection time can easily be measured in a computerized version of the Π-paradigm in which subjects are asked to decide which leg of the Π-figure is longest. Visual inspection time is generally thought to reflect speed of apprehension

or perceptual speed. A meta-analysis conducted by Kranzler and Jensen (1989) including virtually all studies until 1989 investigating the relation between inspection time and intelligence indicated that inspection time and IQ correlate around –0.50: the less time a person needs to make an accurate decision on an obvious stimulus, the higher his score on an IQ test. The overall consensus on the relation between inspection time and IQ is given by Deary and Stough (1996): "inspection time accounts for approximately 20% of intelligence-test variance".

Recently two large twin studies have investigated whether the relation between inspection time and IQ is mediated by shared genetic factors or by shared environmental factors (Luciano *et al.*, 2001a; Posthuma *et al.*, 2001; Chapter 9 of this thesis). These two studies were also the first to report on the heritability of inspection time per se. Using 184 monozygotic (MZ) pairs and 206 dizygotic (DZ) pairs aged 16, Luciano *et al.* (2001a) reported a heritability estimate of inspection time of 36% and of IQ-measures between 73-81%. In chapter 9 (Posthuma *et al.* 2001a) we reported a slightly higher heritability estimate of inspection time (46%) and similar heritability estimates of IQ measures (WAIS-IIIR) ranging from 69-85%. The latter sample consisted of 102 MZ pairs and 525 DZ/sib pairs belonging to two age cohorts (mean ages 26 and 50).

Luciano *et al.* (2001a) reported a correlation between inspection time and performance IQ of -0.35 and between inspection time and verbal IQ of -0.26. In this thesis slightly lower correlations were reported; -0.27 and -0.19 respectively (Posthuma *et al.*, 2001a). Both studies unanimously found that the phenotypic correlations between inspection time and performance IQ/verbal IQ were completely mediated by common genetic factors. This meant that in the study by Luciano *et al.* (2001a) the genetic correlation between inspection time and performance IQ was -0.65 and between inspection time and verbal IQ was -0.47 . In our own study (Posthuma *et al.*, 2001) the genetic correlations were -0.47 and -0.31 respectively. Thus, the genes shared with inspection time are, across studies, estimated to explain between 10 and 42% of the total genetic variance in IQ.

Speed of premotor and motor response selection activation

Speed of premotor and motor response selection activation can be measured with the lateralized readiness potential (LRP). The LRP is mathematically derived from the readiness potential (RP, Kornhuber and Deecke 1965), an evoked potential that can be observed in an EEG registration. The LRP onset is considered to reflect the output of the response selection stage (Coles, 1989; Eimer, 1998) and to be closely time-related to central decision processes. The time of maximal LRP amplitude, LRP peak latency, is thought to additionally reflect central motor processes that take place after response selection has taken place (Falkenstein *et al.*, 1994). The LRP can be calculated from the EEG registration during the execution of any task that requires the selection of either right or left hand responses.

In a dataset of 102 MZ pairs and 525 DZ/sib pairs from two age cohorts (mean ages 26 and 50 years), we determined LRP onset and peak latencies (see Chapter 10 of this thesis and Posthuma *et al.*, (2002b). An inconsistent pattern of heritabilities (ranging from 0 – 62%) was found, and no correlations between these and IQ as measured with the WAIS-IIIR.

Reaction times

Galton (1883) was the first one to propose that reaction time is correlated with general intelligence and may be used as a measure of it. His observations and the results of empirical studies afterwards led to the general belief in the speed of processing theory of intelligence; the faster the accomplishment of basic cognitive operations the more intelligent a person will be (Eysenck, 1986; Vernon, 1987). Since then reaction times have consistently been negatively related to intelligence (e.g. Vernon, 1987; Deary *et al.*, 2001), i.e. a shorter reaction time corresponds to a higher IQ. Correlations with IQ generally range between –0.20 and –0.40, but can be as high as –0.60 (Fry and Hale, 1996). Increasing the information processing load of a task results in prolonged reaction times within in the same subject (Hick, 1952; Eriksen and Eriksen, 1974) and decreases performance. Higher correlations between reaction times and IQ, therefore, are more likely to be found when more complex reaction time tasks are used, although this effect is not unequivocally confirmed in empirical studies (Mackintosh, 1986).

Results from twin studies suggest heritabilities for reaction time of the same magnitude as those for IQ. McGue and Bouchard (1989) observed heritabilities of 54 and 58% for basic and spatial speed factors in a sample of MZ (N=49) and DZ (N=25) twins reared apart. For a general speed factor based on eight complex reaction time tests Vernon (1989) found a heritability of 49% in 50 MZ and 52 DZ twins. In the same study it was also found that reaction time tests requiring more complex mental operations show higher heritabilities. A bivariate analysis of these data with IQ in 50 MZ and 32-SS DZ pairs (15 to 57 years) was reported by Baker *et al.* (1991). Phenotypic correlations of Verbal and Performance IQ with general speed were both -0.59 and were entirely mediated by genetic factors. Genetic correlations were estimated at -0.92 and -1.00. This is in line with results from an earlier study in which phenotypic correlations between reaction time (measured as the total number of correct responses on a timed task) and IQ ranged between 0.37 and 0.42, from which 70-100% was attributed to genetic factors influencing both reaction time and IQ (Ho *et al.*, 1988).

More recently, Rijsdijk *et al.* (1998) conducted a multivariate genetic analysis on reaction time data and IQ data, using 213 twin pairs measured at ages 16 and 18. Heritabilities were reported for age 16 of 58%, 57%, and 58% for simple reaction time, choice reaction time and IQ (RAVEN) respectively. Phenotypic correlations of simple reaction time and choice reaction time with IQ were –0.21 and –0.22 respectively and were completely mediated by common genetic factors. Virtually the same picture was shown at age 18 where the same reaction time battery was correlated with IQ as measured with the WAIS.

Finkel and Pedersen (2000) investigated the underlying covariance structure of measures of speed and measures of cognition in a sample of 292 reared together and reared apart MZ and DZ twins (aged 40-84). Speed was measured by oral versions of the Digit Symbol and Picture Identification subtests of the WAIS. A cognitive factor was constructed based on several standard IQ tests. The phenotypic correlation between the speed factor and the cognitive factor was 0.66, of which 61% was due to correlated genetic factors between the two. Also, they reported that 70% of the genetic variance in the cognitive factor was shared with the speed factor.

Neubauer *et al.* (2000) reported heritability estimates of reaction time data and IQ (RAVEN) ranging from 11-61% and 39-81% respectively. Phenotypic correlations between reaction time data and IQ (RAVEN) data were between –0.08 to –0.50, where higher correlations with IQ were found for more complex reaction time tasks. Common genetic influences on reaction time and IQ accounted for 65% of the observed phenotypic correlation.

Evidence for a genetic mediation between reaction time and IQ also emerges from a recent large twin study by Luciano *et al.* (2001b). Using reaction time data from a two-choice reaction time (2CRT) task, a four-choice reaction time task (4CRT), an eight-choice reaction time task (8CRT) and IQ data from 166 MZ pairs and 190 DZ pairs Luciano *et al.* (2001b) report high heritabilities for reaction times (CRT: 52%, 4CRT: 59%, 8CRT: 70%) and IQ (81%), and moderately high phenotypic (–0.32 to –0.55. The genetic contributions to the observed correlations were all 100% (i.e. were as high as the observed correlations; from –0.32 to –0.49) except for the 4CRT where the genetic contribution covered 89% of the observed correlation. In other words, common genetic influences explained at least 89% of the observed phenotypic correlation between reaction times and IQ.

In Chapter 10 of this thesis the heritability of reaction time (as indexed by decision time) was investigated in a large sample of twins and siblings (see also Posthuma *et al.*, 2002b). To be comparable to reaction times measures used in previous studies, reaction times are best studied in the congruent condition of the Eriksen Flanker Task that was described earlier. The heritability of reaction time was moderate (43%), but no correlation with cognitive ability was found on the phenotypic, genetic or environmental level.

In summary, non-genetic studies have shown a consistent and stable relation between reaction time and IQ; interindividual variance in reaction time seems to explain at most 30% of IQ test variance. Results from previous genetic studies, have suggested that between 65 and 100% of this covariance is explained by a common underlying genetic mechanism. Results from our own, relatively large, study show no

evidence of a covariance between reaction time and IQ, possibly because accuracy may have been stressed more than speed.

Indices of components of working memory

Working memory is considered a central component of cognitive functioning (e.g. Kyllonen and Chrystal, 1990; Baddely, 1986). Some behavioural measures of working memory functioning have already been investigated in a multivariate genetic design simultaneously with a measure of IQ. For instance, Ando *et al.*, (2001) used a spatial and verbal working memory task which were revised versions of the working memory tasks developed by Shah and Miyake (1996). These two tasks generate performance scores on four conditions: spatial storage (Ss), spatial executive (Se), verbal storage (Vs) and verbal executive (Ve). IQ was assessed with the Kyodai NX15 Japanese intelligence scale, form which two components were calculated: Verbal cognitive ability (VCA) and Spatial cognitive ability (SCA). Their data set consisted of 143 MZ's and 93 DZ's aged 16 – 29 years. The working memory performance measures were all moderately heritable (Ss 45%; Se 49%; Vs 48%; Ve 43%) and heritability of IQ was slightly higher (VCA: 65%; SCA 65%). VCA correlated around 0.30 to the working memory measures, whereas SCA correlated around 0.40 to the working memory measures. Genetic contributions predominantly (>85%) explained the observed correlation between IQ and working memory performance.

Luciano *et al.* (2001) determined the heritability and relation with IQ of accuracy on a delayed response task. This task has been adapted from animal research and is widely used as an index for working memory (Goldman-Rakic, 1996). Accuracy on this task was moderately heritable (48%), correlated around 0.20 with IQ, and 100% of this correlation was explained by common genetic factors. Similar results were recently obtained by Wright *et al.*, (2002) after this dataset had been extended by many newly added twin pairs.

In Chapter 10 we reported on the heritability of latency of response selection, reaction times and performance on the incongruent condition of the Flanker task. This task requires a left or right hand response depending on the stimulus. The stimulus can either be congruent with the response or incongruent with the response. Stimulus-response incongruency slows response times and LRP latencies and increases the number of incorrect responses. (Turken and Swick, 1999: Botvinick *et al.*, 1999; Awh and Gehring, 1999). Latencies and performance in the incongruent condition are often thought to reflect inhibition ability which is one of the major function of the frontal executive component of working memory (Baddeley, 1996). Latencies in the incongruent condition showed no or low heritability, whereas decision time was moderately heritable (48%). LRP latencies and decision time in the incongruent condition did not correlate with cognitive ability. However, performance on the incongruent trials was moderately heritable (54% in young

adults aged 26; 41% in older adults aged 50) and correlated well to IQ (around 0.30 – 0.40). This correlation was completely explained by common genetic factors.

Follow up research

Endophenotypes of cognitive ability

Summarizing the above it can be stated that genetic variability in brain volume, perceptual speed and frontal inhibitory control is related to genetic variability in cognitive ability.

Insight into the pathways between genes and cognitive ability is not only important in understanding individual differences in normal cognitive functioning, but may also provide clues into the underlying mechanisms of impaired cognitive ability. Diverging conditions as reading disorder, schizophrenia, ADHD, depression, alcoholism, and dementia all share significant deficits in cognitive ability (Willcutt *et al.*, 2001; Harvey 2001; Bray and Owen, 2001; Austin, Mitchell and Goodwin, 2001: Braver, Barch, Cohen, 1999; Goldman-Rakic, 1999).

Another important advantage of identifying pathways between genes and cognitive ability is that they may aid in the detection of the actual genes or quantitative trait loci (QTL's) that influence cognitive ability. Although cognitive ability shows very high heritability, it is still a complex trait, likely to be influenced by a number of QTL's (Plomin and Crabbe, 2000). Its high heritability may reflect the summed genetic effects of a number of QTL's which each exert only small effects (in the order of explaining less than 2% of the total variance in cognitive ability). Biological substrates that are genetically correlated with cognitive ability each explain part of the variance in cognitive ability (10-20%), and may thus each mediate the influence of a small subset of the genes that influence cognitive ability. These subsets of genes will explain a large part of the variance in the biological substrate or endophenotype and a relatively smaller part of the genetic variance in cognitive ability (De Geus and Boomsma, 2002). Including these endophenotypes in studies aimed at the detection of genes that influences cognitive ability may therefore enhance the chances of detecting these genes (Boomsma *et al.*, 1997; de Geus & Boomsma, 2001; Leboyer, 1998).

Especially when a genetic correlation between the genes that influence the endophenotype and cognitive ability reflects an underlying causality from endophenotype to cognitive ability, (as opposed to reflecting pleiotropic effects of genes), QTL effects on endophenotypes will be greater than effects of these QTLs on cognitive ability, as the latter are a function of the QTL effects on the endophenotype and the effect of the endophenotype on cognitive ability. As stated in Chapter 2, genetic correlations do not provide information on causal relations. Direction of causation, however, can be assessed using longitudinal designs, but also with cross-sectional twin designs, provided the two traits show different heritabilities, and provided the validity and reliability of the measurements are known (Heath *et*

al., 1993; Neale *et al.*, 1994; Duffy and Martin, 1994). For example, perceptual speed showed moderate heritability while cognitive ability was highly heritable. Direction of causation between these measures has not been resolved yet. Combining the datasets on perceptual speed and cognitive ability from the Australian researchers (Luciano *et al.*, 2001) and ourselves (Posthuma *et al.*, 2001) will provide an excellent opportunity to resolve the direction of causation between perceptual speed and cognitive ability.

Candidate genes for cognitive ability

Results from human linkage and associations studies for IQ have not yet identified "genes for IQ", although results from the IQ-QTL project (Plomin *et al.*, 1994, 1995; Daniels *et al.*, 1998) have initially provided some evidence for the association of cognitive ability with the alcohol dehygrenase 5 marker (ADH5), nerve growth factor beta polypeptide marker (NGFB), dystrophia myotonica marker (DM) (Petrill *et al.*, 1996), insulin-like growth factor-2 receptor marker (IGF2R) (Chorney *et al.*, 1998), and markers D4S2943, MSX1 and D4S1607 on chromosome 4 (Fisher *et al.*, 1999). Recently, however, Plomin *et al.*, 2001, conducted a genome scan for cognitive ability, using 1842 markers across the genome. They employed an extremely conservative approach (i.e. a five stage design with three samples) to guard against false positives and false negatives. Such a conservative approach seems necessary given the large number of tests that are employed and the problems in replicating QTL association studies (Cardon and Bell, 2001). Using these criteria, Plomin *et al.*, (2001) could not replicate any of the previously found QTL associations, and did not detect new QTL associations.

An alternative approach for screening the genome for possible QTL's is to pre-select candidate genes (de Geus *et al.*, 2001). The results from this thesis suggest that genes important for cerebral grey matter volume, cerebral white matter volume, cerebellar volume, perceptual speed and frontal inhibition may also be important genes for cognitive ability. The genetic connection between brain volumes, neural speed and cognitive ability fits very well in the myelination hypothesis as formulated by Miller (1994). According to this hypothesis, generally, the relation between speed and intelligence can be explained if part of the interindividual variance in cognitive ability can be ascribed to interindividual variance in the degree of myelination of cortico-cortical connections. If true, this could explain why more intelligent brains show faster nerve conduction, faster reaction times and faster inspection times. And, all other things equal, thicker myelin sheaths will result in larger brain volume, thus explaining the positive relation between brain size and IQ (Miller, 1994).

Although it is unlikely that the myelination hypothesis explains all observed anatomical, behavioural and neurophysiological relations with cognitive functioning (e.g. it does not directly explain the link with frontal inhibition), it may provide theoretical guidance in the choice of candidate genes for cognition. As also became

evident from Chapters 7 and 9, genes important for myelination and development of the brain may also be of importance for cognitive ability. Candidate genes important for myelination as mentioned in those Chapters were the *Plp* gene (Boison and Stoffel, 1994; Griffiths *et al*, 1995, Ikenaka and Kagawa, 1995; Lemke, 1993), the *cgt*-gene (Stoffel and Bosio, 1997), the *MAG* gene (Fujita *et al.*, 1998, Sheikh *et al.*, 1999), and the *tn-r* gene (Weber *et al.*, 1999).

An important new finding in this thesis was that human cerebellar volume is highly heritable and that its relation to cognitive ability is mainly mediated through a common genetic pathway. Some genes known to be important for cerebellar development derived from mouse studies were reported in Chapters 6 and 7; the Pax2 locus and the En-2 locus. Favor *et al.* (1996) showed that in mice, functioning of the Pax2 locus, which has its counterpart in the human PAX2 locus, is absolutely necessary for the normal development of the cerebellum. Millen *et al.* (1994) reported a reduction in cerebellar volume in mice due to dysfunctioning of the En-2 locus.

More recently Airey, Lu, and Williams (2001) conducted a full genome screen for cerebellar size in mice and reported linkage with five QTL's. They proposed a set of candidate genes lying within the linkage regions, which also confirmed the role of the Pax2 gene in cerebellar development. Human homologous chromosomal regions of the five QTL's in mice as reported by Airey, Lu, and Williams (2001), are 1q23-43, 10q11-23, 9q13-q24, 11q12-q13, 10q23-qter, 16q12-22.

Genes that may be important for frontal inhibitory control cannot be directly derived from genes investigated with the help of animal models, as there are no animal behavioural models of frontal inhibition. The COMT gene (Lachman *et al.*, 1996) has repeatedly been linked to frontal executive functioning in humans (Egan *et al.*, 2001; Weinberger *et al.*, 2001 for a review), which encompasses frontal inhibition functioning. This gene has also been associated with increased risk of schizophrenia (see Weinberger *et al.*, 2001) which is characterized by impaired executive functioning.

Morley and Montgomery (2001) extensively reviewed the existing (human and animal) literature for candidate genes for human cognition. They indexed published results from *Drosophila melanogaster*, mice and human studies in four phenotypic categories: memory, learning, cognition, and mental retardation. Over 150 candidate genes were derived that may all influence aspects of human cognition. Morley and Montgomery (2001) selected 36 candidate genes for which the literature review provided strongest evidence. Most of these candidate genes are associated with aspects of memory or learning. Very promising candidate genes are the N-methyl-D-aspartate (NMDA) receptor genes NMDA1, NMDA2A, NMDA2B. NMDA receptors are widely distributed in the brain and play a major role in long term potentiation (LTP) (Bear and Malenka, 1994), which is thought to be a cellular substrate of memory (Miller and Mayford, 1999; Eichenbaum and Harris, 2000).

Several studies have shown that mice lacking NMDA induced LTP show substantial learning deficits and spatial memory impairments (Tsien, Huerta en Tonegawa, 1996; McHugh *et al.*, 1996). Alternatively, Tang *et al.*, (1999) showed that overexpression of NMDA2B in the forebrains of transgenic mice results in a superior ability in learning and memory.

Other studies have recently identified genes or found evidence for linkage for human abilities related to IQ: speech and dyslexia. Lai *et al.*, (2001) found that a point mutation in a coding region within the FOXP2 gene on chromosome 7 is related to severe disruption of speech and language. In fact, Lai *et al.* (2001) suggested that this mutation is *causally* related to development of the neural substrate that underlies language and speech. The FOXP2 gene may also be related to aspects of IQ, or at least reveal genetic pathways important for e.g. verbal abilities.

In summary, candidate genes may be selected using an endophenotype approach, using animal models, or may be derived from human studies on traits related to cognitive ability. Whatever candidate genes are selected, they need to be analysed using optimal statistical methods. In Chapter 11 an existing powerful method for the simultaneous analysis of linkage and association (Fulker *et al.*, 1999) was extended to include variable sibship sizes, estimates of spurious and non-spurious dominance effects, and situation where parental genotypes are unavailable. This method also allows fine mapping, as the effect of linkage will be reduced when estimated in the presence of association, thereby providing information on the distance between the marker and the QTL (Cardon and Abecasis, 2000). An explicit test for the effects of population stratification is also incorporated in this method, thereby allowing to distinguish spurious allele effects from genuine allele effects. In Chapter 11 the translation of this method from the theoretical level to the practical implementation in structural equation modelling software was discussed.

With our current armament of behavioural and biological indices of cognitive ability, obtained in a dataset optimal for linkage and association analyses, we are well equipped to start searching for genes for cognition.

Samenvatting

GENETISCHE VARIATIE EN COGNITIEVE VAARDIGHEDEN

De twee voornaamste doelen van het onderzoek waarop dit proefschrift is gebaseerd waren: het kwantificeren van de erfelijkheid van cognitieve vaardigheden in een Nederlands jong volwassen en ouder volwassen leeftijdscohort en het verkrijgen van meer inzicht in de individuele verschillen in hersenfunctie en structuur die cognitieve vaardigheden onderliggen.

Om deze beide doelen optimaal te verwezenlijken is gebruik gemaakt van een zogenaamd *extended twin design*, oftewel het uitgebreide tweelingen design. In dit design zijn de deelnemers tweelingen of broers en zussen van tweelingen. Een dergelijk onderzoeksdesign is statistisch zeer krachtig (zie Hoofdstuk 3) en biedt de mogelijkheid om een aantal aannames van het klassieke tweelingenmodel te onderzoeken. Eén van die aannames is dat tweelingen niet systematisch verschillen van 'eenlingen'. Er wordt wel eens gezegd dat deze aanname onterecht is en dat tweelingpopulaties niet geheel representatief zijn voor de normale, niet-tweeling populatie. Het is bekend dat slechte omstandigheden in de baarmoeder negatieve effecten op latere leeftijd kunnen hebben (Barker, 1998). Tweelingen zitten altijd samen in de baarmoeder en zouden daarom gemiddeld genomen in slechtere baarmoederlijke omstandigheden verkeren dan eenlingen. Tweelingen hebben bijvoorbeeld ook vaker een lager geboortegewicht dan eenlingen, zelfs wanneer wordt gecorrigeerd voor de gestatietijd. Het verschil in gewicht met eenlingen wordt echter meestal snel weer ingelopen. Niettemin, wanneer deze verschillen niet worden ingelopen en tweelingen systematisch verschillen van eenlingen zouden erfelijkheidsschattingen gebaseerd op tweelingpopulaties, niet zonder meer gelden voor de 'normale' populatie.

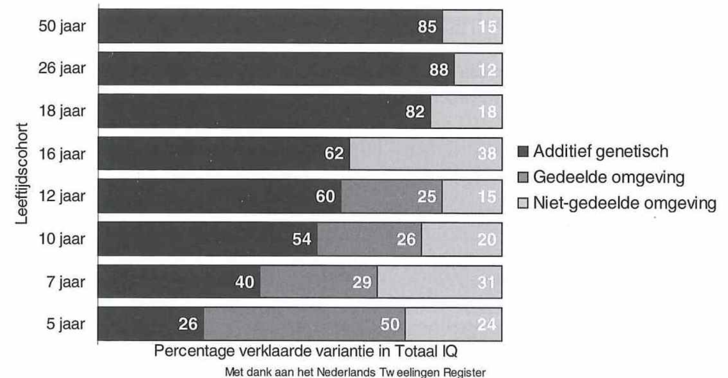
In een aantal buitenlandse studies is het IQ van tweelingen vergeleken met het IQ van eenlingen. In deze studies werd gevonden dat tweelingen een significant lager IQ hebben dan eenlingen (zie bijvoorbeeld Record *et al.*, 1970). Echter, in deze eerdere studies werden tweelingen vergeleken met eenlingen uit een *ander* gezin. Zoals werd beargumenteerd in de hoofdstukken 4 en 5 is dit geen optimale vergelijking; naast het verschil tweeling-eenling zijn er nog meer factoren die de verschillen in IQ zouden kunnen verklaren. De situatie van gezinnen waar een tweeling voorkomt kan in meerdere opzichten anders zijn dan die van gezinnen met alleen eenlingen. Pas wanneer tweelingen en eenlingen uit *hetzelfde gezin* met elkaar worden vergeleken kan worden onderzocht of het 'zijn van een tweeling' invloed heeft op een eigenschap als IQ.

Het *extended twin design* is een optimaal design om tweelingen en eenlingen te vergelijken. De gewone broers en zussen groeien op in hetzelfde gezin als de tweeling en hebben zelfs in dezelfde baarmoeder gezeten (al is het niet op hetzelfde tijdstip). In de huidige studie deden 688 mensen mee die allemaal deel waren van een gezin met daarin een tweeling. Deze 688 mensen kwamen uit 271 gezinnen, waarvan 149

gezinnen behoorden tot het 'jong volwassenen cohort' (gemiddeld 26.2 jaar) en 122 gezinnen tot het 'ouder volwassen cohort' (gemiddeld 50.4 jaar). Met zo'n grote dataset kunnen zelfs zeer kleine verschillen in IQ-scores tussen tweelingen en eenlingen worden aangetoond. De volwassen tweelingen en hun volwassen broers en zussen in de huidige studie verschilden niet in gemiddeld IQ. Bovendien werd expliciet getest of er verschillen waren in de overeenkomst tussen IQ scores voor DZ tweelingparen en paren van gewone broers of zussen. Ook dit was niet het geval. Ook was er geen sprake van een verschil in spreiding van de IQ scores voor tweelingen of gewone broers of zussen. We kunnen dus concluderen dat erfelijkheidsschattingen van IQ die worden gedaan op grond van tweelingpopulaties representatief zijn voor de gehele populatie.

Het *extended twin design* bleek ook in statistische zin een optimaal design te zijn voor het schatten van de bijdrage van erfelijke en omgevingsfactoren aan individuele verschillen. Ook het onderscheid tussen additieve genetische effecten en dominant genetische effecten bleek beter te maken.

Na het vaststellen van de statistische en inhoudelijke voordelen van het *extended twin design* werd onderzocht in welke mate individuele verschillen in cognitieve vaardigheden kunnen worden toegeschreven aan verschillen op genetisch niveau. Cognitieve vaardigheden werden gemeten door middel van de score op de nieuwste versie van de Nederlandse vertaling van de WAISIIIR (WAISIII, 2000).



Figuur 1:
Percentage verklaarde variantie in IQ.

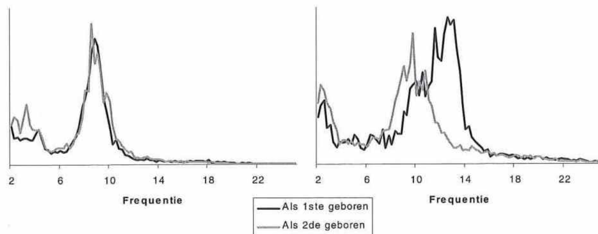
De erfelijkheid van de Totale IQ-score in Nederlandse jonge volwassenen en oudere volwassenen was zeer hoog. Voor de jonge volwassenen werd 88% van de verschillen tussen mensen in IQ score verklaard door verschillen op genetisch niveau. Bij de oudere volwassenen was dat 85%.

De erfelijkheidsschattingen uit de huidige studie kunnen we combineren met erfelijkheidsschattingen voor cognitieve vaardigheden in Nederlandse jonge kinderen en tieners uit voorgaand onderzoek in het Nederlandse Tweeling register. Wanneer we dit doen ontstaat een mooi beeld van een toename van de invloeden van genetische factoren en een afname van de invloed van omgevingsfactoren met de leeftijd (zie Figuur 1).

Tevens werd de erfelijkheid van een aantal andere eigenschappen, die mogelijk samenhangen met de IQ-score, onderzocht. De onderzochte eigenschappen kunnen worden onderverdeeld in twee domeinen, waarvan vooraf werd verwacht dat ze mogelijk gerelateerd zijn aan cognitieve vaardigheden. De domeinen zijn *Snelheid* en *Werkgeheugen/Aandacht*.

Snelheid werd ondermeer in kaart gebracht met de Alfa Piek Frequentie (Hoofdstuk 8). De Alfa Piek Frequentie is de dominante frequentie van de electroencefalografische (EEG) activiteit die te zien is tijdens een rust situatie waarbij de ogen gesloten zijn. Het is tevens de frequentie die het meest afneemt wanneer de ogen geopend worden. Bij de meeste mensen ligt deze frequentie rond de 10 Herz, maar hierin bestaan grote individuele verschillen.

De Alfa Piek Frequentie wordt wel vergeleken met de kloksnelheid van een computer processor. Een hogere kloksnelheid zou er dan voor zorgen dat er meer eenheden kunnen worden opgenomen in het geheugen. In Figuur 2 is te zien dat de twee mensen van een eeneiig tweeling paar een vrijwel identiek frequentie spectrum (en Alfa Piek Frequentie) hebben, terwijl de leden van een twee-eiig tweelingpaar veel minder op elkaar lijken.

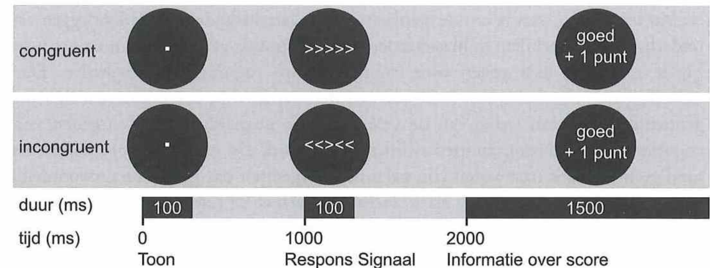


Figuur 2:
Links het EEG frequentie spectrum van een eeneiig tweelingpaar, rechts het EEG frequentie spectrum van een twee-eiig tweelingpaar.

Een dergelijk patroon is indicatief voor een hoge erfelijkheid, omdat eeneiige tweelingen genetisch identiek zijn en twee-eiige tweelingen gemiddeld voor 50% in genetisch opzicht op elkaar lijken. Uit de huidige studie bleek dan ook dat de Alfa Piek Frequentie zeer erfelijk is (71% in het jong volwassen cohort en 83% in het oudere volwassen cohort). In onze studie vonden we echter geen enkel verband tussen de Alfa Piek Frequentie en de IQ-score. De hoge erfelijkheid van zowel de Alfa Piek Frequentie als de totale IQ-score, maar tegelijkertijd de afwezigheid van een verband tussen beide, impliceren dat *verschillende* genen van belang zijn voor Alfa Piek Frequentie en IQ.

Een andere, gedragsmatige maat, waarmee *Snelheid* werd gemeten was de inspectietijd (Hoofdstuk 9). Dit is de tijd die iemand nodig heeft om een simpele beslissing te nemen. In de huidige studie werd dit gemeten door middel van een π-paradigma waarbij besloten moest worden welke poot van de π-figuur het langst was. De aanbiedingstijd van de π was variabel. Inspectietijd bleek redelijk erfelijk te zijn (46% voor beide leeftijdsgroepen) en bleek tevens een relatie met IQ te hebben. Het belangrijkste was echter dat deze relatie geheel toe te schrijven was aan erfelijke factoren. Er zijn dus genen die zowel inspectietijd als IQ beïnvloeden.

Voor het domein *Werkgeheugen/Inhibitie* werd de Eriksen Flanker Taak gebruikt (Hoofdstuk 10). Inhibitie kan worden gemeten in een taak waarbij de aanwezigheid van afleidende elementen de uitvoering van een taak bemoeilijkt. Ofwel het negeren van deze afleidende elementen, ofwel het richten van de aandacht op het doel van de taak zijn nodig om tot een goede taakprestatie te komen. In de Eriksen Flanker taak worden vijf pijlen aangeboden op een computer scherm (zie Figuur 3).



Figuur 3:
Weergave van de Eriksen Flanker Taak.

De pijlen wijzen óf allemaal dezelfde kant op (congruente conditie), óf de middelste pijl wijst een andere kant op (incongruente conditie). De instructie is om op grond van de richting van de middelste pijl met de linker of rechter hand zo snel mogelijk een knop in te drukken. Beide handen rusten op een startknop die niet mag worden losgelaten wanneer niet met die hand mag worden gereageerd. Wanneer alle pijlen dezelfde kant op wijzen is de taak vrij makkelijk. Wijst echter de middelste pijl de andere kant op, dan wordt de taak moeilijker, omdat de aandacht wordt afgeleid door de flankerende pijlen die de andere kant op wijzen. Er worden dan meer fouten gemaakt en de reactietijd gaat omhoog. Ook in het EEG is dan een tijdsverschuiving van het signaal zichtbaar. Het aantal fouten dat werd gemaakt in deze moeilijke conditie bleek door genetische aanleg te worden beïnvloed. Opnieuw bleek dat deze genetische aanleg voor een deel overlapt met de genetische aanleg die de IQ-score beïnvloedt.

Bij een deel van de deelnemers is, dankzij een samenwerking met de onderzoeksgroep van Prof. Kahn van het Medisch Centrum Utrecht, tevens gekeken naar anatomische eigenschappen van het brein (Hoofdstukken 6 en 7).

De gemeten *Brein volumes* zijn intracraniaal volume, grijze massa van de grote hersenen, witte massa van de grote hersenen en het volume van het cerebellum (de kleine hersenen). We vonden dat deze brein volumes zeer erfelijk zijn (80-90%) en dat ze een positieve relatie met de IQ-score hebben. Deze relatie tussen brein volume en IQ-score kon geheel worden toegeschreven aan genen die zowel van invloed zijn op de grootte van het brein als de hoogte van de IQ-score, en niet door omgevingsfactoren die beide beïnvloeden.

Samenvattend kan worden gezegd dat cognitieve vaardigheden op volwassen leeftijd zeer erfelijk zijn en dat een aantal van de genen die van invloed zijn op deze vaardigheden tevens van invloed zijn op brein volume, inspectietijd en de mate waarin iemand in staat is om de aandacht niet te laten afleiden. Het blootleggen van onderliggende verschillen in hersenfunctie en structuur is van belang omdat het helpt bij de zoektocht naar genen voor (stoornissen in) cognitieve vaardigheden. Deze onderliggende eigenschappen (ook wel 'endofenotypes' voor cognitieve vaardigheden genoemd) geven aan welke van de vele genen als mogelijke kandidaat-genen voor cognitieve vaardigheden kunnen worden beschouwd. De endofenotypes die uit het huidige onderzoek naar voren zijn gekomen suggereren dat genen verantwoordelijk voor de groei van de hersenen en de snelheid waarmee de zenuwen informatie in het brein verwerken van belang zullen zijn voor IQ. Een aantal van deze genen is reeds via dier-experimenteel onderzoek geïdentificeerd en zullen in vervolgonderzoek worden getoetst op hun mogelijke relatie tot cognitieve vaardigheden in de mens. Onderzoek in tweelingfamilies is hierbij opnieuw een zeer krachtige aanpak. In Hoofdstuk 11 van dit proefschrift werd een statistische methode besproken waarmee

een mogelijke associatie tussen kandidaat-genen en een IQ-score kan worden onderzocht in tweelingfamilies. Via deze methode hopen we in de toekomst daadwerkelijk de genen te identificeren die van belang zijn om individuele verschillen in cognitieve vaardigheden te verklaren.

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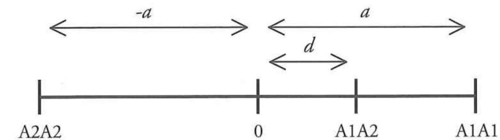
Appendices

Appendix I: Full set of measures collected in (part) of the members of the extended twin families participating in the study on which this thesis was based.

DOMAIN	TASK	MEASURE
Psychometric intelligence	Wechsler Adult Intelligence Scale-revised (WAISIIIR)	Block design, Letter-number sequencing, Information, Matrix reasoning, Similarities, Picture completion, Arithmetic, Vocabulary, Digit symbol-coding, Digit-symbol pairing and Digit symbol-free recall.
	Groninger Intelligence Test (GIT)	Vocabulary
Processing speed	Simple Reaction Time Task	Reaction time, movement time, decision time
	Choice Reaction Time Task	Reaction time, movement time, decision time, efficiency
	Inspection Time Task	Inspection time
	Oddball Task - EEG	P3 latency
	Delayed Response Task -EEG	N1/P2, P3 latency
	Eriksen Flanker Task - EEG	Lateralized Readiness Potential (LRP) – Onset, amplitude, slope, peak latency, decision time, movement time, accuracy
Working memory	Delayed Response Task - EEG	N2-amplitude and latency, Slow wave amplitude, P3 amplitude and latency, intrahemispheric EEG Coherence, EEG Power, Induced Band powers (ERD/ERS), performance (%correct, spatial accuracy)
	Sternberg Task	%correct, Reaction time, Sternberg slope
Brain volume	Magnetic Resonance Imaging (MRI)	Intracranial space, gray matter volume, white matter volume, cerebellar volume, ventricular volume. Head circumference, nasion-inion distance
Resting brain activity	Eyes closed - EEG	EEG Power, Coherence, Alpha peak frequency
	Eyes open - EEG	EEG Power, Coherence
Other physiological Parameters	Eyes open with finapress - EEG	Continuous finger blood pressure
		Arm cuff Systolic pressure, diastolic pressure (DynaMap) Heart rate, heart rate variability, baroreflex sensitivity Weight, height
Lipids, hormones etc.		Cholesterol, high density lipoprotein, triglycerides apolipoproteines A1, A2, B, E apoE genotype C-Reactive Protein, Tissue type Plasminogen Activator, Plasminogen Activator Inhibitor, Fibrinogeen, v Willebrandt Insulin, Glucose,
Other	Questionnaire	Mental effort Scale Demographics Health, life-style, addiction, personality, psychopathology, religion, SES and educational attainment

Appendix II: Biometric model describing the uni- and bivariate situation where $d = a$.

Consider a diallelic trait with alleles A1 and A2. Let *a* be the effect of genotype A1A1 on the phenotypic mean, *-a* the effect of A2A2, and *d* the effect of A1A2 on the phenotypic mean.



Assuming equal allele frequencies of A1 and A2, the mean genotypic effect on the phenotypic mean is ½*d*. The total genetic variance (σ_g^2) equals $½\, a^2 + ¼\, d^2$, $= V_a + V_d$

For complete dominance $d = a$. Substituting d for a in the formulae for the genetic variances, gives: $V_a = ½\, a^2$ and $V_d = ¼\, a^2$, thus $V_a = 2\, V_d$

Now consider a *bivariate* model with latent variances scaled to unity, and

- uniform genetic influences over traits: $V_{a,1} = V_{a,2}$ and $V_{d,1} = V_{d,2}$
- assumption of uniform *d* to *a* ratio over traits
  $(a_{11})^2 / (d_{11})^2 = (a_{21})^2 / (d_{21})^2 = (a_{22})^2 / (d_{22})^2$
- $r_A = a_{11} \times a_{21} / \sqrt{\{(a_{11})^2 \times [(a_{21})^2 + [(a_{22})^2]\}}$ which simplifies to $r_A = a_{21} / a_{11}$
- $r_D = d_{11} \times d_{21} / \sqrt{\{(d_{11})^2 \times [(d_{21})^2 + [(d_{22})^2]\}}$ which simplifies to $r_D = d_{21} / d_{11}$.

This implies that the additive genetic correlation equals the dominant genetic correlation.

Appendix III: Sample size (in subjects) needed to detect *additive genetic influences* in full univariate ACE models under varying levels of variation due to common environmental sources for three different sibshipsizes.

	V_a = 10%			V_a = 20%			V_a = 30%			V_a = 40%			V_a = 50%			V_a = 60%			V_a = 70%			V_a = 80%		V_a = 90%
$V_c \rightarrow$	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	0%
design 1	24896	23084	20110	5908	5230	4332	2406	2026	1588	1192	950	700	644	482	328	360	248	150	198	124	60	104	52	48
design 2	22047	19557	16365	5151	4395	3540	2079	1707	1320	1032	813	600	567	426	294	324	228	144	186	120	63	105	57	51
design 3	26836	23560	19460	6256	5280	4208	2520	2048	1572	1252	976	716	688	512	356	396	280	176	228	148	80	132	72	68

Note: MZ/DZ ratio = 1/1, significance level $\alpha = .05$, power $(1-\beta) = .80$, design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings. To calculate the total number of families needed, all cells from design 1 need to be divided by 2, all cells from design 2 need to be divided by 3, and all cells from design 3 need to be divided by 4. See also Figures 3.2 a, b and c.

Appendix IV: Sample size (in subjects) needed to detect *common environmental influences* in full univariate ACE models under varying levels of variation due to additive genetic sources for three different sibshipsizes.

	V_c = 10%			V_c = 20%			V_c = 30%			V_c = 40%			V_c = 50%			V_c = 60%			V_c = 70%			V_c = 80%		V_c = 90%
$V_a \rightarrow$	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	20%	0%	10%	0%
design 1	15504	14860	13934	3646	3398	3104	1468	1334	1190	722	640	560	390	340	290	222	188	156	126	104	84	70	54	36
design 2	8220	7746	7140	1860	1704	1542	720	651	579	345	309	273	186	162	144	105	90	78	60	51	45	36	27	18
design 3	8220	7628	6908	1808	1632	1448	684	608	536	320	284	248	148	148	128	96	84	72	56	48	40	32	24	16

Note: MZ/DZ ratio = 1/1, significance level $\alpha = .05$, power $(1-\beta) = .80$, design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings. To calculate the total number of families needed, all cells from design 1 need to be divided by 2, all cells from design 2 need to be divided by 3, and all cells from design 3 need to be divided by 4. See also Figures 3.3 a, b and c.

Appendix V: Sample size (in subjects) needed to detect *common environmental influences* in full univariate ACE models under varying levels of variation due to additive genetic sources in the realistic situation that sources of variation due to A are larger than sources of variation due to C for three different sibshipsizes.

	V_a = 20%		V_a = 30%		V_a = 40%		V_a = 50%		V_a = 60%		V_a = 70%		V_a = 80%
V_c →	10%	20%	10%	20%	10%	20%	10%	20%	10%	20%	10%	20%	10%
design 1	13940	3104	12806	2786	11558	2466	10280	2158	9042	1876	7912	1628	6934
design 2	7143	1542	6471	1377	5790	1224	5151	1089	4590	978	4137	894	3822
design 3	6912	1448	6148	1276	5408	1120	4736	988	4172	884	3748	816	3496

Note: MZ/DZ ratio = 1/1, significance level α = .05, power (1-β) = .80, design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings. To calculate the total number of families needed, all cells from design 1 need to be divided by 2, all cells from design 2 need to be divided by 3, and all cells from design 3 need to be divided by 4. See also Figure 3.4.

Appendix VI:
Samplesize (in subjects) needed to detect *additive genetic and dominance influences* in ADE-models.

	V_a = 20% V_d = 10%		V_a = 30% V_d = 15%		V_a = 40% V_d = 20%		V_a = 50% V_d = 25%		V_a = 60% V_d = 30%	
Effect detected→	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d	V_a & V_d	V_d
design 1	148	22808	76	11036	42	5958	42	5958	22	3518
design 2	156	11790	84	5631	48	3081	48	3081	27	1950
design 3	148	11328	84	5236	48	2784	48	2784	28	1776

Note: MZ/DZ ratio = 1/1, significance level α = .05, power (1-β) = .80, design 1 = twins only, design 2 = twins and one additional sibling, design 3 = twins and two additional siblings. To calculate the total number of families needed, all cells from design 1 need to be divided by 2, all cells from design 2 need to be divided by 3, and all cells from design 3 need to be divided by 4. See also Figures 3.5 a, and b.

Appendix VII: Mx job for combined linkage and association analyses, *parental genotypes unavailable*

```
!Fulker et al.(1999) method
!Extended to sibship sizes > 2,
!Additive and dominance association
!Multiple alleles
!Using sibling genotypes to calculate mean genotypic value of sibship

#define n 5                    !number of alleles is 5
#define nvar 1                 !univariate
#define nsibs 3                !sibshipsize = 3
#ngroups 4                     !one precalculation, one data group, two constraint

G1: calculation group between and within effects
Data Calc
  Begin matrices;
  A Full 1 n  free             !will contain additive allelic effects within
  C Full 1 n  free             !will contain additive allelic effects between
  D Sdiag n n free             !will contain dominance deviations within
  F Sdiag n n free             !will contain dominance deviations between
  I Unit 1 n                   !unit vector to multiply allelic effects [1 1 1 1 1]
 End matrices;

 Begin algebra;
  K = (A'@I) + (A@I') ;
  L = D + D' ;
  W= K+L ;
  M = (C'@I) + (C@I') ;
  N = D + D' ;
  B= M+N ;
 End algebra ;
st .2 all
end

G2: datagroup: sibship size three
  Data NInput=12
  Missing =-99.00
  Rectangular File=myfile.dat
  Labels ph1 ph2 ph3 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23
  Select ph1 ph2 ph3 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
        !selects 3 phenotypes; one for each sib
        !selects 6 allele variables, a1s1 is allele #1 from sib #1
        !selects pi's and z's
  Definition_variables
        a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
        !declare the allele variables, pi and z as definition variables
 Begin Matrices;
  F Lower nvar nvar Free        !familial variance
  Q Lower nvar nvar Free        !QTL additive variance
  R Lower nvar nvar Free        !QTL dominance variance
  E Lower nvar nvar Free        !non-shared environmental variance
  B Computed n n  = B1          !spurious and genuine genotypic effects
  W Computed n n  = W1          !genuine genotypic effects
  I Ident nsibs nsibs Fix!
  P Sym nsibs nsibs Fix         !To contain pi-hats
  Z Sym nsibs nsibs Fix         !To contain pIBD2's
  T Stand nsibs nsibs Fix
  K Full 1 4 Fix                !First  and second allele of sib1
  L Full 1 4 Fix                !First  and second allele of sib2
  M Full 1 4 Fix                !First  and second allele of sib3
  S Full 1 1 Fix                !to contain nsibs
  G Full 1 nvar Free            !grand mean
 End Matrices;

 Matrix S 3                     ! sibship size 3
 Matrix K 1 1 1 1
 Matrix L 1 1 1 1
 Matrix M 1 1 1 1
```



```
Matrix P
        0
        1 0
        1 1 0
Matrix Z
        0
        1 0
        1 1 0
 Specify K a1s1 a2s1 a1s1 a2s1    !genotype sib1 to be used for \part
 Specify L a1s2 a2s2 a1s2 a2s2    !genotype sib2 to be used for \part
 Specify M a1s3 a2s3 a1s3 a2s3    !genotype sib3 to be used for \part
 Specify P 1
          pi12 1
          pi13 pi23 1
 Specify Z 1
          z12 1
          z13 z23 1
 Specify T .5                     !when familial variance is modelled as add genetic
          .5 .5

 Begin Algebra;
  V = (\part(B,K) + \part(B,L) + \part(B,M) ) % S ; !"B"
  D = (\part(W,K) + \part(W,L) + \part(W,M) ) % S ; !used for deviation: W
 End Algebra;

 Means G+V+(\part(W,K)-D)|G+V+(\part(W,L)-D)|G+V+(\part(W,M)-D);
 Covariance T@(F*F') + P@(Q*Q') + Z@(R*R') + I@(E*E') ;

 End

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
  A full 1 n = A1          !
  0 zero 1 1
End Matrices;
Begin algebra;
  B = \sum(A) ;
End Algebra;
Constraint 0 = B ;
end

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
 C full 1 n = C1 !
 0 zero 1 1
End Matrices;
Begin algebra;
 B = \sum(C) ;
End Algebra;
Constraint 0 = B ;
option multiple issat            !this is saturated model for submodel comparison
end

save full.mxs

!test for spurious association W=B
Specify 1 A 101 102 103 104 105
Specify 1 C 101 102 103 104 205   !first 4 equal to within; last unequal but because
                                  !of second constrain 205 will be equal to 105
Specify 1 D 801 802 803 804 805 806 807 808 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
end

!Drop dominance : non-conservative test (i.e. genuine and spurious)
Specify 1 D 801 802 803 804 805 806 807 808 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
Drop @0 801 802 803 804 805 806 807 808 809 810
end
```

```
!Drop all allelic effects : non-conservative test (i.e. genuine and spurious)
Specify 1 A 101 102 103 104 105
Specify 1 C 101 102 103 104 205
Specify 1 D 801 802 803 804 805 806 807 808 809 810
Specify 1 F 801 802 803 804 805 806 807 808 809 810
Drop @0 101 102 103 104 105 801 802 803 804 805 806 807 808 809 810
end

get full.mxs

!drop QTL linkage effect while keeping association effects in the model
Drop Q 2 1 1 !QTL additive variance
Drop R 2 1 1 !QTL dominance variance
end
```


Appendix VIII: Mx job for the conduction of the combined linkage and association method, *parental genotypes available.*

```
!Fulker et al.(1999) method
!Extended to sibship sizes > 2,
!Additive and dominance association
!Multiple alleles
!Using parental genotypes to calculate mean genotypic value of sibship

#define n 5                     !number of alleles is 5
#define nvar 1                  !univariate
#define nsibs 3                 !sibshipsize = 3
#ngroups 4                      !one precalculation, one group, two constraint

G1: calculation group between and within effects
Data Calc
 Begin matrices;                !start declaration of matrices
  A Full 1 n  free              !will contain additive allelic effects within
  C Full 1 n  free              !will contain additive allelic effects between
  D Sdiag n n free              !will contain dominance deviations within
  F Sdiag n n free              !will contain dominance deviations between
  I Unit 1 n                    !unit vector to multiply allelic effects [1 1 1 1 1]
 End matrices;
Begin algebra;
  K = (A'@I) + (A@I') ;
  L = D + D' ;
  W= K+L ;
  M = (C'@I) + (C@I') ;
  N = F + F' ;
  B= M+N ;
 End algebra ;
st .2 all
end

G2: datagroup: sibship size three
  Data NInput=12
  Missing =-99.00
  Rectangular File=myfile.dat
  Labels ph1 ph2 ph3 a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23
        z12 z13 z23
  Select ph1 ph2 ph3 a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23
        z12 z13 z23;
        !selects 3 phenotypes; one for each sib
        !selects 6 allele variables for sibs, a1s1 is allele #1 from sib #1
        !selects 4 allele variables for parents a1p1 is allele #1 parent #1
        !selects pi's and z's
  Definition_variables
        a1p1 a2p1 a1p2 a2p2 a1s1 a2s1 a1s2 a2s2 a1s3 a2s3 pi12 pi13 pi23 z12 z13 z23;
        !declare the allele variables, pi and z as definition variables
 Begin Matrices;
  F Lower nvar nvar Free        !familial variance
  Q Lower nvar nvar Free        !QTL additive variance
  R Lower nvar nvar Free        !QTL dominance variance
  E Lower nvar nvar Free        !non-shared environmental variance
  B Computed n n  = B1          !spurious and genuine genotypic effects
  W Computed n n  = W1          !genuine genotypic effects
  I Ident nsibs nsibs Fix       !To multiply E
  P Sym nsibs nsibs Fix         !To contain pi-hats and to multiply Q
  Z Sym nsibs nsibs Fix         !To contain pIBD2's and to multiply R
  T Stand nsibs nsibs Fix       !To multiply F
  K Full 1 4 Fix                !First  and second allele of sib1
  L Full 1 4 Fix                !First  and second allele of sib2
  M Full 1 4 Fix                !First  and second allele of sib3
  N Full 1 4 Fix                !a1p1 a1p2
  O Full 1 4 Fix                !a1p1 a2p2
  X Full 1 4 Fix                !a2p1 a1p2
  Y Full 1 4 Fix                !a2p1 a2p2
  S Full 1 1 Fix                !to contain 4: maximum of 4 possible genetically
                                !different offspring
  G Full 1 nvar Free            !grand mean
```

```
End Matrices;
Matrix S 4
Matrix K 1 1 1 1
Matrix L 1 1 1 1
Matrix M 1 1 1 1
Matrix N 1 1 1 1
Matrix O 1 1 1 1
Matrix X 1 1 1 1
Matrix Y 1 1 1 1
Matrix P
       0
       1 0
       1 1 0
Matrix Z
       0
       1 0
       1 1 0
Specify K a1s1 a2s1 a1s1 a2s1     !genotype sib1
Specify L a1s2 a2s2 a1s2 a2s2     !genotype sib2
Specify M a1s3 a2s3 a1s3 a2s3     !genotype sib3
Specify N a1p1 a1p2 a1p1 a1p2     !parental alleles
Specify O a1p1 a2p2 a1p1 a2p2     !parental alleles
Specify X a2p1 a1p2 a2p1 a1p2     !parental alleles
Specify Y a2p1 a2p2 a2p1 a2p2     !parental alleles
Specify P 1
         pi12 1
         pi13 pi23 1
Specify Z 1
         z12 1
         z13 z23 1
Specify T .5                      !when familial variance is modelled as add genetic
         .5 .5

Begin Algebra;
 V = (\part(B,N) + \part(B,O) + \part(B,X) + \part(B,Y)) % S ; !Between effects
 D = (\part(W,N) + \part(W,O) + \part(W,X) + \part(W,Y)) % S ; !for Within effects
End Algebra;

Means G+V+(\part(W,K)-D)|G+V+(\part(W,L)-D)|G+V+(\part(W,M)-D);
Covariance T@(F*F') + P@(Q*Q') + Z@(R*R') + I@(E*E') ;

End

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
  A full 1 n = A1
  O zero 1 1
End Matrices;
Begin algebra;
  B = \sum(A) ;
End Algebra;
Constraint O = B ;
end

Constrain sum allelic effects = 0
Constraint ni=1
Begin Matrices;
 C full 1 n = C1
 O zero 1 1
End Matrices;
Begin algebra;
 B = \sum(C) ;
End Algebra;
Constraint O = B ;
end
```


Appendix IX: Overview of multivariate genetic studies relating behavioural and neurophysiological indices of brain structure / function to measures of cognitive abilities. List is restricted to neurophsyiological indices that have been described in this thesis

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Brain size / volume								
Posthuma et al., 2002a	24 MZ 31 DZ 25 additional siblings	Cerebral gray matter volume (GMV) Cerebral white matter volume (WMV) Cerebellar volume (CV)	WAISIIIR (FSIQ, VC, WM, PO, PS)	Multivariate genetic model fitting (including age and sex correction on means)	GMV 82% WMV 87% CV 87%	VC 84% WM 65% PO 68% PS 63%	VC – GMV/WMV/CV: n.s./n.s./n.s. WM – GMV/WMV/CV 0.27/0.28/0.27 PO – GMV/WMV/CV 0.20/n.s./0.18 PS– GMV/WMV/CV: n.s./0.25/ns	100%, except for PO and CV (66%)
Thompson et al., 2001	10 MZ 10 DZ	Voxel based gray matter density	Spearman's 'g' (derived from Vocabulary, Block Design, Digit Symbol subtetst of WAIS-R)	Intraclass correlations	80-90%	70%	0.45 (twin 1) 0.37 (twin 2)	Not calculated, suggestive evidence of genetic mediation
Pennington et al., 2000	Con- or discordant for reading disorder / control 25 / 9 MZ 23 / 9 DZ	Brain volumes measured with Magnetic Resonance Imaging	WISC WAIS-3R	Correlational study	MZ correlations (0.78 – 0.98) > DZ correlations (0.32 – 0.65) in both samples	not reported	Reading disorder / healthy sample 0.42 / 0.31	Genetic contribution not reported, cannot be derived. But genetic correlation 0.48 (both samples combined),

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Wickett et al., 1997	68 adult males (34 sibships)	Total brain volume measured with Magnetic Resonance Imaging	MAB Trail Making Test Factor Referenced Tests	Correlation within families compared with correlation between families	not reported	not reported	Correlation IQ-brain volume within families 0.24; between families 0.50	Suggestive of genetic mediation
EEG- alpha peak frequency								
Posthuma et al., 2001b	Crossectional, mean age 26 / 50: 47 / 44 MZ 253 / 192 DZ and sibpairs	Individual alpha peak during resting condition	WAIS-IIIR – dimensions (VC, WM. PO, PS)	Multivariate genetic model fitting (including age and sex correction on means)	71% aged 26 83% aged 50	Aged 26/aged 50: VC 83% / 83% WM 71% / 67% PO 68% / 68% PS 66% / 66%	no correlation	-
Inspection Time								
Luciano et al., 2001a	184 MZ 206 DZ age 16 years	Inspection time (pi-paradigm)	MAB	Multivariate genetic model fitting	36%	VIQ 81% PIQ 73%	-0.26 with VIQ -0.35 with PIQ	100%; Genetic correlations –0.47 with VIQ –0.65 with PIQ
Posthuma et al., 2001a	Crossectional, mean age 26 / 50: 47 / 44 MZ 253 / 192 DZ and sibpairs	Inspection time (pi-paradigm)	WAIS-3R	Multivariate genetic model fitting (including age and sex correction on means)	46%	VIQ 85% PIQ 69%	-0.19 with VIQ -0.27 with PIQ	100% Genetic correlations –0.31 with VIQ –0.47 with PIQ

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Latency of premotor and motor response selection activation								
Posthuma et al., 2002b	Crossectional, mean age 26 / 50: 47 / 44 MZ 253 / 192 DZ and sibpairs	Onset and peak latency (PL) of the LRP in congruent condition	WAIS-IIIR	Uni- and multivariate genetic model fitting (including age and sex correction on means)	Young/Older Onset-Congruent 65%/0% PL-Congruent 39%/39%	VIQ 85% PIQ 69%	No correlation	-.
Reaction times/speeded responses								
Posthuma et al., 2002	Crossectional, mean age 26 / 50: 47 / 44 MZ 253 / 192 DZ and sibpairs	Decision time in Flanker Task (congruent condition)	WAIS-IIIR	Uni- and multivariate genetic model fitting (including age and sex correction on means)	Young/Older Dec-Congruent 43%/43%	VIQ 85% PIQ 69%	No correlation	-.
Luciano et al. 2001b	166 MZ 190 DZ	Choice Reaction Time tasks: 2-choice (2crt), 4-choice (4crt), 8 choice (8crt)	MAB -FSIQ	Multivariate genetic model fitting	2crt 52% 4crt 59% 8crt 70%	81%	Mean of males and females) FSIQ-2crt -0.32 FSIQ 4crt –0.55 FSIQ 8crt -0.48	Genetic contributions: FSIQ-2crt -0.32 100%) FSIQ 4crt -0.49 (89%) FSIQ 8crt -0.47 (100%)
Neubauer et al. 2000	169 MZ 131 DZ	Sternbergs Memory Scanning Posner's Letter Matching	RAVEN Leistungs Pruf System	Multivariate genetic model fitting	11 - 61%	39 - 81%	-0.08 to -0.50 (More difficult tasks -> higher correlation)	65%

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Finkel and Pedersen, 2000	45 MZA 67 MZT 94 DZA 86 DZT	Oral version of Digit Symbol and Figure Identification subtest ('perceptual speed')	Cognitive factor constructed from 11 cognitive measures (a.o. WAIS, Thurstone's Picture Memory, Card Rotations)	Multivariate genetic model fitting	56%	cognitive factor 61% (not age corrected) 85% (age corrected)	0.66	61%
Rijsdijk et al., 1998	82 MZ 109 DZ age 16	Donders simple/two choice reaction time Sternbergs Memory Scanning Posner's Letter Matching	RAVEN - Advanced Progressive Matrices	Multivariate genetic model fitting	40 – 58%	58%	around -0.20	Genetic correlations around –0.40
Rijsdijk et al., 1998	74 MZ 100 DZ age 18	Donders simple/two choice reaction time Sternbergs Memory Scanning Posner's Letter Matching	WAIS	Multivariate genetic model fitting	22-57%	33- 74%	-0.14 to -0.28	Genetic correlations – 0.46 and –0.42
Baker et al., 1991	50 MZ 32 SS DZ ages 15-57	Battery of eight different reaction time tasks	MAB	Multivariate genetic model fitting	45%	68-85%	-0.59	Genetic correlations - 0.92 – -1.00

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Ho et al. 1988	30 MZ 30 SS DZ ages 8-18	Rapid Automatic Naming tests Colorado Perceptual Speed test	WISC-R WAIS-R	Multivariate genetic model fitting, using IQ score and composite scores of reaction time tests	49-52%	78%	0.37 - 0.42	70 - 100% of the phenotypic correlation is due to common genetic factors
Indices of components of working memory								
Posthuma et al., 2002	Crossectional, mean age 26 / 50: 47 / 44 MZ 253 / 192 DZ and sibpairs	Onset, peak latency (PL), decision time and performance derived from the incongruent condition of the Flanker task	WAIS-IIIR	Uni- and multivariate genetic model fitting (including age and sex correction on means)	Young/Older Onset0%/0% PL 0%/45% Dec 48%/48% Perfomance 54% / 41%	VIQ 85% PIQ 69%	0.24 – 0.43	100%
Wright et al., 2002	218 MZ 256 DZ (including sibpairs)	DR accuracy	MAB	Multivariate genetic model fitting (including educational level and sex correction on means)	45%	81%	0.22	100%

Study	Subjects	Behavioural or Neuro-physiological measure	Cognitive measure	Statistical method	Heritability of (neuro-) physiological measure	Heritability of cognitive measure	Phenotypic correlation	Percentage of the phenotypic correlation explained by genetic contribution. (genetic correlation also provided, if reported)
Luciano et al., 2001	166 MZ 190 DZ	DR accuracy	MAB	Multivariate genetic model fitting (including educational level and sex correction on means)	48%	81%	0.26 (females) 0.13 (males)	100%
Ando et al., 2001	143 MZ 93 DZ aged 16 – 29 yrs.	Performance on spatial and verbal WM span task, tapping storage and efficacy (Ss Se Vs Ve)	Kyodai Japanese IQ test – SCA (spatial cognitive ability) and VCA (verbal cognitive ability)	Multivariate genetic model fitting	Ss 45% Se 49% Vs 48% Ve 43%	SCA 65% VCA 65%	SCA with WM performance: 0.34 – 0.44 VCA with WM performance :0.26 – 0.37	Genetic contributions (0.36 – 0.38 for SCA and WM performances and 0.23-0.24 for VCA and WM performances) predominantly (around 85%) explain phenotypic correlations

Note for Appendix IX
MZ = monozygotic twins ; DZ = dizygotic twins ; MZA = MZ raised apart ; MZT = MZ raised together; DZA = DZ raised apart ; DZT = DZ raised together ; SS = same sex; WAIS = Wechsler Adult Intelligence Scale ; WISC = Wechsler Intelligence Scale for Children ; RAVEN = Raven's Advanced Progressive Matrices ; MAB = Multidimensional Aptitude Battery ; RAKIT = Revisie Amsterdamse Kinder Intelligentie Test ; VIQ = verbal IQ ; PIQ = performance IQ; P3A-f = P3 amplitude frontal; P3A-p = P3 amplitude parietal; P3L-f= P3 latency frontal; P3L-p = P3 latency parietal; PL = peak latency; Ss = Spatial storage; Se = Spatial executive; Vs = Verbal storage; Ve = Verbal executive; SCA = Spatial Cognitive Ability; VCA = Verbal Cognitive Ability; DR = Delayed Response; PL = Peak Latency; Dec = decision time; LRP – Lateralized Readiness Potential; VC = Vrebal Comprehension; WM = Working Memory; PO = Perceptual Organization; PS = Processing Speed; GMV = Grey Matter Volume of the cerebrum; WMV = White Matter Volume of the cerebrum; CV = Cerebellar volume.

List of Publications

Baaré WFC, Hulshoff Pol HE, Boomsma DI, Posthuma D, de Geus EJC, Schnack HG, van Haren NE, van Oel CJ, Kahn RS. (2001). Quantitative genetic modeling of human brain morphology variation. Cerebral Cortex, 11, 816-824.

Boomsma DI, Vink JM, van Beijsterveldt CEM, de Geus EJC, Beem AL, Mulder EJCM, Riese H, Willemsen AHM, Bartels M, van den Berg M, Derks EM, de Graaff SC, Kupper HM, Polderman JC, Posthuma D, Rietveld MJH, Stubbe JH, Knol LI, Stroet Th, van Baal GCM (2002). Netherlands Twin Register, *Twin Research,* submitted.

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