Summary

In order to identify common DNA polymorphisms explaining variation in cognitive ability, a family-based association study was conducted among a set of putative candidate genes (see Table 1.1 in Chapter 1). The use of two age-dependent cohorts allowed us to detect genetic effects exerted by genetic variants located on putative candidate genes, and more specifically, those polymorphisms that might be involved in variation across different stages of life. Finally, the results of the present work as well as the possibilities of further research are discussed.

Sample description

For this thesis, data from two different age cohorts was available: a young cohort (Polderman *et al., 2006a; 2006b)* and an adult cohort (Posthuma *et al., 2005)*. Both cohorts consist of twins and their siblings and were recruited from the Netherlands twin Register (Boomsma et al. 2006; Boomsma *et al*. 2002) (see Table 8.1). Participation in this study included a voluntary agreement to provide buccal swabs for DNA extraction. For all same-sex twin pairs, zygosity was assessed using 11 polymorphic microsatellite markers (Heterozygosity > 0.80). Genotyping was performed blind to familial status and phenotypic data.

Table 8.1 Zygosity status among individuals for young and adult cohort participating in this study

		Young Cohort		Adult Cohort	
		Families	Subjects	Families	Subjects
MZM	twin pair	35	80	25	66
	single twin	-	-	15	17
MZF	twin pair	28	65	20	46
	single twin	-	-	15	16
DZM	twin pair	48	116	15	40
	single twin	-	-	14	17
DZF	twin pair	23	47	28	71
	single twin	-	-	28	45
DOS	twin pair	26	60	23	59
	single twin	-	-	-	-
Single twin		3	3	12	14
Total cohort		163	371	195	391

IQ Phenotypes

The WISC-R (1986) (Wechsler 1986) was used to assess psychometric IQ in the young cohort. Six subtests were used: similarities, arithmetic, vocabulary, digit span, object assembly and block design. Psychometric IQ within the adult cohort was assessed with the Dutch version of the WAIS-III (1997) (Wechsler 1997). Eleven subtests were used:

Information, Similarities, Arithmetic, Vocabulary, Digit –symbol pairing, Digit-symbol coding, Digit-symbol free recall, Picture completion, Block design, Matrix reasoning and Letter-number sequencing (see Table 8.2a). Heritability estimates for Full Scale IQ (FSIQ), Verbal IQ (VIQ) and Performance IQ (PIQ) are given in Table 8.2b. These heritability estimates are comparable to those reported previously for the young and adult cohorts in the Dutch population (Bartels *et al*. 2002; Posthuma *et al*. 2001).

Table 8.2a Subtests comprising the WAIS[A] and WISC[B]

		Subtest	Description
Verbal	1	Information [A]	General factual knowledge, long term memory
	2	Similarities [A, B]	Abstract reasoning, categories, relationships
	3	Arithmetic [A, B]	Attention, concentration, numerical reasoning
	4	Vocabulary [A,, B]	Word knowledge, verbal fluency
	5	Comprehension [A]	Social judgment, common sense reasoning
	6	Digit Span [A, B]	Short term auditory memory, concentration
Performance	7	Picture Completion [A]	Alertness to essential detail
	8	Coding [A]	Visual motor co-ordination, speed, concentration
	9	Picture Arrangement [A]	Sequential, logical thinking
	10	Block Design [B]	Spatial, abstract visual problem solving
	11	Object Assembly [B]	Visual analysis, construction of objects
	12	Symbol Search [A]	Speed of processing novel information
	13	Mazes [A]	Fine motor co-ordination, planning, following directions

Table 8.2b Heritability estimates (h^2) for Full-Scaled IQ (FSIQ), Verbal IQ (VIQ), and Performance IQ (PIQ) for young and adult cohorts

Cognitive Phenotypes (subtests)	Subtests	Heritability estimates (h^2)
FSIQ Young	2, 3, 4, 6, 10, 11	0.80 (0.72-0.85)
FSIQ Adult	1, 2, 3, 4, 10, 12, 13	0.78 (0.72-0.83)
VIQ Young	2, 3, 4, 6	0.70 (0.59-0.78)
VIQ Adult	1, 2, 3, 4	0.78 (0.72-0.83)
PIQ Young	10, 11	0.73 (0.63-0.80)
PIQ Adult	10,12,13	0.71 (0.62-0.77)

The Muscarinic acetylcholine receptor type 2 (CHRM2) gene and variation in cognitive ability

Muscarinic acetylcholine receptors (mAChR) belong to a group of seven transmembrane-spanning receptors that includes the adrenergic receptors, whose signals are transduced across membranes via interaction with GTP-binding proteins. Several macromolecular interactions are involved in the response triggered by activation of muscarinic receptors (Hulme 1990), ranging from inhibition of adenylyl cyclase, stimulation of phosphoinositide hydrolysis and

regulation of potassium channels (Caulfield 1993). It is also known that promoters of neuronal proteins contain consensus sequences for various transcription factors whose expression is increased by mAChR activation in different neuronal cells (Nitsch *et al*. 1998; Von der Kammer *et al*. 1999). Genotypic variation within the muscarinic acetylcholine receptors type 2 (*CHRM2*) gene was investigated in relation to variation in cognitive phenotypes (chapters 2 and 3). What makes this putative candidate gene particularly interesting is its autoreceptor activity at brain structures such as the hippocampal formation, which is fundamentally related to memory and learning processes (Iannazzo & Majewski 2000). The *CHRM2* gene may be involved in fine-tuning of feedback inhibition after memory formation (Miranda *et al*. 2000; Orsetti *et al*. 1996).

The first putative region we found associated with IQ variation (i.e. Performance IQ (PIQ): χ^2=9.14, *P=0.003;* 6.89 IQ points increase in PIQ) among the adult cohort is located in intron 4. The second region encompassing intron 5 – the last intron before the coding sequence – was found associated with variation in IQ (i.e. Full scaled IQ (FIQ): χ^2=7.14, *P=0.008;* 5.35 IQ points increase in FIQ, Verbal IQ (VIQ): χ^2=9.50, *P=0.002;* 5.30 IQ points increase in VIQ) among the young cohort. Although preliminary expression analysis of *CHRM2* in relation to genotypic variation did not reveal differential transcript expression, our association results confirmed previous independent results that have evidenced a putative role of the *CHRM2* gene in cognition (Comings *et al*. 2003; Dick *et al*. 2006; Dick *et al*. 2007).

Because association results were found among young and adult cohorts in different regions of the *CHRM2* gene; one could propose that both associated regions contain regulatory elements that are differently used during early and adult life. *How can these differential associations can fit in a plausible biological model ?* A large body of literature has shown the importance of choline availability during brain development, and more specifically during hippocampal development (for review see Blusztajn and Wurtman 1983, Glenn *et al*., 2007; Mellot *et at.,* 2007). In line with this, Cermak and colleagues (Cermak *et al.* 1998; Cermak *et al.,* 1999) reported a significant association between choline availability and performance in hippocampal-related tasks. Moreover, the rate of Ach turnover (i.e. synthesis, degradation and choline utilization) underlying this association seemed to be programmed early in life in relation to the amounts of Ach availability during critical developmental stages. For example, the hippocampus of prenatally choline-deficient animals is characterized by accelerated Ach turnover, possibly indicating an adaptative response to the reduce availability of choline *in utero* (Cermak *et al.,* 1998). Such a "metabolic imprinting" hypothesis, is attractive from the

epigenetic point of view since it takes into account environmental factors occurring prenatally that may explain the lack of replication of association studies when only genetic factors are considered (Niculescu *et al.* 2006).

The role of the synaptosomal protein of 25 kDa (SNAP-25) gene in synaptic plasticity underlying cognitive ability

The synaptosomal protein of 25 kDa (*SNAP-25*) gene was one of the putative candidate genes that was investigated in the present study (chapters 4 and 5). Several studies have associated this gene to a wide range of cognitive impairment disorders, ranging from ADHD phenotypes to schizophrenia. The activity of *SNAP-25* is exerted across different organs and tissues. At the brain level, its expression seems to be critical in the hippocampus, in relation to long term potentiation (LTP) and memory consolidation (Hou *et al*. 2004; Hou *et al*. 2006). Whitin the brain alternative splicing of exon 5 gives rise to two protein isoforms: *SNAP-25a* and *SNAP-25*b (Bark & Wilson 1991). During development, the *SNAP-25*a isoform is known to be the main isoform present, which, in turn, is involved in synaptogenesis. In the adult brain, however, *SNAP-25b* is the predominant isoform which forms a fusion machinery complex (SNARE) together with syntaxin and the synaptic vesicle proteins (synaptobrevin and synaptotagmin). This complex mediates exocytosis of neurotransmitters from the synaptic vesicle into the synaptic cleft. These isoforms are fundamental for keeping a balanced trade-off between synaptic formation and neurotransmitter vesicle release. Our results (chapters 4 and 5) showed genomic variation in intron 1 of the *SNAP-25* gene to be associated with variation in IQ phenotypes (i.e. Full scaled IQ (FIQ): χ^2= 15.99 *P*= 0.0001, 3.28 IQ points increase in FIQ, Verbal IQ (VIQ): χ^2= 13.01 *P*= 0.0003, 2.76 IQ points increase in VIQ, and Performance IQ (PIQ): χ^2= 11.22 *P*= 0.0008, 3.21 IQ points increase in PIQ).

These reported genetic (non)coding variants present in intron 1 might be involved in regulation of protein isoform expression, since all associated SNPs were located in putative transcription factor binding sites (TFBS). In line with the idea that quantitative trait loci (QTL) with small effect sizes can be expected to explain complex traits, it is likely that (non)coding variants affecting regulatory sequences (e.g. TFBS, promoters, enhancers) may regulate gene expression and function in a more subtle way than do polymorphisms in coding regions. Although the associated variants reported could indeed be the biologically relevant variants, another possibility is that they might be associated variants, in high LD with the

causal variant(s). Therefore, further functional studies are required in order to corroborate these polymorphisms as functional variants underlying variation within cognitive phenotypes.

Functional non-synonymous polymorphisms under positive evolutionary selection underlying phenotypic differences in cognitive abilities

Using a comparative genomics approach, a two stage design was proposed in which genes ascertained for enhanced protein evolution in primates were subsequently searched for the presence of non-synonymous coding SNPs in the current human population at amino acid sites that differ between humans and chimpanzees (Chapter 6). In this study, as our primary interest was to find genes involved in phenotypic differences in cognitive abilities, we focused on genes expressed in the central nervous system (CNS). Because positively selected genes among primates are generally presumed to determine phenotypic differences between humans and non-human primates, amino acid substitutions segregating in humans at positively selected amino acid sites are expected to affect phenotypic differences among humans. After selecting candidate genes harboring such amino acid substitutions, an association study between cognitive ability and the β-2 adrenergic receptor (*ADRB2*) gene was performed. The β-adrenergic receptors belong to the G-protein-coupled receptor superfamily and mediate some of the physiological actions of catecholamines (noradrenaline and adrenaline) in a variety of tissues (Liggett 2000) via activation exerted by noradrenaline (NA) (Kobayashi & Kobayashi 2001).

Several lines of evidence postulate the *ADRB2* gene as having a fundamental role in memory and learning formation. Hippocampal long-term potentiation (LTP) has been shown to increase after the activation of the β-2 receptor, that in turn, induces LTP in neurons in the hippocampus (Hillman *et al*. 2005). In line with this, the use of β2- agonists (Gibbs & Summers 2000) has been shown to increase performance related to memory and learning tasks, while β2- antagonists have been shown to impair memory consolidation (Gibbs & Summers 2005). Two non-synonymous coding SNPs under positive selection (rs1042713 and rs1042714) in the β-2 adrenergic receptor were selected to conduct a genetic association study for IQ in two independent family-based Dutch cohorts. Interestingly, the derived, human-specific, allele of the beta-2 adrenergic receptor (rs1042713) conferred an 8 IQ point increase in verbal IQ. However, a word of caution must be given due to possible inflation of the estimated genetic effect size due to the relatively small sample size.

Positive heterosis and gene-gene interaction on working memory functioning

In chapter 7, we tested the association of the *Catechol-O-methyl transferase (COMT)* Val$^{108/158}$Met polymorphism with working memory (WM) performance. The association reflected positive heterosis such that the Met/Val heterozygotes performed better than each homozygous counterparts on the WM tasks. The association was found in the adult but not in the young cohort. Interestingly, an age-dependent positive heterosis pattern has previously been reported in a longitudinal study by Harris and colleagues (Harris *et al*. 2005). Because the *COMT* polymorphism has been hypothesized to have a non-linear effect on DA availability in the prefrontal cortex (PFC) (Mattay *et al*. 2003), the finding of heterosis is in keeping with the idea that the relationship between DA signalling and cognitive performance follows an inverted U-shaped curve, with both suboptimal and supra-optimal DA activity impairing prefrontal function (Cools & Robbins 2004). Nevertheless, DA activity is not only dependent on its catabolism rate (*COMT*) but also on the presence of DA receptors. In line with this, cognitive performance may be also critically dependent on the *D1/D2* binding ratio, with a relative lack of *D1*-signalling causing impulsivity, distractibility and poor working memory performance with schizophrenia at the extreme end (Winterer *et al*. 2004). On the other hand, a relative lack of D2-signalling may fail to signal the presence of reward information, a signal that is required to engage the PFC in updating its working memory system (Weinberger *et al*. 2001). Consequently, individual differences in DA availability as well as *D2* receptor sensitivity may come into play during the performance of WM tasks. This expectation was tested in a secondary analysis in which a *DRD2* tagging SNP (rs2075654) was tested for an interactive effect with the *COMT* polymorphism. Our results suggest that the interaction is stronger in the adult than in the young cohort. Although p-values in neither cohort reach formal significance levels, this age difference may be real. Although no significant main effect on WM was found for the rs2075654 tag-SNP, either for the young cohort or for the adult cohort, the *DRD2* and *COMT* polymorphisms had a significant interactive effect on WM performance when both samples are combined. The interaction suggested that the Met/Val heterozygotes perform better than both Met/Met and Val/Val homozygotes only when they carry at least one A1 allele. Evidence for age-related changes regarding DA metabolism within the PFC has been reported in both animal (Lee *et al*. 2001) and human studies (Kaasinen & Rinne 2002), with increased DA metabolism (e.g *MAO, COMT*) thought to be present at a more mature age (Gottfries 1990) as well as a decrease of DA receptors with age (Suhara *et al*. 1991; Volkow *et al*. 1996; Wong *et al*. 1984). Such a pattern has been previously described by Reuter *et al.* (2005), who reported a significant

interactive effect between the *DRD2* Taq IA and the *COMT* polymorphisms on the amount of response interference in the Stroop color-word conflict task. Nevertheless, full genetic contribution to dopaminergic variation in frontal executive function will rely on far more complex interactions between multiple receptors (e.g *DRD1, DRD2, DRD4*), transporters and enzymatic polymorphisms (e.g. *DAT, COMT, MAO*) (Berman & Noble 1995; Bertolino *et al*. 2006; Tsai *et al*. 2002; Williams & Castner 2006). Further studies involving such interactions are needed in order to obtain a clearer overview of the dopaminergic pathway underlying working memory tasks.

Dopaminergic and serotonergic candidate genes

Common variants among other dopaminergic (*DBH, DRD2, DRD3*, and *TH*) and serotonergic (*HTR2A* and *SERT*) system were also investigated in the context of a family-based association study for cognitive ability. Since the preliminary results were not significant after multiple testing correction, no further investigation was performed within these candidate genes. It is worth noting that preliminary results based on these candidate genes may not be conclusive and a more extensive coverage of these genes may be required (see Tables in Appendix I).

In summary, two types of polymorphisms within a candidate-gene design were found associated with variation among cognitive ability phenotypes: coding or so-called functional polymorphisms (*COMT* and *ADRB2* genes) and non-coding polymorphisms (*CHRM2*, *SNAP-25,* and *DRD2* genes). While effects of the former type imply changes that can be observed at the protein level (i.e. enzymatic activity), the later type of polymorphisms should be considered as part of a wider range of regulatory elements, whose genetic contribution, although small in size, is necessary for the final phenotypic outcome. Future functional studies in combination with analysis of gene expression profiles at different brain regions may aid in understanding the role of genetic variants and their relation with synaptic plasticity underlying cognition, learning and attention traits.