A developmental perspective on the etiology of alcohol use and comorbid traits

Lot Geels

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A developmental perspective on the etiology of alcohol use and comorbid traits

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General introduction

General Introduction

Alcohol use is widespread in the Netherlands, with as much as 88% of the adult population having consumed alcohol in the past year (European Commission, 2010). The general drinking culture is characterized by frequent but moderate alcohol consumption, and public intoxication is not socially accepted. As in most western European countries, beer is the most preferred beverage, especially among men (Anderson et al., 2012; Room, 1992). Despite the general pattern of moderate drinking, problematic alcohol use is not uncommon in the Netherlands; the proportion of problem drinkers in the adult population is about 9%, and alcohol use disorders occur in 5% of men and 1% of women (Ouwehand et al., 2011; World Health Organization, 2011). In the literature, various forms of normative and problematic forms of alcohol use are described. Recent drinking refers to having consumed alcohol in the past month, which may be considered as an indicator of regular drinking (van Laar et al., 2010). Binge drinking is defined as having 5 or more alcoholic drinks at a single occasion and heavy drinking as binge drinking at least once a week (van Laar et al., 2011a; Verdurmen et al., 2012). Excessive drinking involves consuming more than 14 glasses of alcohol per week for women, and more than 21 glasses for men (Health Council of the Netherlands, 2006). Alcohol abuse is described in the DSM-IV as a maladaptive pattern of alcohol use with negative consequences, such as the inability to perform work- or family-related duties due to alcohol use. The criteria for alcohol dependence additionally involve aspects of increased tolerance to alcohol and withdrawal symptoms (American Psychiatric Association, 1994). Hazardous drinking is described by the World Health Organization (WHO) as a pattern of alcohol use that puts the drinker or his/her environment at increased risk for harmful consequences, without meeting the criteria of alcohol abuse or dependence (Babor et al., 2001).

Alcohol use patterns throughout the lifespan

Alcohol use is typically initiated in adolescence. Dutch laws regarding adolescent drinking are relatively lenient; the minimum legal age for buying soft alcoholic drinks (wine, beer, and distilled drinks containing less than 15% alcohol by volume) is 16 years, and for strong alcoholic drinks (distilled drinks with at least 15% alcohol by volume) 18 years (Ministry of Health Welfare & Sport, 2009). Before age 16, 72-85% of Dutch adolescents has used alcohol, and about 40% drank alcohol in the past month (Van Laar et al., 2011; Verdurmen et al., 2012). The prevalence of recent binge drinking (in the past month) is 30% among adolescents ages 12-18 years (Verdurmen et al., 2012). Heavy drinking occurs most often in young adulthood (18-24 years), with an incidence of 30% in

men and 12% in women (Van Laar et al., 2011). Among young adults, college students are at increased risk for alcohol abuse and alcohol-related problems, especially when they are members of a fraternity or sorority (Netherlands Institute on Mental Health and Addiction, 2009). Across adolescence and adulthood, the prevalence of binge drinking declines (from 37% to 27% between ages 15 - 64 years), while the prevalence of recent drinking increases from 72% to 79%. The average alcohol consumption among drinkers between ages 30-65 years varies between 1.1 and 1.5 glasses a day (Van Laar et al., 2011). Above age 65 years, the number of drinkers is relatively low, but in recent years, increases in frequency of alcohol use and alcohol use disorders have been observed in the elderly population, especially among women (Weingart, 2009; Zantinge & van Laar, 2011). Across all age groups, the prevalence of alcohol use is higher among men than women and men have higher incidence of recent drinking and binge drinking, but the gap between male and female alcohol use is narrowing (Van Laar et al., 2011; Verdurmen et al., 2012).

Genetic and environmental influences on alcohol use and comorbid traits

Just as patterns of alcohol use change across the lifespan, so does the etiology of alcohol use. Twin studies have demonstrated that the most striking changes in the genetic architecture of alcohol use occur in adolescence, as illustrated by Figure 1.

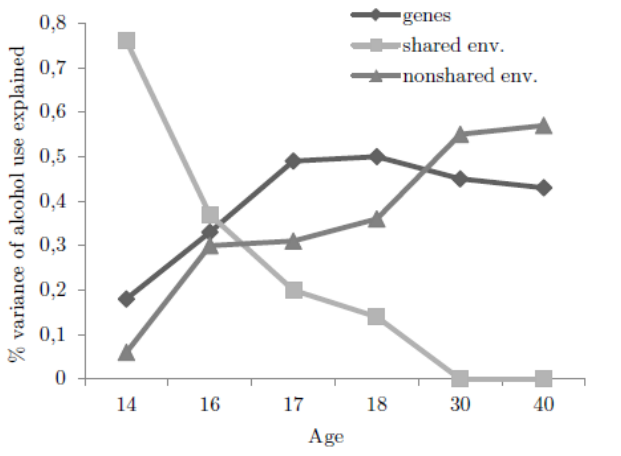


FIGURE 1 Relative contributions of genes, shared environment and nonshared environment to alcohol use at age 14 through 40 years

Figure 1 shows relative contributions of genes, shared environment, and nonshared environment to variation in alcohol initiation at age 14 years, frequency of alcohol use at ages 16-18 years, and monthly alcohol consumption at ages 30 and 40 years. These estimates were selected from the studies of Rose et al. (2001b), Rose et al. (2001a), and Kendler et al. (2008), to illustrate the age pattern as it is commonly observed for alcohol use. At age 14, individual differences in alcohol initiation and use are mainly explained by shared environmental factors (estimates range between 47-88%), while a minor part of the variance is explained by genetic factors (between 14-40%). The importance of genetic factors however increases with increasing age, while the influence of shared environment declines in parallel (Bergen et al., 2007; Dick et al., 2007a; Hopfer et al., 2003; Kendler et al., 2008). At age 30-40 years, the influence of genetic factors on monthly alcohol consumption is about 45%, while shared environmental factors play no role; the remaining 55% of individual differences are explained by nonshared environment (Kendler et al., 2008). The influence of genetic factors on alcohol dependence in adult populations is slightly higher still, with estimates varying between 50-60% (Kendler & Prescott, 2006).

When studying the etiology of alcohol use, an important consideration is that alcohol use is strongly associated with externalizing behavior and use of other substances, e.g. cigarettes. This co-morbidity likely results from a common, highly heritable vulnerability to disinhibitory behavior (Hicks et al., 2011; Kendler et al., 2008; Meyers & Dick, 2010; Stephens et al., 2012). Support for an underlying vulnerability comes from the common finding that childhood externalizing problems, e.g. impulsivity, hyperactivity, and aggressiveness, are strongly related to alcohol initiation and use later in life (reviews by Meyers & Dick, 2010; Zucker et al., 2008). Alcohol use also co-occurs with internalizing psychopathology, but these associations are often weaker and may be due to different underlying mechanisms (Hussong et al., 2011). On the one hand, childhood internalizing psychopathology has been linked to substance use in adolescence, and evidence has been found for a shared liability resulting in comorbid alcohol use and depression in Finnish adolescents (Edwards et al., 2010; Hussong et al., 2011). In contrast, in a review and meta-analysis of 15 studies in adolescent and adult samples, Boden and Fergusson (2011) concluded that a common vulnerability cannot completely explain the associations between alcohol use disorder and major depression, and that the association may be causal, such that alcohol use disorders increase the risk of developing major depression.

Environmental stressors

After birth, the human brain continues to develop and may therefore be more vulnerable to harmful effects of environmental stressors (Bava & Tapert, 2010). Prenatal alcohol exposure has been associated with childhood externalizing problems, adolescent conduct-disorder symptoms, and alcohol disorders (Alati et al., 2006; D'Onofrio et al., 2007; Disney et al., 2008). Studies in mice and rats suggest that such behavioral problems are due to disruptions in the formation of neural structures and alterations in the dopaminergic and serotonergic systems, as a result of moderate maternal alcohol consumption during pregnancy (Valenzuela et al., 2012). Moderate prenatal alcohol exposure also activates the hypothalamic-pituitary-adrenal (HPA) axis, thereby sensitizing it to later stressors. A dulled HPA axis stress response in turn is related to various types of psychopathology and substance use (Enoch, 2012; Valenzuela et al., 2012). Maternal prenatal smoking has similarly been related to adolescent and adult behavioral (externalizing) problems, and early alcohol initiation (Cornelius & Day, 2009; Goldschmidt et al., 2011; Knopik, 2009; Paradis et al., 2011). The teratogenic effects of prenatal tobacco exposure and its compounds, most importantly nicotine, include disruption of neural development, mainly since nicotine interacts with nicotinic acetylcholine receptors; fetal hypoxia and malnutrition; and exposure to many other toxic chemicals, such as carbon monoxide, lead, and ammonia (Dwyer et al., 2009; Ernst et al., 2001; Hellström-Lindahl, 2000; Huizink & Mulder, 2006; Rogers, 2009). Moreover, prenatal tobacco exposure may affect the HPA axis and thereby lead to increased risk for psychopathology and substance use later in life (Huizink & Mulder, 2006). Causal effects of prenatal maternal smoking on offspring substance use and comorbid externalizing/internalizing problems may be mediated through birthweight, since prenatal tobacco exposure has a well-established decreasing effect on birthweight, and lower birthweight has in turn been associated with delays in neurobehavioral development in childhood, independently of effects of prenatal tobacco exposure (Cnattingius, 2004; Ernst et al., 2001; Hayes & Sharif, 2009). Prenatal tobacco and alcohol exposure may co-occur and have independently, adverse effects on the fetus, leading to offspring ADHD (Mick et al., 2002). Associations between prenatal substance exposure and offspring substance use and comorbid disorders are commonly observed, but to which extent they reflect causal, teratogenic effects of prenatal exposure or confounding effects of genetic or shared environmental factors, remains unclear (Thapar & Rutter, 2009).

Childhood stressors, e.g. parental divorce, maltreatment, and stressful life events, are related to early alcohol initiation and adult alcohol use disorders

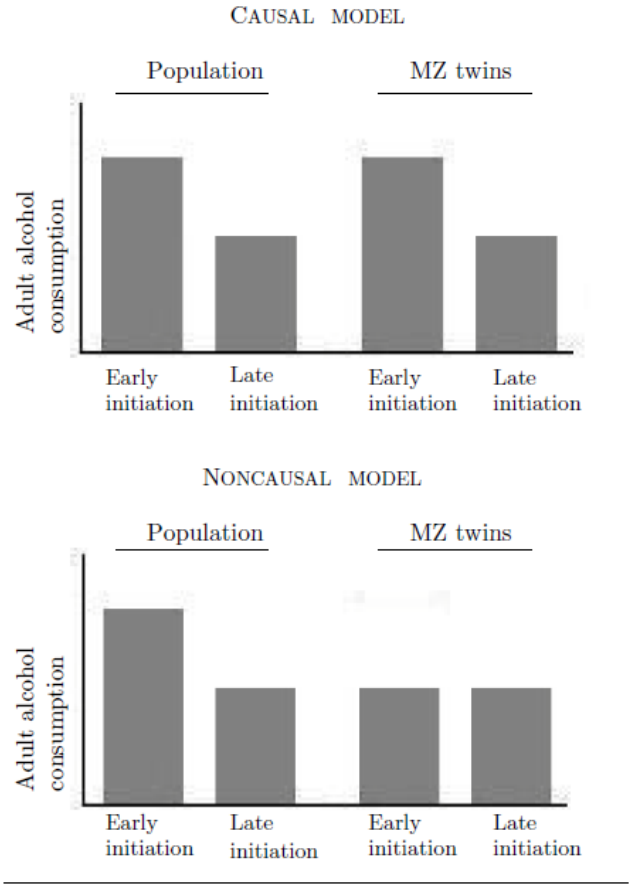
(review by Enoch, 2012; McCarty et al., 2011; Sartor et al., 2007). Experiencing stress in early life may lead to long-lasting changes to the hypothalamic-pituitary-adrenal (HPA) axis and the mesolimbic dopamine reward pathway, which is also involved in stress response, but additionally plays an important role in the brain reward circuitry, which is vital for how the brain responds to effects of substance exposure (Enoch, 2012). Neural development continues throughout adolescence, and alcohol use during this period has been linked to various adverse outcomes, such as increased alcohol consumption and dependence in adulthood (e.g. Agrawal et al., 2009; Guttmannova et al., 2011; Lee et al., 2012; Sartor et al., 2011). Effects of early alcohol use on the brain may include changes in dopamine and glutamate transmission, as well as in epigenetic mechanisms, which in turn may lead to increased alcohol consumption and risk of alcohol dependence (reviews by Bava & Tapert, 2010; Guerri & Pascual, 2010; Witt, 2010). As with prenatal alcohol and tobacco exposure, the extent to which these associations reflect causal effects of early alcohol use or result from an underlying vulnerability, remains to be resolved (Buchmann et al., 2009).

Aim of this thesis

The aim of this thesis is to gain insight in the etiology of alcohol use and comorbid externalizing and internalizing behavior throughout the lifespan. Specifically, I examine alcohol initiation and drinking patterns in adolescence, various indicators of adult alcohol consumption and dependence, and transmission of risk for comorbid externalizing and internalizing problems to the next generation. Various types of risk factors are examined, including genetic risk, familial and social environmental factors. Moreover, I investigate if longitudinally observed associations between alcohol or cigarette use and comorbid externalizing and internalizing problems are the result of causal, environmental effects of substance exposure, or of an underlying vulnerability for these behaviors. For this thesis, I use twin and family data that are collected longitudinally and across generations in participants of the Netherlands Twin Register (NTR). The NTR data collection and samples are described in more detail in the next section of this chapter. Several twin and family-based designs are applied to examine causes of variation in alcohol use. Classical twin modeling is performed in chapter 2 to investigate the genetic architecture of alcohol use in various stages of adolescence. Twin models enable estimating relative contributions of genetic and environmental factors to alcohol use by relating the similarity between twin pairs on alcohol use to their genotypic similarity (Plomin, 2008). In chapter 3, I use data on alcohol initiation and

comorbid psychopathology from one twin as an index of genetic risk for these traits for his or her co-twin (c.f. Kendler et al., 2011b). In following chapters, two family-based designs are used, which can help to disentangle shared genetic and environmental influences from causal effects of exposure to alcohol or tobacco. These include a co-twin control design, which is applied in chapter 4 to examine whether the association between early alcohol initiation and adult alcohol consumption is due to causal effects, or to an underlying vulnerability for alcohol use. The co-twin control design makes use of monozygotic (MZ) twin pairs who are discordant for early alcohol initiation. Within MZ pairs, twins who started drinking early are compared to their co-twins on adult alcohol consumption. Figure 2 shows that early alcohol initiation is associated with adult alcohol consumption at the population level. If the association between early alcohol initiation and adult alcohol consumption is due to causal effects of early alcohol initiation, or to environmental factors that make twins discordant, the association will be observed within monozygotic discordant twins as well. If, on the other hand, the association is due to an underlying vulnerability for alcohol use (non-causal model), the association will be present in the population, but within MZ twins, early drinkers will not differ from their co-twins, since MZ twins share 100% of their DNA material and their shared family environment, and have thus been equally exposed to risk factors under a non-causal model (e.g. Kendler et al., 1993; Ligthart & Boomsma, 2012; Lynskey et al., 2003; Vink et al., 2007).

The second family-based design is applied in chapter 6, and addresses possible causal effects of prenatal smoking on offspring externalizing problems and internalizing psychopathology. Effects of prenatal smoking are examined by comparing associations of prenatal maternal smoking with offspring externalizing/internalizing problems to associations with paternal prenatal smoking. Maternal prenatal smoking often co-occurs with paternal prenatal smoking. Mothers and fathers may both transmit genetic and environmental factors that may cause them to smoke and predispose their offspring to externalizing or internalizing problems (Boomsma et al., 1994; Homish et al., 2012). However, mothers also expose the child to tobacco in the prenatal environment (the putative causal effect). If a stronger association is observed between maternal smoking and offspring externalizing/internalizing problems than between paternal smoking and offspring problems, this suggests a causal effect of prenatal tobacco exposure, although it cannot be ruled out that maternal factors, like diet or stress during the pregnancy, confound the effect (Alati et al., 2008; Brion et al., 2010; Huizink, 2009; Smith, 2008). If the associations are equally strong, this suggests that they are explained by genetic



FIGUUR 2 Expected alcohol consumption in the population and in MZ twins under a causal/noncausal model

and environmental factors that influence smoking behavior in parents and externalizing or internalizing problems in their children.

In summary, this thesis examines the etiology of alcohol use in various stages of development. Moreover, causal effects of tobacco and alcohol exposure in developmentally sensitive periods are investigated. In the next section of this chapter, the NTR data collection and samples used in this thesis are described in more detail. The third section provides an outline of the following chapters.

Netherlands Twin Register survey collection

The Netherlands Twin Register (NTR) was established at the VU University Amsterdam in 1987. Longitudinal survey collection in twins and their family members started in 1991 and has been ongoing since (Boomsma et al., 2006). The NTR data collection is organized into two lines of research, as shown in Figure 3. For this thesis, existing data from both lines of research were used and new data collected. The data used in this thesis are shown in black in Figure 3 and include:

1. The ANTR 1993 survey
2. YNTR age 1, age 3, age 7, age 10, and age 12 surveys
3. DHBQ14, -16, and -18 surveys

In addition, genotype data on young twins and their mothers (serotonin transporter gene; 5-HTTLPR) are analyzed in a gene x environment interaction model.

NTR data collection in young twins and their siblings (YNTR)

The left column of Figure 3 shows the longitudinal survey collection in young twins (YNTR). Recruitment into the YNTR is an ongoing process; parents of newborn twins are invited to participate via national birth felicitation services and the Dutch Association for Parents of multiples (NVOM) (Bartels et al., 2007b). Upon registration, mothers complete a survey containing questions about the pregnancy and health of the twins. After that, twins are followed throughout childhood, by collecting maternal reports at age 2 and reports from both parents at ages 3, 5, 7, 10, and 12. Teacher reports are collected at ages 7, 10, and 12. Parents and teachers report on the twins' zygosity, health, internalizing and externalizing problems, socioeconomic status, religion, and school grades (Bartels et al., 2007b; van Beijsterveldt et al., 2012). From 2005 onwards, self-report surveys have been collected in adolescent YNTR twins (ages 14, 16, and 18 years; Table 1). Twins and their non-twin siblings were invited to complete the Dutch Health Behavior Questionnaire (DHBQ), which included questions on health, lifestyle, internalizing and externalizing problems, well-being and school performance (Bartels et al., 2011). In addition, self-reports have been collected in subsamples of twins and siblings aged 11-12 years who participated in neuroimaging studies and studies on cognition or ADHD (van Beijsterveldt et al., 2012).

NTR data collection in adult twins and their family members (ANTR)

The second line of NTR survey collection focuses on adult twins and their

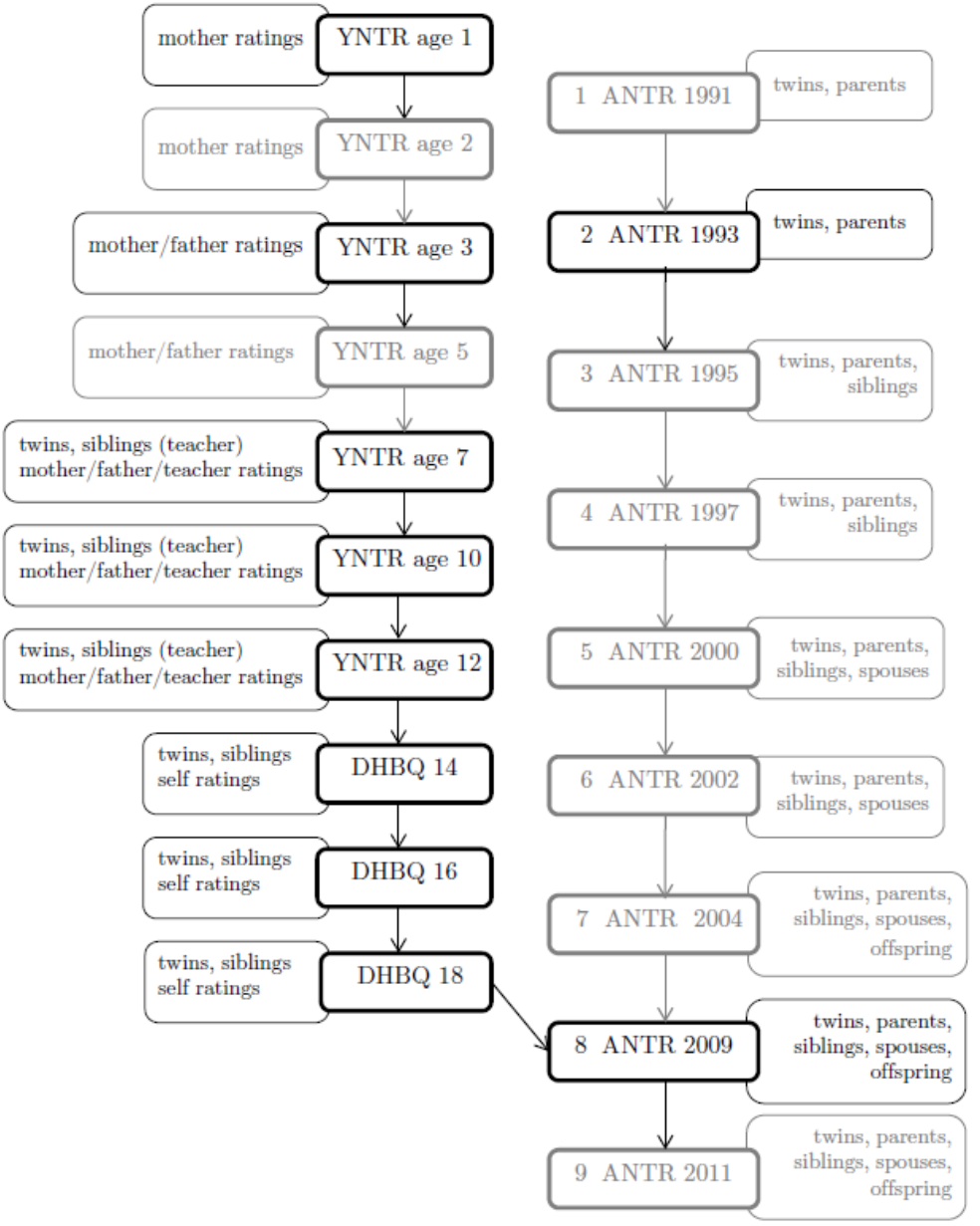


FIGURE 3 Longitudinal NTR survey collection in young (YNTR; left column) and adult (ANTR; right column) twins and their family members. All ANTR surveys are self-ratings. Surveys shown in black are used in this thesis.

Table 1 Number of reports on YNTR twins and their siblings at each age of survey collection, by rater

	N pairs/siblings	*Birth cohort*
Age 1		
Mother	31,879	1986 – 11
Age 2		
Mother	22,233	1986 – 09
Age 3		
Mother	18,805	1986 – 08
Father	11,357	1986 – 08
Age 5		
Mother & father[1]	15,781	1986 – 06
Teacher	1117/117	1990 – 91/2002 – 03
Age 7		
Mother	11,625	1986 – 00/2002 – 04
Father	8,276	1986 – 00/2002 – 04
Teacher	11,359/853	1992 – 04/1999 – 04[2]
Age 10		
Mother	9,036	1986 – 97/2000 – 02
Father	5,006	1986 – 97/2000 – 02
Teacher	10,634/717	1989 – 02/1996 – 02[3]
Age 12		
Mother	7,857	1986 – 00
Father	3,775	1986 – 00
Teacher	7,949/338	1986 – 00/1994 – 00[4]
Self	1,714/135	1986 – 87/1989 – 91/1993 – 94
DHBQ pilot		
Self	580/142	1987 – 88
Age 14		
Self	8,631/1,717	1990 – 98
Age 16		
Self	5,254/1,380	1988 – 95
Age 18		
Self	1,177/311	1986 – 88

[1]mother and father survey in same booklet; [2]cohorts 1999 – 04 are siblings; [3]cohorts 1999 – 02 are siblings; [4]cohorts 1994 – 00 are siblings

family members (ANTR; shown in the right-hand column of Figure 2). Twins and their parents were initially recruited into the ANTR by writing to all city councils in the Netherlands and requesting the addresses of families with twins between ages 13-22 years (Koopmans et al., 1994). In later years, additional recruitment efforts have been made, which include contacting city councils, and advertisements in the media and in the yearly NTR newsletter (Boomsma et al., 2002b). Instead of being age driven, all ANTR participants aged 18 years or older, who are registered at a valid address and willing to participate in survey studies, are invited about every 3 years to complete a survey containing questions on health, lifestyle, personality, and psychopathology (Boomsma et al., 2006). ANTR surveys have been collected in 1991, 1993, 1995, 1997, 2000, 2002, and 2004, and for this project, the 2009 ANTR survey was collected (Figure 2). This was the first ANTR survey that included YNTR participants, since in 2009, the first cohort of YNTR twins who had been followed from birth (starting in 1991) had reached age 18 years and thus were eligible to participate in ANTR studies (Figure 2). All subjects aged 18 years and older, who were registered at a valid address and willing to participate in survey studies, were invited to complete the survey. Thus, both ANTR and YNTR twins above age 18 years were invited, as well as their spouses, parents, siblings and children above age 18 years. Participants first received a written invitation including a link to the webpage where they could log on to a web-based version of the survey with a unique, personal login name and password. If subjects did not access the web-based survey in the 6 weeks after the invitation, they received a hard copy of the survey, to be completed by paper and pencil. Between 3-9 months after the paper versions of the survey were sent, subjects who had not responded received a reminder card by post, or a reminder by email (if an email address was available). Additionally, several groups of non-responders of particular interest (e.g. twins from incomplete twin pairs; subjects who took part in biobank studies) were reminded by a phone call. The response rate and sample are described in detail in chapter 5. In 2011, the ninth ANTR survey collection was started, which is ongoing at present (September 2012).

Table 2 shows cross-sectional participation rates of all ANTR surveys. Longitudinal participation rates are presented in Table 3. Not all family members were invited at each wave of data collection; siblings of twins were included from 1995 onwards, spouses from 2000 onwards, and children of twins older than 18 years were invited to participate in 2004 and 2009 (de Moor, 2009). A total of 10,275 individuals has participated once in ANTR surveys, of which 3,675 individuals (36%) are the YNTR twins and their siblings and parents who were only included in the 2009 survey. Table 3 further shows that

20,852 individuals have participated more than once, of whom 239 respondents have completed all 9 ANTR surveys (Willemsen et al., in press).

Genotype data

In addition to survey data, genotype data on the serotonin transporter gene (5-HTTLPR) from young twins and a subset of their mothers are analyzed in this thesis. Genotype data were derived from blood and buccal samples. Genotyping procedures are described in detail by van Beijsterveldt et al. (in press).

Table 2 Cross-sectional participation of twins and their family members in each ANTR survey

	1991	*1993*	*1995*	*1997*	*2000*	*2002*	*2004*	*2009*	*2011*
Multiples	3,391	4,234	3,425	3,232	4,613	4,530	5,723	8,414	5,570
Siblings	3	7	1,482	1,510	1,472	1,455	1,641	1,871	1,224
Parents	3,037	3,671	3,240	5	26	2,840	3,324	5,464	4,040
Spouses	-	-	1	-	682[1]	1,462	961	824	619
Offspring	-	-	-	-	-	-	329	288	186
Total	**6,431**	**7,912**	**8,148**	**4,747**	**6,793**	**10,287**	**11,978**	**16,861**	**11,639**

Note. [1]only spouses of twins between ages 25-30 years were invited.

Table 3 Longitudinal participation of twins and their family members in ANTR surveys

	1x	*2x*	*3x*	*4x*	*5x*	*6x*	*7x*	*8x*	*9x*	***Total***
Multiples	4,522	3,695	2,141	1,278	1,088	750	606	420	238	**14,738**
Siblings	1,348	1,002	532	375	322	266	142	1	1	**3,989**
Parents	3,543	3,122	1,296	685	507	594	423	1	-	**10,171**
Spouses	762	542	341	318	82	-	-	-	-	**2,045**
Offspring	165	136	122	-	-	-	-	-	-	**423**
Total	**10,340**	**8,497**	**4,432**	**2,656**	**1,999**	**1,610**	**1,171**	**422**	**239**	**31,127**

Outline of this thesis

Table 4 shows an overview of the following chapters, including the data and samples used. In chapter 2, I explore whether the prevalence of alcohol initiation, frequency, and quantity in adolescents between ages 13-21 years has changed between 1993 and 2005-2008. The liability structure and genetic architecture of these indicators of adolescent alcohol use are investigated, and changes in genetic architecture are examined over that same period. In chapter 3, a path model is constructed to examine which specific developmental risk factors predict alcohol initiation between ages 13-15 years. Predictors were identified based on the literature and include genetic risk factors, prenatal tobacco and alcohol exposure, childhood risk factors, such as externalizing/internalizing problems and parental divorce, and risk factors in adolescence, such as externalizing/internalizing problems, urbanization, smoking initiation, and family functioning. Chapter 4 examines whether the association between early alcohol initiation and adult alcohol consumption/dependence is causal or due to an underlying vulnerability for alcohol use, by making use of a discordant twin design (co-twin control design). Chapter 5 presents an epidemiological analysis of alcohol use patterns and demographic/lifestyle traits in the adult Dutch population, ranging in age from 18 to 97 years old. Associations between alcohol consumption and demographic/lifestyle traits are examined with regression analyses. In chapter 6, I examine if prenatal smoking has causal effects on offspring externalizing and internalizing problems at age three, by comparing the strength of associations of maternal versus paternal prenatal smoking on offspring outcomes. Chapter 7 describes a replication effort of a recently detected interaction between serotonin transporter genotype and prenatal maternal smoking on offspring internalizing problems at age three in a Dutch cohort study (Generation R; Cents et al., 2012). In chapter 8, the findings are summarized and their implications discussed.

Table 4 Overview of the chapters of this thesis

Ch.	Topic	Method	Data	Subjects
2	Effects of age, sex, and cohort on prevalence and genetic architecture of adolescent alcohol use	Heritability estimation with liability models	ANTR Survey 1993; DHBQ 14-18	Twins
3	Prediction of alcohol initiation at age 13-15	Path modeling	YNTR age 1-age 12; DHBQ 14	Twins
4	Effects of early alcohol initiation on adult alcohol consumption/dependence	Co-twin control design (nonparametric paired-samples tests)	ANTR Survey 2009	Monozygotic twins
5	Epidemiology of adult alcohol consumption in the Netherlands	Descriptive statistics/regression analyses	ANTR survey 2009	Twins, parents, siblings, spouses, offspring
6	Effects of prenatal tobacco exposure on offspring externalizing/internalizing at age 3	Comparison maternal prenatal smoking to paternal prenatal smoking (regression analyses)	YNTR age 1 and age 3	Twins
7	Replication of 5-HTTLPR x prenatal smoking interaction effect on offspring internalizing at age 3	Linear mixed models	YNTR age 1 and age 3; genotype data	Twins and their mothers

2

Trends in adolescent alcohol use: Effects of age, sex and cohort on prevalence and heritability

This chapter is published as:

Geels, LM, Bartels, M, van Beijsterveldt, TCEM, Willemsen, G, van der Aa, N, Boomsma, DI, Vink, JM. Trends in adolescent alcohol use: effects of age, sex and cohort on prevalence and heritability. Addiction 2012*;* 107(3), 518-27.

Abstract

Aims To determine the effect of age, sex, and cohort on the prevalence and genetic architecture of adolescent alcohol use (AAU). *Design* Survey study in participants registered with the Netherlands Twin Register. *Setting* Twins from the general population. *Participants* Two cohorts (data collected in 1993 and 2005-8) of twins aged 13-15, 16-17 and 18-21. In 1993 and 2005-8 a total of respectively 3269 and 8207 twins took part. *Measurements* Survey data on initiation and frequency of alcohol use and quantity of alcohol consumed. *Findings* The prevalence of alcohol initiation increased between 1993 and 2005-8, for both males and females. The largest difference was observed at age 13-15, where the prevalence increased from 62.5% to 73.7%. We also found increases in prevalence across cohorts for quantity of alcohol consumed and non-significant increases for frequency of alcohol use. From age 16 onwards, boys drank more frequently and larger quantities than girls. Genetic model fitting revealed that the genetic architecture of AAU does not differ between birth cohorts, nor were there differences between boys and girls. Genetic factors explained between 21% and 55% of individual differences in alcohol measures throughout adolescence. Shared environment explained between 17% and 64% of variance in alcohol use, across different age groups and alcohol measures. *Conclusions* The prevalence of alcohol initiation, frequency and quantity has increased in adolescents over a 15 year period, but there are no changes in the genetic architecture of adolescent alcohol use.

Introduction

Over the past decades, an increase in alcohol consumption has been reported in adolescents in the Netherlands. Both an increase in quantity consumed and an increase in the number of youngsters who start drinking at earlier ages have been described (Ministry of Health Welfare & Sport, 2009; Poelen et al., 2005; Statistics Netherlands, 2003; Trimbos, 2010). In 1992, 69% of Dutch adolescents between ages 12 and 18 had initiated alcohol use. In 2007 this increased to 79%. Over the same period, the percentage of adolescents between ages 12 and 18 who had consumed alcohol in the past month increased from 45% to 51% (Trimbos, 2010). Different European countries have reported minor fluctuations in the prevalence of adolescent alcohol use (AAU) between 1995-2007, but overall the prevalence across Europe remained relatively stable (Hibell et al., 2009; Poelen et al., 2005; Statistics Netherlands, 2003; Trimbos, 2010).

Dutch law prohibits selling alcohol to adolescents under age 16. Selling mildly alcoholic beverages is legal when buyers are 16 or older and for strong alcoholic spirits the buyer has to be over 18 (Ministry of Health Welfare & Sport, 2009). These rules are not always strictly enforced. Moreover, although buying alcohol under age 16 is illegal, Dutch alcohol law does not specify a minimum legal age for alcohol consumption (Dutch Centre for Crime Prevention and Safety, 2010). It is not a taboo for researchers to ask questions about alcohol use in youngsters under age 16.

Studies on the heritability of AAU often obtain heritability estimates as a function of sex and age (e.g. reviews by Hopfer et al. and Dick et al. (Dick et al., 2009; Hopfer et al., 2003)). Studies examining age effects have commonly found that from early adolescence to adulthood, the importance of genetic factors in the etiology of AAU increases, while the influence of environmental factors that are shared by offspring within a family declines (Bergen et al., 2007; Dick et al., 2007a; Hopfer et al., 2003; Kendler et al., 2008; Rose et al., 2001a). Twin studies on sex differences in the genetic architecture of AAU have yielded mixed results. Some observed a higher heritability in boys than in girls (Han et al., 1999; Hopper et al., 1992; McGue et al., 2001a), others did not find sex differences in AAU heritability (Rhee et al., 2003; Rose et al., 2001b; Viken et al., 1999) and higher heritability in girls has also been reported (Heath & Martin, 1988; Rose et al., 2001b; Viken et al., 1999). Secular changes in the heritability of AAU and whether these interact with age and gender, has been examined in an early study by Kaprio et al. in Finnish twins (Kaprio et al., 1991). Between 1975-1981 there were no changes in alcohol prevalence. For the youngest of two age groups (age 18-24 in 1975) heritability increased in both sexes across the six year study period.

In this paper we explore changes in prevalence and heritability of alcohol traits and possible interactions with age and gender in Dutch adolescents aged 13-21 . Data were collected in longitudinal survey studies of the Netherlands Twin Register (NTR) (Bartels et al., 2007b; Boomsma et al., 2006). We first describe to what extent the prevalence of AAU has changed over a period of 15 years by contrasting the alcohol use in a cohort of twins who were adolescents in 1993 to the alcohol use of a cohort of twins who were adolescents between 2005-2008. Data on alcohol initiation, frequency, and quantity are analyzed as a function of sex, age of the twins at data collection (13-15, 16-17 and 18-21), and cohort. Secular differences in AAU can be assessed because in both cohorts identical questions about alcohol use were asked.

Secondly, we describe whether there are secular changes in the genetic architecture of AAU. A change in the genetic architecture of AAU, occurring simultaneously with an increase in prevalence, would constitute evidence for moderation of heritable influences by environmental conditions (genotype x environment interaction).

Methods

Subjects

Participants come from the Netherlands Twin Registry (NTR), established in 1987 at the VU University in Amsterdam. Twins and their family members registered with the NTR are invited about every two years to participate in longitudinal survey studies (Bartels et al., 2007b; Boomsma et al., 2006). Data from two cohorts are analyzed: the first cohort participated in the 1993 survey study (Boomsma et al., 2006; Koopmans et al., 1994). They were born between 1954-1980. At the time of measurement they were on average 17.7 years old (SD=4.13, range 12-40). They were recruited via Dutch City Councils. Recruitment and participation rates have been described in Koopmans et al. (1994). For this study subjects between ages 13-21 were selected, resulting in a sample of 3269 twins. The second twin cohort participated between 2005-2008. They were born between 1987-1994 and registered at the NTR at birth by their parents. At the time of assessment they were on average 15.7 years old (SD=1.51, range 13-21). Recruitment and participation rates have been described in Bartels et al. 8207 twins. IRB approval was obtained for both studies.

Within both cohorts, twins were stratified by age: 13-15, 16-17 and 18-21 years. In all groups, slightly more girls than boys participated (54.2%-63.3%). The longitudinal data collection resulted in some overlap (8%-17% of individuals) between age groups in the 2005-8 cohort because individuals

participated at multiple ages. Information on zygosity and number of complete/incomplete twin pairs is given in Table 1. In same-sex twin pairs zygosity was determined based on DNA-polymorphisms or on parental/self-report items about physical resemblance between twins. Zygosity classification based on these items has shown over 93% agreement with DNA-polymorphisms (Rietveld et al., 2000; Willemsen et al., 2005). Zygosity was based on DNA-polymorphisms for 39.5% of individuals in same-sex twin pairs in the 1993 cohort, and 27.6% in the 2005-8 cohort. In this cohort, an additional 11.3% of individuals in same-sex pairs had zygosity typed from blood polymorphisms.

Table 1 Sample size and number of complete twin pairs stratified by zygosity in each age group and cohort

	1993		2005-8	
Age 13-15	N	n complete pairs	N	n complete pairs
MZM	202	98	580	274
DZM	148	70	542	253
MZF	281	138	917	433
DZF	171	83	587	274
DOS	157/155	150	604/674	576
Total	1114		3904	
Age 16-17				
MZM	133	65	526	248
DZM	119	56	388	175
MZF	200	99	748	348
DZF	161	80	540	243
DOS	105/105	104	392/464	358
Total	823		3058	
Age 18-21				
MZM	230	112	171	77
DZM	191	91	148	61
MZF	325	160	325	147
DZF	207	101	281	127
DOS	189/190	182	138/182	123
Total	1332		1245	

Note. MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic opposite sex

Measures

Three alcohol measures were analyzed: initiation and frequency of alcohol use and quantity of alcohol consumed. Subjects were asked if they had ever used alcohol, to which they responded 'no', 'a few times' or 'yes'. The categories 'a few times' and 'yes' were collapsed, creating a binary initiation variable. Additionally, subjects were asked about their frequency of alcohol use over the past year. This question had eight response categories, ranging from 'never' to 'daily'. Because alcohol use obviously increases with age during adolescence, the response distribution of alcohol frequency differed substantially between age groups. Moreover, across all groups the response distributions showed considerable positive skewness (see supplemental Table 1). Therefore, the eight categories of alcohol frequency were combined into three. The most appropriate and meaningful categorization was applied at each age. At age 13-15, the resulting categories were 'once a year or less', 'several times a year-monthly' and 'several times a month–daily'. At age 16-17 the categories were 'several times a year or less', 'monthly-several times a month' and 'weekly–daily' and at age 18-21: 'monthly or less', 'several times a month-weekly' and 'several times a week–daily'. Subjects who had initiated alcohol use were asked about the quantity of alcohol consumed per week, scored in seven categories (ranging from 'less than one glass' to 'more than20 drinks'). Quantity was also collapsed into three categories because of age differences and the overall positive skewness of the response distributions (see suppl. Table 1). Again, the most meaningful categorization was applied to each age group. For age 13-15, this resulted in categories 'less than 1
glass', '1-2 glasses' and '3 glasses or more'. At age 16-17, the categories were 'less than 1 glass', '1-5 glasses' and '6 glasses or more' and at age 18-21: '1-2 glasses or less', '3-10 glasses' and '11 glasses or more'.

Missing data on alcohol initiation ranged from 0-2.2% across cohorts, age and sex. For alcohol frequency missing values ranged from 0-12.4%. Alcohol quantity was not observed in all cases (61.5%-95.1% observed), because this question was not asked in those who had not started drinking alcohol.

Analyses

Prevalence of alcohol use

Prevalences of alcohol initiation and frequencies of alcohol use and alcohol quantity were reported as a function of cohort, age and sex. Data management and preliminary analyses were done in SPSS 15.0 (Inc, 2006).

Twin correlations

Structural equation modeling was used to test cohort- and sex differences in prevalence and twin correlations of alcohol initiation, frequency and quantity, within age groups. Analyses were done under the assumption that these categorical measures have an underlying continuous, normally distributed liability which can be influenced by genetic and non-genetic factors. Thresholds divide this continuous liability into discrete categories (Falconer & Mackay, 1996). Sex and cohort effects on the thresholds were tested by estimating separate thresholds and subsequently constraining them to be equal across sex or cohort for all zygosity groups simultaneously. In each age group , twin resemblances were summarized into tetrachoric (initiation) and polychoric (frequency/quantity) correlations. Cohort effects were tested by constraining all five twin correlations across cohorts simultaneously. Quantitative sex differences in twin correlations were tested by equating the correlations across sex (within mono- and dizygotic same-sex groups). Qualitative sex differences were examined by equating the dizygotic opposite-sex correlation to the dizygotic same-sex correlation (see page 3 of supplementary materials for a more detailed description of the model testing procedure). Models were fitted on raw data.

For alcohol quantity, defined as number of glasses per week, the prevalence of any weekly alcohol use was very low at age 13-15. For alcohol initiation from age 16 onward, the prevalence approached 100%. For these two phenotypes there was no meaningful population variance to analyze.

Relationship between alcohol initiation and frequency/quantity of alcohol use

In the young adolescents, three genetic models were considered to describe the relationship between alcohol initiation and frequency, within each cohort. These analyses addressed the question how to handle subjects who have not initiated drinking in the analysis of frequency/quantity, because in those subjects, alcohol frequency/quantity are unobserved (Heath et al., 1991; Koopmans, 1997). This is necessary in young adolescents (age 13-15), as not all of them have started using alcohol yet. As the prevalence of alcohol initiation increases with age and approaches 100%, the distinction becomes unnecessary. If alcohol frequency/quantity are determined by a single liability, subjects who have not initiated drinking are excluded from the analysis (because we cannot observe their scores on frequency/quantity) and the liability distribution for frequency/quantity is truncated (Heath et al., 1991). If, on the other hand, frequency/quantity are determined by separate liabilities, including those who have not initiated alcohol use in the analysis of frequency/quantity may lead to biased heritability estimates (Heath et al., 1991). We compared three liability

models: 1) the single liability dimension (SLD) model; in which initiation and frequency/quantity are modelled on a single underlying liability. 2) The independent liability dimension (ILD) model which assumes that initiation and frequency/quantity have separate, unrelated liabilities, and 3) the combined model (CM) which postulates separate but related liabilities for initiation and frequency/quantity. The combined model allows for people to be non-drinkers either because they have never started or because they have started but are low on the frequency/quantity liability. Detailed descriptions of these models can be found in Vink et al. (2005) and in Koopmans et al. (1999a). For these models, model fit was determined based on the χ^2 goodness-of-fit statistic and on the AIC (Akaike, 1987).

The combined models were fitted to 4x4 contingency tables of the drinking behavior of the firstborn twin cross-classified with the co-twin. Thus only data from complete twin pairs were included in these analyses (N=1078 in the 1993 cohort and N= 3620 in the 2005-8 cohort). No bias was found when results from univariate genetic models on all raw data and on the data excluding incomplete twin pairs were compared (data available on request from corresponding author). Frequency and quantity of alcohol use previously consisted of three categories. For these analyses frequency and quantity data were divided in four categories, with subjects who had not initiated alcohol use in the lowest category.

Genetic architecture of alcohol use

The genetic architecture of alcohol initiation was analyzed in univariate models. For frequency of alcohol use, the best fitting liability model was explored in the youngest group, while univariate models were used in the two older groups. The total variance in alcohol initiation, frequency and quantity was partitioned into an additive genetic component (A), shared environmental (C) and non-shared environmental component (E) (Neale & Cardon, 1992). Shared environment represents environmental factors that cause twins to become more similar, whereas non-shared environment refers to environmental influences that make twins less similar (Hopfer et al., 2003).

If the pattern of twin correlations indicated qualitative sex differences, genetic models were specified accordingly. At age 13-15, qualitative sex differences were evaluated for the shared environment based on previous literature (Koopmans & Boomsma, 1996; Rose et al., 2001b; Viken et al., 1999). Specifically, the correlation between shared environments of dizygotic opposite twin pairs was estimated as a free parameter. At age 18-21 qualitative sex differences were modeled in the genetic component because that is where the

qualitative sex difference was significant. It was not significant for the shared environment (results available on request from corresponding author). The significance of genetic, shared environmental and unique environmental components was examined by constraining them at zero one at a time.

Statistical testing

All statistical testing was done by comparing nested submodels and evaluating the difference in minus twice log-likelihood of the restricted model and the more general model (likelihood-ratio test). If models are nested, this difference is χ^2-distributed. The degrees of freedom equal the difference in estimated parameters (Rhee et al., 2003). Analyses were done in Mx (Neale et al., 2006). Because of the large number of tests (multiple variables, age groups, and cohorts), all tests throughout the study were evaluated at a .01 significance level.

Results

Prevalence of alcohol use

The prevalence of initiation, frequency, and quantity of alcohol use was examined as a function of age and sex within the two cohorts (Table 2). At age 13-15, more boys had started drinking alcohol than girls (for model fitting results see supplementary Table 3, model 3). In all age groups, boys outnumbered girls in the highest category of alcohol frequency (for model fitting results see supplementary Table 4, model 3). In the older age groups, boys consumed larger quantities than girls (see supplementary Table 5). Table 2 shows an increase in alcohol initiation, frequency, and quantity between 1993 and 2005-8, across sex, although for alcohol frequency, the increases were not significant at alpha=.01 in the older age groups.

Twin correlations

Twin correlations for alcohol initiation, frequency and quantity were examined for cohort- and sex differences in univariate saturated models, as a function of age (see supplementary Tables 2-5). Significantly lower correlations were observed in the dizygotic opposite-sex twins than in the dizygotic same-sex twins for frequency of alcohol use at age 13-15 and for quantity at age 18-21 (for model fitting results see supplementary Tables 4-5, model 6). No quantitative sex differences in the correlation structure of alcohol use were found. No differences between cohorts were observed either. Twin correlations were therefore estimated on combined cohorts for mono- and dizygotic twins, stratified by age (Table 3). Correlations suggest that at age 13-15, individual differences in alcohol use were mainly explained by shared environmental factors

Table 2 Frequencies (percentages) of alcohol initiation, alcohol use and quantity as a function of cohort, age and gender

	Male		Female	
Initiation	1993	2005-8	1993	2005-8
Age 13-15				
Initiated alcohol use	65.5	75.0	59.5	72.4*†
	N=507	*N=1698*	*N=607*	*N=2130*
Age 16-17				
Initiated alcohol use	91.0	94.9	91.6	94.9
	N=357	*N=1306*	*N=466*	*N=1752*
Age 18-21				
Initiated alcohol use	96.7	97.6	94.9	96.2
	N=610	*N=457*	*N=722*	*N=788*
Frequency				
Age 13-15				
Once a year or less	52.9	41.4	63.6	42.7*†
Several times a year – monthly	37.8	37.2	30.2	38.4*†
Several times a month – daily	9.2	21.5	6.2	18.9
	N=444	*N=1666*	*N=563*	*N=2093*
Age 16-17				
Several times a year or less	33.9	19.3	43.1	26.6†
Monthly - Several times a month	23.0	24.9	31.3	34.9†
Weekly - daily	43.1	55.8	25.5	38.5
	N=357	*N=1287*	*N=466*	*N=1722*
Age 18-21				
Monthly or less	18.8	15.0	40.3	33.8†
Several times a month - weekly	34.8	41.2	41.8	46.5†
Several times a week - daily	46.5	43.8	17.9	19.7
	N=607	*N=447*	*N=720*	*N=775*
Quantity				
Age 13-15				
Less than 1 glass per week	85.1	77.9	88.3	78.2*†
1-2 glasses per week	9.5	14.3	7.1	13.2*†
3 glasses or more per week	5.4	7.8	4.6	8.6
	N=349	*N=1185*	*N=367*	*N=1467*
Age 16-17				
Less than 1 glass per week	39.2	25.4	60.0	36.8*†
1-5 glasses per week	33.7	40.5	31.6	46.0*†
6 glasses or more per week	27.2	34.0	8.5	17.2
	N=309	*N=1187*	*N=412*	*N=1601*
Age 18-21				
1-2 glasses a week or less	30.7	25.2	62.4	53.5*†
3-10 glasses per week	38.8	45.5	31.1	38.3*†
11 glasses or more per week	30.5	29.3	6.5	8.3
	N=580	*N=437*	*N=657*	*N=737*

Note. * significant cohort difference within age group; † significant sex difference within age group For frequency and quantity each test was done on both thresholds simultaneously. All tests were evaluated at $\alpha = .01$.

whereas in later adolescence genetic factors became more important because with age, the difference between the monozygotic and dizygotic twin correlations increased.

Table 3 Tetra- and polychoric twin correlations with 95% confidence intervals for alcohol frequency and quantity in each age group, estimated in best fitting saturated models

	Age 13-15	Age 16-17	Age 18-21
Initiation			
MZ	.86 (.81 - .90)	-	-
DZ	.71 (.65 - .76)	-	-
Frequency			
MZ	.83 (.80 - .86)	.79 (.74 - .82)	.65 (.58 - .71)
DZ	.74 (.68 - .78)	.59 (.53 - .64)	.41 (.33 - .49)
DOS	.59 (.52 - .66)		
Quantity			
MZ	-	.76 (.71 - .81)	.68 (.60 - .74)
DZ	-	.51 (.44 - .57)	.56 (.44 - .66)
DOS			.31 (.17 - .44)

Note. MZ=monozygotic twins, DZ=dizygotic twins, DOS=dizygotic opposite sex twins. Correlations were not computed for alcohol initiation at age 18-21, because the prevalence was close to 100%.

Genetic architecture of alcohol use

In each age group, the genetic architecture of AAU was explored. At age 13-15, a combined model was specified for alcohol initiation & frequency because a combined model fit the data best (see supplementary Table 6 for model fitting results). Based on results described above (and in supplementary Tables 3-4), separate prevalences (thresholds) were estimated for each cohort and gender. Variance components were constrained to be equal across gender and cohorts (because no sex-/cohort differences in correlation structure were observed). For frequency, the correlation of the shared environmental component between twins in the DOS group was estimated as a free parameter (based on different DZ/DOS correlation in Table 3). This correlation was .75 (.51-.94).

Table 4 shows that at age 13-15, alcohol initiation and frequency were mainly influenced by shared environment (55%, 64%) while genetic influences were less important (31%, 21%). At age 16-17 a single liability (SLD) model was fitted for alcohol frequency and quantity, because at this age most subjects had

initiated alcohol use, making a bivariate model (CM/ILD) unnecessary. Individual differences in alcohol frequency and quantity were explained by genetic factors (42%, 55%) and shared environment (36%, 22%). At age 18-21, alcohol frequency and quantity were also analyzed under the single liability model.

Table 4 Estimates of genetic and environmental variance components with 95% confidence intervals for frequency and quantity of alcohol use in each age group

Initiation	A	C	E
Age 13-15	.31 (.17 - .45)	.55 (.43 - .67)	.14 (.10 - .19)
Frequency			
Age 13-15	.21 (.03 - .42)	.64 (.44 - .79)	.15 (.11 - .22)
Age 16-17	.42 (.29 - .54)	.36 (.25 - .47)	.22 (.19 - .26)
Age 18-21	.47 (.27 - .67)	.17 (.00 - .34)	.36 (.30 - .42)
Quantity			
Age 16-17	.55 (.42 - .69)	.22 (.10 - .34)	.23 (.19 - .27)
Age 18-21	.36 (.20 - .56)	.35 (.17 - .48)	.29 (.24 - .36)

Note. A=genetic factors, C=shared environment, E=nonshared environment. For initiation, variance components were estimated in univariate models. Variance components of frequency were estimated under a combined model at age 13-15 and variance components of frequency and quantity were estimated under single liability models at ages 16-17 and 18-21.

Table 4 shows that genetic factors explained 47% and 36% of individual differences in alcohol frequency and quantity respectively. Shared environment explained 17% to 35% of the variance in frequency and quantity. For quantity, the genetic correlation in DOS twin pairs was freely estimated, at .00 (.00-.48). The picture that emerges from Table 4 is that generally, models including a genetic, shared environmental and non-shared environmental factor best explained individual differences in AAU (see also supplementary Tables 7-10). Alcohol initiation at age 13-15 was mainly explained by shared environmental factors. For alcohol frequency, genetic influences increased between age 13-15 and the older two age groups, while shared environmental influences decreased. For alcohol quantity, only analyzed at ages 16-17 and 18-21, this pattern was not observed.

Discussion

We report a comparison of adolescent alcohol use (AAU), identically assessed in two cohorts of young twins, in 1993 and 2005-8. The prevalence and genetic architecture of alcohol initiation, frequency, and quantity were compared across cohort and sex, as a function of age. Over a 15-year period an increase in AAU was observed. A larger number of young adolescents initiated drinking in 2005-8 than in 1993. In the more recent cohort they also drank larger quantities. Frequency of alcohol use also increased across cohorts, but this increase was non-significant. The increase in prevalence led to the question whether the genetic architecture of AAU differed as a function of environmental exposure. Changes in social environment with respect to AAU can moderate the genetic influence on drinking behavior (genotype x environment (GxE) interaction (Boomsma et al., 2002a)). This has been observed for several environmental factors, such as peer substance use (Agrawal et al., 2010a; Guo et al., 2009) religiosity (Button et al., 2010; Koopmans et al., 1999b), socio-regional factors (Legrand et al., 2008; Rose et al., 2001a) and parental monitoring (Dick et al., 2007b), suggesting that in an environment where alcohol is more readily available to adolescents their alcohol use is more heritable. In the current study no specific environmental variable was tested, but instead changes in adolescent drinking patterns (prevalence) were used as proxy for environmental changes.

An increased prevalence of AAU was seen for the period under study, however, no change in the genetic architecture of AAU was observed. This finding is analogous to what has been observed for human height for example, which has increased substantially over the past 150 years, due to improved environmental circumstances (McEvoy & Visscher, 2009; Silventoinen, 2003). The heritability of height however, has not changed over this time-period (Silventoinen, 2003). Several circumstances possibly led to the increase in AAU over the 15 years under study. Teenagers have more money to spend and more adolescents in high school worked (35%-58% increase) (Boelens & Sinkeldam, 2000; Nibud Scholierenonderzoek 2008-2009," 2009). Also, the variety of pre-mixed alcoholic drinks offered by stores has increased. These drinks are especially popular among teenagers (Dutch Institute for Alcohol Policy (STAP), 2008). These factors have led to a widespread availability of alcohol; which can be compared to the universal improvement in environmental circumstances leading to increases in height. Analogous to the human height example, these environmental changes seem to affect different genotypes in a similar manner: heritability did not change (no genotype x cohort interaction) with an increase in the prevalence of alcohol use. This observation may imply that as 'interventions' that modify drinking behavior towards larger consumption do

not depend on genotype, the reverse is also true. If a reduction in alcohol use would be a desirable target, intervention could be equally effective for different genotypes.

Within cohorts sex effects on prevalence and genetic architecture were explored as well. At age 13-15, more boys than girls had started to drink alcohol and drank more frequently. From age 16 onwards, boys drank larger quantities. These findings agree with the 2009 report of the Netherlands Institute of Mental Health and Addiction (2010). No quantitative sex differences in the genetic architecture of AAU were observed. Previous results on sex differences in the genetic architecture of AAU are conflicting: some studies found a higher heritability of alcohol use in boys [13-15] while others did not (Maes et al., 1999; Rhee et al., 2003; Rose et al., 2001b; Viken et al., 1999) and higher heritability in girls has also been reported (Heath & Martin, 1988; Rose et al., 2001b; Viken et al., 1999). We found evidence of qualitative sex differences at ages 13-15 and 18-21, i.e. genetic or shared environmental factors that influence alcohol frequency and quantity differed across sex. This has been observed before in Dutch twins (aged 15-24) and in large studies in adolescent Finnish twins (Koopmans & Boomsma, 1996; Rose et al., 2001b; Viken et al., 1999).

The liability structure of alcohol use at age 13-15 was best described by separate but related liabilities for alcohol initiation and frequency. AAU liability structure was previously examined by Fowler et al. (2007), Koopmans et al. (1997) and Heath et al. (1991), who observed the same structure for alcohol initiation and frequency/quantity (Fowler et al., 2007; Heath et al., 1991; Koopmans, 1997). From age 16 onward, the prevalence of initiation approached 100%, so there were no differences between subjects in alcohol exposure.

Genetic modeling suggested that within cohorts, the heritability of alcohol frequency increased throughout adolescence, while the influence of shared environment declined, in line with what is commonly found in adolescents declines (Bergen et al., 2007; Dick et al., 2007a; Hopfer et al., 2003; Kendler et al., 2008; Rose et al., 2001a). The increase was most apparent when comparing ages 13-15 and 16-17.

Most importantly, in recent years adolescents in the Netherlands consumed more alcohol, drank more frequently and started drinking at a younger age than in the early 1990s, but the relative contributions of genes and environment to individual differences in AAU have not changed across this period.

Supplement to chapter 2

1. he frequencies of alcohol frequency and quantity in the original categories are shown in Table 1, as a function of cohort, age and gender.

Table 1 Frequencies (percentages) of alcohol frequency and quantity in the original categories for each cohort, age group and gender

	Male		Female	
Frequency	1993	200 5-8	1993	2005-8
Age 13-15				
Never	32.5	28.9	40.7	28.7
Once a year or less	14.6	11.0	18.5	12.3
Several times a year	27.7	26.1	24.3	26.6
About once a month	7.6	10.3	4.8	11.3
Several times a month	9.4	11.1	6.0	10.5
Once a week	5.8	8.1	5.0	6.2
Several times a week	2.4	1.7	.8	1.6
Daily	-	.1	-	.0
	N=501	*N=1679*	*N=605*	*N=2116*
Age 16-17				
Never	12.9	6.8	10.3	7.0
Once a year or less	4.8	2.8	9.0	3.7
Several times a year	16.2	8.7	24.1	14.6
About once a month	9.2	6.8	13.7	12.0
Several times a month	13.7	17.0	17.5	21.5
Once a week	25.2	29.1	17.1	26.3
Several times a week	17.4	23.4	8.3	10.3
Daily	.6	.8	-	.2
	N=357	*N=1290*	*N=468*	*N=1729*
Age 18-21				
Never	4.0	3.2	7.8	5.3
Once a year or less	3.8	1.5	6.0	2.7
Several times a year	7.2	5.4	15.3	12.1
About once a month	3.8	4.3	11.3	12.4
Several times a month	12.0	14.8	18.1	22.1
Once a week	22.7	24.7	23.8	22.6
Several times a week	42.3	39.1	16.7	18.3
Daily	4.1	3.2	1.3	.7

	N=607	*N=448*	*N=720*	*N=775*
	Male		Female	
Quantity	1993	2005-8	1993	2005-8
Age 13-15				
Less than 1 glass	85.1	53.5	88.1	52.7
1-2 glasses	9.4	9.8	7.0	8.9
3-5 glasses	3.4	3.7	3.8	4.1
6-10 glasses	1.7	1.3	.5	1.4
11-16 glasses	.3	.3	.3	.3
17-20 glasses	-	.1	-	.0
More than 20 glasses	-	.1	-	-
	N=350	*N=1185*	*N=369*	*N=1467*
Age 16-17				
Less than 1 glass	39.2	25.4	60.0	36.7
1-2 glasses	17.2	18.7	18.9	21.5
3-5 glasses	16.5	21.9	12.6	24.5
6-10 glasses	16.2	20.9	6.3	13.2
11-16 glasses	4.4	8.2	1.0	3.1
17-20 glasses	4.3	4.8	.9	1.1
More than 20 glasses	2.3	.2	.2	-
	N=309	*N=1190*	*N=413*	*N=1603*
Age 18-21				
Less than 1 glass	17.8	12.7	44.7	30.2
1-2 glasses	12.9	10.9	17.7	18.8
3-5 glasses	16.2	21.2	17.7	22.1
6-10 glasses	22.6	21.7	13.4	12.9
11-16 glasses	9.0	16.5	2.6	4.6
17-20 glasses	9.8	10.5	2.1	3.0
More than 20 glasses	11.8	.4	1.8	-
	N=580	*N=438*	*N=657*	*N=737*

2. Univariate saturated models were fitted to alcohol initiation, frequency and quantity in each age group and cohort to test for cohort- and sex differences in thresholds and correlation structure. In all full models, the following parameters were estimated:

- threshold(s) for males, threshold(s) for females in the 1993 cohort
- threshold(s) for males, threshold(s) for females in the 2005-8 cohort
- twin correlations for all five zygosity groups in the 1993 cohort (see Table 2 below)
- twin correlations for all five zygosity groups in the 2005-8 cohort (see Table 2 below)

Table 2 Tetra- and polychoric twin correlations with 95% confidence intervals for alcohol initiation, frequency and quantity in each age group and cohort, estimated in full saturated models

	1993			2005-8		
	Age 13-15	Age 16-17	Age 18-21	Age 13-15	Age 16-17	Age 18-21
Initiation						
MZM	.83 (.65 - .94)	-	-	.81 (.71 - .89)	-	-
DZM	.72 (.43 - .90)	-	-	.68 (.51 - .81)	-	-
MZF	.88 (.76 - .95)	-	-	.88 (.82 - .93)	-	-
DZF	.83 (.63 - .94)	-	-	.81 (.68 - .89)	-	-
DOS	.62 (.40 - .78)	-	-	.66 (.55 - .75)	-	-
Frequency						
MZM	.90 (.79 - .96)	.83 (.67 - .92)	.63 (.45 - .76)	.77 (.69 - .83)	.67 (.58 - .75)	.69 (.49 - .82)
DZM	.81 (.61 - .91)	.77 (.56 - .89)	.46 (.21 - .65)	.66 (.55 - .75)	.53 (.38 - .65)	.35 (.04 - .60)
MZF	.87 (.77 - .94)	.80 (.68 - .88)	.68 (.56 - .78)	.86 (.81 - .89)	.78 (.72 - .84)	.60 (.44 - .72)
DZF	.75 (.53 - .87)	.67 (.47 - .81)	.67 (.50 - .79)	.79 (.72 - .85)	.54 (.41 - .65)	.36 (.14 - .55)
DOS	.62 (.42 - .75)	.43 (.19 - .62)	.34 (.15 - .51)	.59 (.51 - .66)	.53 (.43 - .61)	.33 (.11 - .51)
Quantity						
MZM	-	.82 (.63 - .92)	.60 (.41 - .74)	-	.79 (.70 - .85)	.54 (.28 - .72)
DZM	-	.46 (.13 - .70)	.53 (.29 - .71)	-	.49 (.30 - .64)	.39 (.05 - .64)
MZF	-	.79 (.62 - .89)	.77 (.63 - .86)	-	.73 (.64 - .79)	.73 (.60 - .83)
DZF	-	.66 (.42 - .82)	.62 (.39 - .78)	-	.61 (.49 - .71)	.63 (.41 - .78)
DOS	-	.43 (.17 - .63)	.25 (.04 - .43)	-	.42 (.29 - .54)	.39 (.18 - .57)

Note. MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic opposite sex. Correlations for alcohol initiation were only computed at age 13-15, because at older ages the prevalence was nearly 100%.

Saturated model testing

The full models (model 1) were the starting point from which nested models were tested. Whenever the deterioration in model fit of a nested model was nonsignificant, the restrictions of that model were also applied to the following models nested under that.

Model 2: Thresholds were equated across the cohorts, but still estimated separately for males and females. No restrictions were done on the twin correlations.

Model 3: Thresholds were equated across males and females, no restrictions were done on the twin correlations.

Model 4: Twin correlations in all zygosity groups were equated across cohorts, but still estimated separately for males and females.

Model 5: The monozygotic male twin correlation was equated to the monozygotic female twin correlation, and the dizygotic male twin correlation was equated to the dizygotic female twin correlation and to the dizygotic opposite sex correlation. In case equating the dizygotic twin correlations resulted in worsening of model fit, the DOS correlation was estimated, and equating it to the dizygotic correlation was tested separately in an additional submodel (model 6).

Model 6: The dizygotic opposite sex twin correlation was equated to the dizygotic twin correlation (which was already equated across males and females in model 5).

Table 3 Initiation of alcohol use: test for cohort- and sex differences in thresholds and correlation structure using univariate saturated models

		n par	-2 LL (df)	X^2 (Δ df)	P	vs
Age 13-15						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	14	5199.996 (4930)			
model 2	As 1 plus thresholds equated across cohorts	12	5238.884 (4932)	38.888 (2)	<.0001	1
model 3	As 1 plus thresholds equated across sex	12	5209.887 (4932)	9.891 (2)	.0071	1
model 4	As 3 plus correlations equated across cohorts	7	5200.408 (4935)	.412 (7)	.9997	1
model 5	As 4 plus correlations equated across sex	4	5208.597 (4938)	8.189 (3)	.0423	4

Note. n par: number of parameters estimated; -2LL: -2 loglikelihood. χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01.
Initiation of alcohol use was not examined in age groups 16-17 and 18-21 because the prevalence was close to 100%.

Table 4 Frequency of alcohol use: test for cohort- and sex differences in thresholds and correlation structure using univariate saturated models

		n par	-2 LL (df)	X^2 (Δ df)	P	vs
Age 13-15						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	18	8657.526 (4748)			
model 2	As 1 plus thresholds equated across cohorts	14	8743.615 (4752)	86.089 (4)	<.0001	1
model 3	As 1 plus thresholds equated across sex	14	8673.834 (4752)	16.308 (4)	.0026	1
model 4	As 3 plus correlations equated across cohorts	13	8664.310 (4753)	6.784 (5)	.2372	1
model 5	As 4 plus correlations equated across sex (not DOS)	11	8672.277 (4755)	7.967 (2)	.0186	4
model 6	As 5 plus DOS correlation equated to DZ correlation	10	8683.761 (4756)	11.484 (1)	.0007	5

Age 16-17						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	18	7481.531 (3814)			
model 2	As 1 plus thresholds equated across cohorts	14	7491.107 (3818)	9.756 (4)	.0477	1
model 3	As 1 plus thresholds equated across sex	12	7587.057 (3820)	95.950 (2)	<.0001	2
model 4	As 1 plus correlations equated across cohorts	9	7496.276 (3823)	5.169 (5)	.3956	2
model 5	As 1 plus correlations equated across sex	6	7499.020 (3826)	2.744 (3)	.4328	4
Age 18-21						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	18	5032.899 (2531)			
model 2	As 1 plus thresholds equated across cohorts	14	5043.393 (2535)	10.494 (4)	.0329	1
model 3	As 1 plus thresholds equated across sex	12	5244.100 (2537)	200.707 (2)	<.0001	2
model 4	As 1 plus correlations equated across cohorts	9	5050.306 (2540)	6.913 (5)	.2272	2
model 5	As 4 plus correlations equated across sex	6	5053.792 (2543)	3.484 (3)	.3228	4

Note. n par: number of parameters estimated; -2LL: -2 loglikelihood. χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01.

Table 5 Quantity of alcohol use: test for cohort- and sex differences in thresholds and correlation structure using univariate saturated models

		n par	-2 LL (df)	X^2 (Δ df)	P	vs
Age 16-17						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	18	6785.585 (3491)			
model 2	As 1 plus thresholds equated across cohorts	14	6858.556 (3495)	73.006 (4)	<.0001	1
model 3	As 1 plus thresholds equated across sex	14	6925.546 (3495)	139.961 (4)	<.0001	1
model 4	As 3 plus correlations equated across cohorts	9	6786.615 (3496)	1.030 (5)	.9601	1
model 5	As 4 plus correlations equated across sex	6	6794.950 (3499)	8.335 (3)	.0396	4
Age 18-21						
model 1	Full model: thresholds equated across birth order and zygosity within sex and cohort	18	4401.021 (2393)			
model 2	As 1 plus thresholds equated across cohorts	14	4418.124 (2397)	17.103 (4)	.0018	1
model 3	As 1 plus thresholds equated across sex	14	4657.316 (2397)	239.192 (4)	<.0001	1
model 4	As 1 plus correlations equated across cohorts	13	4403.084 (2398)	2.063 (5)	.8404	1
model 5	As 4 plus correlations equated across sex (not DOS)	11	4410.051 (2400)	6.967 (2)	.0307	4
model 6	As 5 plus DOS correlation equated to DZ correlation	10	4417.717 (2401)	7.666 (1)	.0056	5

Note. n par: number of parameters estimated; -2LL: -2 loglikelihood. χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01.

3. The liability structure of alcohol initiation and frequency/quantity was examined in the 13-15 age group by comparing three multivariate saturated models specifying different liability structures (single liability, independent liabities or combined liabilities). Analyses were done within cohorts. The best fitting model has the least significant χ^2-value and the lowest AIC.

Table 6 Goodness-of-fit of the SLD, ILD and Combined Model to alcohol frequency and initiation at age 13-15, within cohorts

1993				
Age 13-15	n par	χ^2 (df)	P	AIC
Single liability dimension (SLD)	11	86.648 (64)	.0302	-41.352
Independent liability dimension (ILD)	16	371.185 (59)	<.0001	253.185
Combined model (CM)	18	62.040 (57)	.3012	-51.960
2005-8				
Age 13-15				
Single liability dimension (SLD)	11	152.357 (64)	<.0001	24.357
Independent liability dimension (ILD)	16	736.273 (59)	<.0001	618.273
Combined model (CM)	18	80.599 (57)	.0215	-33.401

Note. n par: number of parameters estimated; χ^2 (df): tests goodness of fit; AIC = χ^2 - 2df, a measure of parsimony of the model

4. Univariate variance decomposition of alcohol initiation at age 13-15 on combined cohorts (thresholds and variance components constrained based on twin correlations).

Table 7 Univariate variance decomposition of alcohol initiation: model fitting results in age groups 13-15 and 16-17, on pooled cohorts

		n par	-2LL (df)	χ^2 (Δdf)	p	vs
Age 13-15						
model 1	ACE model without sex difference	6	5208.597 (4941)			
model 2	CE model	5	5226.436 (4942)	17.839 (1)	<.0001	1
model 3	AE model	5	5264.534 (4942)	55.937 (1)	<.0001	1

Note. A: additive genetic variance component; C: common environmental variance component; E: unique environmental variance component; n par: number of parameters estimated; -2LL: -2 loglikelihood; χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01. Separate thresholds were estimated for each cohort and gender.

5. Variance decompositions were done under the best fitting liability models, with thresholds and correlation structure specified according to results from univariate saturated models.

In the 13-15 age group variance components were estimated under combined models. This model is optimized through a user-defined fit function and does not compute a -2 loglikelihood of the data. Determining the significance of the variance components was therefore done by comparing the χ^2-goodness-of-fit statistic and the AICs and choosing the best fitting model from the submodels.

Table 8 Variance decomposition under the Combined model: model fitting results for alcohol frequency combined with initiation, age group 13-15, on pooled cohorts

	Initiation	*Frequency*	n par	χ^2 (df)	p	AIC
1.	ACE	ACE	20	169.989 (130)	.0106	-90.011
2.	CE	ACE	19	173.471 (131)	.0077	-88.529
3.	AE	ACE	19	184.971 (131)	.0013	-77.029
4.	ACE	CE	19	179.624 (131)	.0031	-82.376
5.	ACE	AE	19	223.122 (131)	<.0001	-38.878

Note. ACE = full model without sex differences; AE = additive genetic model; CE = shared environmental model; n par: number of parameters estimated; χ^2 (Δdf): tests goodness of fit; AIC = χ^2 - 2df, a measure of parsimony of the model. Thresholds were estimated separately for each cohort and gender.

In the 16-17 and 18-21 age groups, a single liability model was used for the variance decomposition, which amounts to a univariate analysis (initiation and frequency/quantity are on the same liability distribution). Therefore the significance of the variance components was determined by dropping them from the model one at a time and evaluating the difference in model fit.

Table 9 Variance decomposition under the Single Liability Dimension model: model fitting results for alcohol frequency and quantity, age group 16-17, on pooled cohorts

		n par	-2LL (df)	χ^2 (Δdf)	p	vs
Frequency						
model 1	ACE model without sex difference	8	7696.415 (3490)			
model 2	CE model	7	7740.270 (3491)	43.855 (1)	<.0001	1
model 3	AE model	7	7729.914 (3491)	33.499 (1)	<.0001	1
Quantity						
model 1	ACE model without sex difference	14	7111.956 (3222)			
model 2	CE model	13	7176.908 (3223)	64.952 (1)	<.0001	1
model 3	AE model	13	7123.571 (3223)	11.615 (1)	.0007	1

Note. A: additive genetic variance component; C: common environmental variance component; E: unique environmental variance component; n par: number of parameters estimated; -2LL: -2 loglikelihood; χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01. Incomplete twin pairs were excluded from the analyses. For alcohol frequency, thresholds were equated across cohorts but separate thresholds were estimated for boys/girls. For quantity, thresholds were estimated separately for each cohort and gender.

Table 10 Variance decomposition under the Single Liability Dimension model: model fitting results for alcohol frequency and quantity in age group 18-21, on pooled cohorts

		n par	-2LL (df)	χ^2 (Δdf)	p	vs
Frequency						
model 1	ACE model without sex difference	8	5031.778 (2306)			
model 2	CE model	7	5053.232 (2307)	21.454 (1)	<.0001	1
model 3	AE model	7	5035.596 (2307)	3.818 (1)	.0507	1
Quantity						
model 1	ACE model without sex difference	15	4309.554 (2119)			
model 2	CE model	14	4329.840 (2120)	20.286 (1)	<.0001	1
model 3	AE model	14	4321.560 (2120)	12.006 (1)	.0005	1

Note. A: additive genetic variance component; C: common environmental variance component; E: unique environmental variance component; n par: number of parameters estimated; -2LL: -2 loglikelihood; χ^2 (Δdf): likelihood ratio test value and difference degrees of freedom; vs: the model to which the submodel is compared. Likelihood ratio tests were evaluated at alpha=.01. Incomplete twin pairs were excluded from the analyses. Separate thresholds were estimated for boys and girls and across cohorts, for both frequency and quantity. For quantity, the genetic correlation was estimated freely in the DOS group of the 1993 cohort.

3

Developmental prediction model for early alcohol initiation in Dutch adolescents

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Abstract

Objective Multiple factors predict early alcohol initiation in teenagers. Among these are genetic risk factors, childhood behavioral problems, life events, lifestyle, and family environment. We constructed a developmental prediction model for alcohol initiation below the Dutch legal drinking age (16 years), elaborating on the pathways identified by earlier studies. *Method* A set of 22 prospectively measured variables, previously associated with alcohol initiation, was examined by path analytic techniques in a sample of 1,804 Dutch adolescents (ages 13–15 years, 56% girls). The predictors included genetic risk for alcohol initiation and behavioral/emotional problems; prenatal and childhood stressors and childhood behavioral/emotional problems; and adolescent behavioral/emotional problems, lifestyle, family functioning, and peer-related factors. *Results* The model explained 66% of variance in early alcohol initiation. Subjects at higher genetic risk of alcohol initiation who had friends who drank alcohol and who had started smoking at an early age were at increased risk of initiating alcohol use before age 16. Behavioral (externalizing) problems were moderately and indirectly associated with early alcohol initiation, and emotional (internalizing) problems were marginally and indirectly associated with alcohol initiation. *Conclusions* The Netherlands has relatively lenient alcohol laws. In this permissive environment, early alcohol initiation is explained by alcohol-specific genetic risk, smoking initiation, and peer-related factors, whereas behavioral and emotional problems are only indirectly related to early alcohol initiation.

Introduction

Early initiation of alcohol use is associated with numerous adverse outcomes, such as increased risk of adolescent problem drinking, delinquency, risky sexual behavior, academic problems, and adult alcohol dependence (e.g. Donovan & Molina, 2011). The timing of alcohol initiation is associated with multiple factors occurring throughout development that either increase risk of early initiation or protect against it (Kendler et al., 2011b; Zucker et al., 2008). We aim to determine which factors are the most powerful in predicting whether Dutch adolescents start drinking alcohol before reaching the minimum legal age. The Netherlands has relatively permissive alcohol laws—buying soft alcoholic beverages (beer, wine, and distilled drinks containing under 15% alcohol by volume) is legal from age 16, and to purchase strong alcoholic drinks (distilled drinks containing at least 15% alcohol by volume) the buyer must be 18 years old (Ministry of Health Welfare & Sport, 2009). These laws are not always strictly enforced, and buying alcoholic beverages is often possible for those younger than 16 years (van Hoof et al., 2011). Moreover, parental attitudes toward early drinking are lenient; more than 50% of teenagers younger than 16 years are allowed to drink alcohol at home (van Laar et al., 2010).

Below, we first review the literature on risk and protective factors, ranging from prenatal exposure to adolescence, that have been associated with timing of alcohol initiation. A large set of risk and protective factors was assessed in Dutch adolescents (1,007 girls and 797 boys), and these factors are examined simultaneously in a prediction model for alcohol initiation before age 16.

Sex

Donovan (2004) concluded in a review on predictors of alcohol initiation that there was no convincing evidence that sex influences timing of alcohol initiation. This finding has since been corroborated in several American samples (Donovan & Molina, 2011; Goldschmidt et al., 2011; Malone et al., 2012). However, in an American sample, male sex was associated with earlier alcohol initiation, and in Dutch adolescents, more boys than girls had started drinking before age 16 (Geels et al., 2012; Poelen et al., 2005; Sartor et al., 2007). In contrast, in an Australian sample and in the Finnish Twin studies, girls started drinking earlier than boys (Heath & Martin, 1988; Rose et al., 2001b; Viken et al., 1999).

Genetic risk for alcohol use and comorbid disorders

Alcohol use by family members predicts adolescent early alcohol initiation and use. Early regular drinking of the co-twin is more strongly related to adolescent alcohol use in monozygotic twin pairs than in dizygotic twin pairs (Poelen et al.,

2007). These results indicate that the predictive value of familial alcohol initiation/use is partly attributable to shared genes, in addition to shared family environment. The timing of parental alcohol initiation also predicts when children will start drinking alcohol (Donovan, 2004). Hopfer (2003) reviewed twin studies on alcohol initiation and reported genetic influences between 14% and 40%. In Dutch twins, the genetic influence on alcohol initiation was 31% (Geels et al., 2012), and in an Australian sample, 36% (Sartor et al., 2009). Alcohol initiation is associated with behavioral (externalizing) problems, and this comorbidity likely results from a common, highly heritable vulnerability to disinhibitory behavior (review by Hicks et al., 2011; Kendler et al., 2003; Zucker et al., 2008).

Prenatal exposure and childhood stressors

Prenatal alcohol exposure has been associated with childhood externalizing problems, adolescent conduct-disorder symptoms, and alcohol disorders in Australian and American studies (Alati et al., 2006; D'Onofrio et al., 2007; Disney et al., 2008). Maternal prenatal smoking has been related to adolescent and adult behavioral (externalizing) problems and early alcohol initiation (Cornelius & Day, 2009; Goldschmidt et al., 2011; Knopik, 2009; Paradis et al., 2011). These associations are commonly observed, but to what extent they reflect causal, teratogenic effects of prenatal exposure or confounding effects of genetic or shared environmental factors is unclear (Thapar & Rutter, 2009). Childhood stressors such as parental divorce are related to early alcohol initiation (McCarty et al., 2011; Sartor et al., 2007). There is some evidence that low socioeconomic status (SES) is related to early alcohol use (reviews by Wiles et al., 2007; Zucker et al., 2008), although Donovan (2004) concluded that childhood SES does not affect early alcohol initiation.

Childhood behavioral and emotional problems

Childhood behavioral problems (e.g., impulsivity, hyperactivity, and aggressiveness) are strongly related to alcohol initiation (reviews by Donovan, 2004; Zucker et al., 2008). In samples from the United States, Canada, Finland, and New Zealand, conduct disorder, attention-deficit/hyperactivity disorder (ADHD), and delinquent behavior as early as ages 3–5 years have been related to early alcohol initiation (Mayzer et al., 2009; Sartor et al., 2007). Nonsignificant associations between childhood ADHD and later alcohol initiation/use have also been reported (review by Zucker et al., 2008). The relationship between childhood emotional (internalizing) problems and alcohol initiation is less well established and more ambiguous. Internalizing

psychopathology is associated with early alcohol initiation, but some internalizing symptoms, such as withdrawn behavior, have also been found to be protective against alcohol initiation (review by Donovan, 2004; Hussong et al., 2011; review by Zucker et al., 2008).

Adolescent predictors

Behavioral problems during adolescence (e.g., impulsivity, disinhibition, and attention problems) are highly comorbid with alcohol initiation (Anderson & Brown, 2011; Donovan, 2004; Goldschmidt et al., 2011; Iacono et al., 2008). Alcohol initiation is also related to aspects of sensation seeking (e.g., boredom susceptibility) (Koopmans, 1997). Kendler et al. (2011b) used a path modeling approach to predict adolescent alcohol use and symptoms of alcohol use disorder in young adult male American twins and observed a strong externalizing pathway. Emotional problems in adolescents, such as depression and anxiety, co-occur with alcohol initiation, although associations are often weaker than with externalizing problems. Moreover, some aspects (e.g., withdrawn behavior) may protect against alcohol initiation (Hussong et al., 2011). Kendler et al. (2011b) similarly observed weak and mixed associations of internalizing symptoms on adolescent alcohol use and symptoms of alcohol use disorder. Early alcohol initiation is related to behavioral and emotional problems, and heavy alcohol use has been associated with lower well-being and decreased life satisfaction in Australian and Finnish adults (Dear et al., 2002; Koivumaa-Honkanen et al., 2012). Therefore, general well-being may protect against early alcohol initiation.

Early alcohol initiation is strongly associated with characteristics of friends and peers. Peer group deviancy/delinquency and peer alcohol use are important predictors of early alcohol initiation (Anderson et al., 2011; Donovan & Molina, 2011; Trucco et al., 2011). Another chief predictor of alcohol initiation is the family environment. Positive parental attitudes toward alcohol use and alcohol availability at home predict whether adolescents start drinking early (Donovan & Molina, 2011; Hung et al., 2009). General parenting skills (e.g., less strict, less involved parenting) as well as lower familial support and more family conflict increase risk of early initiation (Donovan & Molina, 2011; Goldschmidt et al., 2011; Hung et al., 2009; Ryan et al., 2010). Living with a single parent or a stepparent also adds to risk of early initiation (review by Donovan, 2004; Donovan & Molina, 2011). In contrast, American and Lithuanian studies show that eating daily dinners with family members and spending time on family activities protect against early alcohol initiation (Fisher et al., 2007; Garmiene et al., 2006). Again, the extent to which these associations reflect causal

mechanisms is unclear.

Lifestyle factors, such as smoking cigarettes, are related to alcohol initiation and early alcohol use (review by Donovan, 2004; Fisher et al., 2007; Koopmans, 1997; MacArthur et al., 2012). Exercise behavior has not been linked specifically to initiation but is protective against adolescent alcohol use (Terry-McElrath et al., 2011). Less religious behavior increases risk of early alcohol initiation in some studies (Donovan & Molina, 2011) but not in others (Koopmans et al., 1999b). School-related factors are associated with timing of alcohol initiation as well. Lower expectations for school achievement, negative attitudes toward school, and lower grades are associated with early alcohol initiation (Donovan, 2004; Donovan & Molina, 2011). Last, degree of urbanization may be associated with alcohol initiation in that living in a more rural environment has been linked to increased alcohol use in American adolescents (Swaim & Stanley, 2011).

Aim of the present study

A predictive model of risk and protective factors—identified from the literature—for alcohol initiation was developed and tested on data that were prospectively collected in Dutch adolescents. We based our approach on the path model proposed by Kendler et al. (2011b), which predicted adolescent alcohol use and symptoms of alcohol use disorder in a sample of American twins. Data on alcohol initiation that were collected in a population-based sample of Dutch adolescents (1,804 twin pairs) ages 13–15 years from the Netherlands Twin Register were analyzed. A set of 22 risk and protective factors, prospectively collected in this group, were evaluated. These included genetic risk factors, and variables measured in childhood and adolescence. By examining all factors simultaneously, we assessed which factors are associated with early alcohol initiation and whether associations reflected direct or indirect effects.

Methods

Sample

Participants were registered with the Netherlands Twin Register at birth. Recruitment for the Netherlands Twin Register started in 1987 at the VU University Amsterdam and is ongoing at present (Boomsma et al., 2006). Survey data are collected longitudinally in young twins, starting with maternal reports on the pregnancy, health, and temperament of the twins during their first 2 years of life. Parental reports on behavioral and emotional problems, health, school performance and SES are collected at ages 3, 5, 7, 10, and 12

years. Data collection and participation rates have been described in Bartels et al. (2007b). When twins are 14, 16, and 18 years old, they are invited to complete self-report questionnaires on topics such as health, lifestyle, behavior problems, well-being, and school performance. Descriptions of data collection and response rates can be found in Bartels et al. (2011).

The data included in this study comprise maternal reports on alcohol use and cigarette smoking during pregnancy; maternal reports on childhood behavioral problems, emotional problems, attention problems, and SES; and adolescent self-reports on behavioral and emotional problems, lifestyle (smoking, exercise behavior), family functioning, well-being, amount of time spent with friends, peer alcohol use, urbanization, religiousness, and school performance. Data from the adolescent survey were available for 6,217 twins (individuals) between ages 13 and 15 years, of whom 5,898 had stated whether they had initiated alcohol use (2,637 complete twin pairs). Data on alcohol initiation and all predictor variables were available for 1,804 complete twin pairs. From each twin pair, one member was randomly selected as the index case, and data from his or her co-twin were used to specify the genetic risk variables. Subjects ranged in age from 13 to 15 (1.6% were 13 years old, 65.3% were 14, and 33.1% were 15 years old). Slightly more girls than boys participated (56%).

Measures

Early alcohol initiation was defined as ever having used alcohol (at age 13–15). Response categories were *no, a few times,* and *yes.* The categories *a few times* and *yes* were collapsed, creating a binary variable. Table 1 shows all predictor variables and their measurement scales.

Genetic risk for alcohol use and co-morbid disorders. Genetic risk for alcohol initiation, internalizing, and externalizing problems were indexed from co-twin data. Internalizing and externalizing problems were assessed with the Youth Self-Report (Achenbach & Rescorla, 2001). The internalizing scale consists of 32 items and the externalizing scale of 30 items. To obtain genetic risk measures for internalizing and externalizing problems, continuous scores were first transformed into z scores. Zygosity was used as a weight factor to correct for the difference in genetic similarity between mono- and dizygotic twins (cf. Kendler et al., 2011b). In regression terms, the outcome variable was predicted differentially for mono- and dizygotic twins: $Y = \beta X$ for monozygotic twins, and $Y = 0.5 \times \beta X$ for dizygotic twins, where X could be externalizing, internalizing, or alcohol initiation.

Prenatal and childhood predictors. Prenatal alcohol and tobacco exposure were obtained shortly after birth of the twins by asking mothers if they had

used cigarettes (ranging from *no* to *more than 10 cigarettes per day*) or alcohol (ranging from *no* to *more than one glass per week*) in the first pregnancy trimester, the last trimester, or during the entire pregnancy. Most mothers had not used any alcohol while pregnant (80%; n = 1,440), 4% had used alcohol in the first trimester (n = 72), 6% in the last trimester (n = 105), and 10% throughout the entire pregnancy (n = 187). A total of 81% of mothers had not smoked while pregnant (n = 1,457), 3% had smoked in the first trimester (n = 60), 2% in the last trimester (n = 41), and 14% had smoked during the entire pregnancy (n = 246). The categories of both variables were collapsed to *no* versus *any alcohol use/smoking* because cross-classification with other variables in the model resulted in empty cells.

Childhood externalizing, internalizing, and attention problems were measured with the Child Behavior Checklist (Achenbach, 1992; Achenbach & Rescorla, 2001), completed by mothers when twins were 3, 7, 10, and 12 years old (Bartels et al., 2007b). For each of these scales, longitudinal measurements were summarized in a single score, which was based on t scores and represented low, middle, or high probability of externalizing, internalizing, or attention problems. Subjects were classified as scoring high if they had $t \geq 65$ at least once and $t \geq 60$ at every available assessment. Subjects scoring $t \leq 55$ at each available time point were classified as low scorers, and if they scored in between they were in the middle category (cf. Lehn et al., 2007). Childhood SES was measured longitudinally between ages 3 and 10 years. The most recent SES data available were used. The coding followed that of Statistics Netherlands (2001), based on the mental complexity of parental occupation (Lehn et al., 2007). SES had six categories, ranging from unemployed to academic, which were collapsed into three categories (low, middle, and high). Subjects were retrospectively asked about parental divorce in the adolescent self-report survey.

Table 1 Overview of model variables, grouped by developmental timing

Genetic risk for alcohol use and co-morbid disorders	
Genetic risk for alcohol initiation	0= having a non-drinking mz co-twin, 1= having a non-drinking dz co-twin, 2=having a drinking dz co-twin, 3= having a drinking mz co-twin
Genetic risk for externalizing	continuous, range -1.53 – 6.85, high scores indicating high risk
Genetic risk for internalizing	continuous, range -1.24 – 4.72, high scores indicating high risk
Sex	0=male, 1=female
Prenatal and childhood predictors	
Smoking during pregnancy	0=not exposed, 1=exposed
Alcohol during pregnancy	0=not exposed, 1=exposed
Childhood externalizing behavior prob.	0=low, 1=middle, 2=high
Childhood internalizing behavior prob	0=low, 1=middle, 2=high
Childhood attention problems	0=low, 1=middle, 2=high
Childhood socioeconomic status	0=low, 1=middle, 2=high
Parental divorce	0=not divorced, 1=divorced
Adolescent predictors	
Family Functioning	continuous, range 1.20 – 4.80, high scores indicating good family functioning*
Adolescent Externalizing	continuous, range .00 – 5.80, high scores indicating more externalizing problems*
Adolescent Internalizing	continuous, range .00 – 5.40, high scores indicating more internalizing problems*
Urbanization	continuous, range 1-5, high score indicating low urbanization level
Well-Being	continuous, range 1.00 – 6.30, high scores indicating higher well-being*
Socializing with friends	continuous, range 3-21, high scores indicating more frequent socializing with friends
Regular Exercise	0=don't exercise regularly, 1= exercise regularly
Peer Alcohol Use	0= none of friends drink alcohol, 1=1-5 friends drink alcohol , 2= >5 friends drink alcohol
Smoking Initiation	0=not initiated smoking, 1=initiated smoking
Religiousness	0=not religious, 1=religious
Secondary school level	0=low, 1=middle, 2=high

*to avoid computational difficulties with model fitting due to large variance differences, all scores on these scales were divided by 10.

Adolescent predictors. Degree of urbanization of the residential area was a continuous variable, ranging between 1 (*highly urban*) and 5 (*not urban*). Data were based on participants' postal code and obtained from Statistics Netherlands (cf. Willemsen et al., 2005). Secondary school level was measured by asking adolescents which level of secondary school they were in or had last been in (low, middle, high) when completing the questionnaire. In the Dutch education system, there are different levels of secondary school, ranging from lower professional education to pre-university education, suited to the students' capabilities (National Reference Point, 2009). Family functioning was measured with the general family functioning subscale of the McMaster Family Assessment Device (De Coole & Jansma, 1983; Epstein et al., 1983). Subjective well-being was indexed with a sumscore of the Satisfaction with Life Scale and the Subjective Well-being Scale (Diener et al., 1985; Lyubomirsky & Lepper, 1999).

Smoking initiation was indexed by asking subjects whether they had ever smoked. Answer categories were *no, a few times,* and *yes.* The latter two categories were collapsed. Religiousness was defined as being religious (yes/no) when completing the survey. Regular exercise was measured by asking subjects if they exercised regularly (yes/no). Subjects were asked about the frequency with which they spent leisure time with friends in their own home, in the homes of friends, and on the street. Answer categories were 1 (*never*), 2 (*once until now*), 3 (*less than once a week*), 4 (*once a week*), 5 (*a few days per week*), 6 (*almost daily*), and 7 (*daily*). Scores on these three items were summed into an overall score for frequency of socializing with friends, ranging from 3 to 21 (cf. van der Aa et al., 2012). Peer alcohol use was measured by asking participants how many of their friends used alcohol. The answer categories were none, one friend, two to five friends, and more than five friends. The two middle categories were infrequently endorsed and were therefore collapsed into *one to five friends.*

Model

A path model was specified in Mplus 5.21 (Muthén & Muthén, 2010) in which variables were grouped in the model according to developmental timing (Table 1). A fully saturated model was specified in which each variable was related to all other variables. Within developmental groups (genetic risk, prenatal, childhood, adolescence), the covariance between each pair of variables was estimated. Between developmental groups, regressions were specified between each pair of variables. The variables in the genetic risk group functioned solely as independent variables, predicting all downstream variables. Alcohol

initiation, the final outcome variable, only functioned as a dependent variable. The variables in the intermediate groups (prenatal, childhood, adolescence) had multiple functions in the model. Each functioned as an independent variable, predicting all downstream variables. These intermediate variables also functioned as dependent variables, being predicted by all upstream variables. The continuous variables (family functioning, internalizing, externalizing, urbanization, well-being, socializing with friends) were predicted with linear regressions. The binary and categorical variables (all prenatal and childhood factors, regular exercise, peer alcohol use, smoking initiation, religiousness, secondary school level) were assumed to reflect an underlying normal distribution. These variables were analyzed with probit regressions and predict probability of the categories of the dependent variable with a linear combination of predictors, multiplied by the cumulative distribution function (Garwood, 1941).

All nonsignificant regression coefficients or covariances were removed (constrained at 0) from the saturated model. Parameter significance was determined by evaluating whether the parameter z value (parameter estimate divided by its standard error) was significant according to the z distribution. Parameters were removed sequentially, starting with those with the smallest z values (cf. Kendler et al., 2011b). While dropping parameters, model fit was evaluated using three statistics: the Tucker–Lewis Index (TLI), comparative fit index (CFI), and root mean square error of approximation (RMSEA). For the CFI and TLI, values greater than .95 indicate good model fit. RMSEA values below .05 reflect good model fit (Schermelleh-Engel et al., 2003; Tucker & Lewis, 1973). A parsimonious model was created by removing nonsignificant parameters until the fit statistics reached these boundaries.

Because the model contained ordinal variables, weighted mean squares estimation with the theta parameterization was used. This parameterization allows estimation of the residual variance of the normally distributed variable assumed to underlie each categorical variable (Muthén & Muthén, 2010).

Results

Sample characteristics

A total of 1,189 (65.9%) adolescents between ages 13 and 15 years stated that they had initiated alcohol use. Table 2 shows the mean and prevalence of all model variables. The distributions of genetic risk for internalizing and externalizing problems were skewed, with more observations in the lower range of genetic risk. A similar distribution was observed for genetic risk for alcohol initiation. A total of 20% of the subjects had been prenatally exposed to alcohol

and 19% to tobacco. Parental divorce was reported by 12% of the subjects. More than half of the subjects had low probability of childhood externalizing problems (55.1%), 41.6% of subjects were classified in the middle category, and 3.3% of the subjects had high probability of childhood externalizing problems. Very similar distributions were observed for childhood internalizing and attention problems (Table 2). Nearly 17% of the subjects had low childhood SES, 44.5% were classified as having intermediate childhood SES, and 38.8% had high childhood SES. About 42% of adolescents stated that they were religious when completing the survey. Low level of secondary school was reported by 41.9%, intermediate school level by 25.9%, and high school level by 32.2%. The average frequency of socializing with friends was 10.91 ($SD = 3.65$), and 42.7% of the subjects had more than five friends who used alcohol. A total of 21% of the subjects had initiated smoking, and 87% exercised regularly (Table 2).

Correlations

Table 3 shows correlations between all predictor variables and alcohol initiation. These correlations show that alcohol initiation was most strongly associated with genetic risk for alcohol initiation, smoking initiation, and peer alcohol use. Moderate positive correlations were observed with prenatal alcohol and tobacco exposure, childhood externalizing behaviors, parental divorce, regular exercise, genetic risk for externalizing, adolescent externalizing, urbanization, and socializing with friends. Alcohol initiation was negatively associated with SES, family functioning, well-being, religiousness, and secondary school level. The correlations further show clustering between externalizing and substance use measures. These variables were weakly related to the variables indexing internalizing psychopathology. Externalizing and internalizing variables were associated with adverse family environment (higher probability of parental divorce, poor family functioning). Higher SES was associated with good family functioning, more regular exercise, and higher secondary school level but lower probability of being religious and of having internalizing and externalizing problems.

Model fitting results

The final, best fitting model had TLI and CFI = .95 and RMSEA = .04 and explained 66% of variance in alcohol initiation.

Table 2 Mean, standard deviation, and range for continuous model variables and frequency distributions/prevalences of categorical/binary model variables

Genetic risk for alcohol use and co-morbid disorders	*Mean*	*SD*	*Range*
Genetic risk externalizing problems	-.03	.74	-1.53 – 6.85
Genetic risk internalizing problems	.00	.73	-1.24 – 4.72
		Distribution	*%*
Genetic risk alcohol initiation	0:	258	14.3
	1:	366	20.3
	2:	740	41.0
	3:	440	24.4
Sex	Girls:	1007	55.8
Prenatal and childhood predictors		*Distribution*	*%*
Childhood externalizing problems	Low	994	55.1
	Middle	750	41.6
	High	60	3.3
Childhood internalizing problems	Low	993	55.0
	Middle	769	42.6
	High	42	2.3
Childhood attention problems	Low	932	51.7
	Middle	832	46.1
	High	40	2.2
Childhood socioeconomic status	Low	301	16.7
	Middle	803	44.5
	High	700	38.8
		Prevalence	*%*
Prenatal alcohol exposure		364	20.2
Prenatal tobacco exposure		347	19.2
Parental divorce		217	12.0
A*dolescent predictors*	*Mean*	*SD*	*Range*
Family Functioning	3.88	.51	1.20 – 4.80
Adolescent Externalizing	.83	.55	.00 – 5.80
Adolescent Internalizing	.85	.70	.00 – 5.40
Urbanization	3.46	1.17	1.00 – 5.00
Socializing with friends	10.91	3.65	3.00 – 21.00
Well-being	5.06	.89	1.00 – 6.30
		Distribution	*%*
Peer alcohol use	None	425	23.6
	1-5 friends	608	33.7
	>5 friends	771	42.7
Secondary school level	Low	756	41.9
	Middle	468	25.9
	High	580	32.2
		Prevalence	*%*
Religiousness		762	42.2
Smoking initiation		376	20.8
Regular exercise		1562	86.6

Note. SD, standard deviation; %, percentage.

Table 3 Observed correlations between all model variables and alcohol initiation.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
1. Gen. risk alc in.																						
2. Gen. risk Ext.	.26																					
3. Childh. Ext.	.09	.20																				
4. Adol. Ext.	.16	.41	.27																			
5. Child. Att. Pr.	.05	.14	.68	.18																		
6. Social. w friends	.20	.16	.08	.23	.04																	
7. Smoking init.	.31	.28	.22	.39	.16	.36																
8. Peer alcohol use	.32	.19	.13	.27	.06	.29	.45															
9. Gen. risk Int.	.03	.44	.14	.23	.10	-.07	.05	.06														
10. Childh. Int.	-.01	.14	.55	.14	.49	-.01	.09	-.03	.20													
11. Adol. Int.	.03	.22	.11	.44	.14	-.07	.17	.07	.39	.22												
12. Parent. divorce	.12	.12	.07	.13	.18	.08	.22	.13	.07	.08	.12											
13. Family Funct.	-.08	-.18	-.13	-.24	-.06	-.03	-.23	-.12	-.20	-.09	-.32	-.17										
14. SES	-.03	-.07	-.14	-.06	-.10	.01	-.08	-.05	-.04	-.08	-.07	-.06	.08									
15. Regular exerc.	.06	-.06	-.03	.00	-.10	.17	-.12	.05	-.11	-.18	-.23	-.10	.05	.16								
16. Second. School	-.07	-.10	-.21	-.10	-.33	-.15	-.25	-.17	.01	-.09	-.05	-.15	.07	.39	.16							
17, Religiousness	.00	-.12	-.07	-.04	-.05	-.09	-.12	-.08	-.05	-.06	.02	-.20	.06	-.07	-.03	.00						
18. Well-being	-.06	-.16	-.12	-.24	-.10	.05	-.22	-.07	-.26	-.14	-.52	-.16	.46	.08	.16	.13	.01					
19. Urbanization	.03	-.09	.01	-.05	.00	-.04	-.02	.09	-.07	-.03	-.05	-.13	-.04	-.10	.06	-.09	.21	.00				
20. Sex	.01	-.05	-.02	-.04	-.03	-.03	-.04	.07	.09	-.05	.31	-.03	-.01	-.03	.01	-.02	.09	-.07	.01			
21. Alcohol preg.	.06	.10	-.04	.04	-.06	.05	.04	.03	.02	-.08	-.04	-.01	-.02	.31	.05	.25	-.15	.06	-.13	-.06		
22. Smoking preg.	.06	.10	.18	.09	.13	.11	.20	.07	.06	.05	.04	.09	-.02	-.25	-.09	-.23	-.19	-.05	-.04	.00	-.01	
Alcohol Initiation	.62	.26	.15	.32	.01	.28	.64	.54	.03	-.03	.05	.17	-.13	-.07	.10	-.10	-.07	-.11	.07	-.01	.15	.18

Note. For each pair of variables where both were binary/ordinal, a tetra- or polychoric correlation was estimated. For each pair where both variables were continuous, a Pearson correlation was estimated and for pairs of variables where one was continuous and the other binary/ordinal, a polyserial correlation was estimated. Gen. = genetic; alc. = alcohol; init. = initiation; extern. = externalizing; child. = childhood; adol. = adolescent; att. = attention; prob. = problems; social. = socializing; w/ = with; intern. = internalizing; SES = socioeconomic status; second. = secondary. Black font: correlation is significant at alpha=.01.

Direct and indirect associations with alcohol initiation

The standardized partial regression coefficients show that genetic risk for alcohol initiation, smoking initiation, and peer alcohol use directly predicted alcohol initiation (Figure 1). The influence of genetic risk for alcohol initiation was partly direct and partly mediated through smoking initiation, peer alcohol use, and socializing with friends.

The correlations, predicted under the best fitting model, reflect the total association between variables (Table 4). Based on these correlations and the standardized partial regression coefficients (Figure 1), the contribution of a direct path (regression coefficient) between two variables in the model can be separated from the total association between those variables (cf. Kendler et al., 2011b). The predicted correlation between alcohol initiation and genetic risk for alcohol initiation was .61 (Table 4). The direct path between these variables was .40 (Figure 1), indicating that 66% (.40 / .61) of the association between alcohol initiation and genetic risk for alcohol initiation was direct, whereas the remaining 34% was mediated through peer alcohol use, socializing with friends, and smoking initiation (Figure 1). The predicted correlation between alcohol initiation and peer alcohol use was .54 and the regression coefficient was .23. This means that 43% (.23 / .54) of the association between peer alcohol use and alcohol initiation was direct, and that 57% of the association was mediated by other factors. The correlation between smoking initiation and alcohol initiation was .67 and the direct path was .44; therefore, 66% (.44 / .67) of the association between smoking and alcohol initiation was explained by the direct path. Genetic risk for internalizing and externalizing problems, and sex, were indirectly associated with alcohol initiation. Genetic risk for externalizing problems predicted smoking initiation and peer alcohol use, which were positively related to alcohol initiation. Genetic risk for internalizing problems was negatively related to socializing with friends, which was indirectly related to alcohol initiation. Genetic risk for alcohol initiation, in addition to predicting alcohol initiation, was associated with peer alcohol use, smoking initiation, and socializing with friends (Figure 1). Within the genetic risk group, genetic risk for alcohol initiation was associated with genetic risk for externalizing problems and genetic risk for internalizing psychopathology.

None of the childhood factors directly predicted alcohol initiation, but some were associated with adolescent factors, which in turn were associated with alcohol initiation (Figure 1). Maternal prenatal smoking and parental divorce were associated with higher probability of smoking initiation, which in turn was strongly related to increased risk of alcohol initiation.

Peer alcohol use and smoking initiation were directly associated with

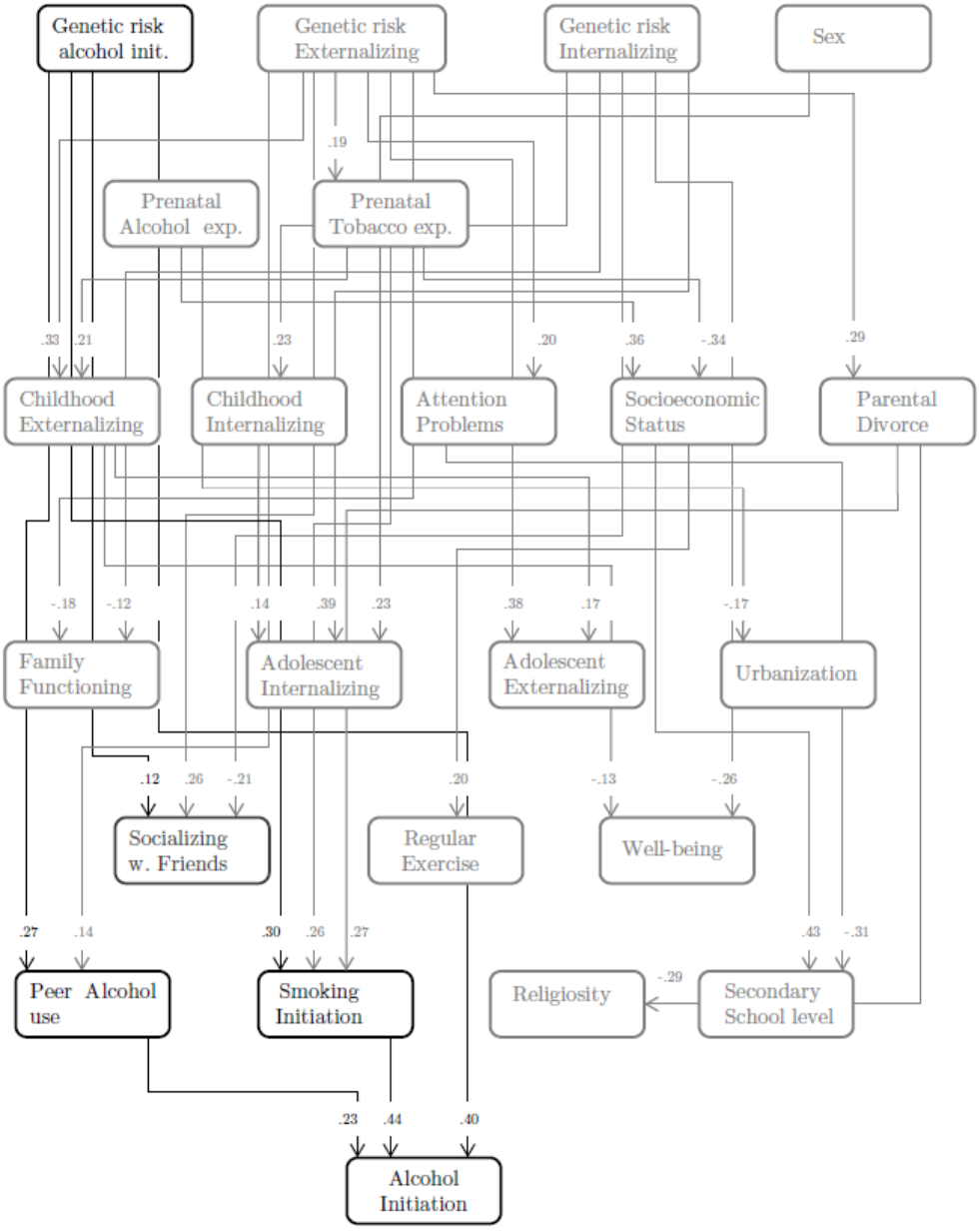


FIGURE 1 Standardized partial regression coefficients estimated under the best fitting model. Variables are grouped by developmental timing (genetic risk, prenatal, childhood, and adolescence). Pathways directly related to alcohol initiation are depicted in black, the indirect pathways are shown in grey.

Table 4 Predicted correlations between all model variables and alcohol initiaton.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
1. Gen. risk alc init																						
2. Gen. risk Extern.	.31																					
3. Childh. Extern.	.08	.26																				
4. Adol. Extern.	.13	.43	.27																			
5. Child. Att. Prob.	.06	.20	.67	.19																		
6. Social. w. friends	.19	.19	.05	.23	.04																	
7. Smoking init.	.34	.22	.11	.40	.04	.36																
8. Peer alcohol use	.31	.23	.06	.22	.05	.29	.44															
9. Gen. risk Intern.	.00	.48	.13	.21	.10	-.09	.06	.07														
10. Childh. Intern.	.00	.11	.55	.13	.48	-.02	.01	.02	.23													
11. Adol. Intern.	.00	.20	.12	.44	.10	-.04	.03	.03	.42	.23												
12. Parental divorce	.09	.29	.08	.10	.06	.05	.31	.06	.14	.03	.06											
13. Family Funct.	-.05	-.23	-.06	-.24	-.05	-.02	-.23	-.05	-.21	-.05	-.32	-.07										
14. SES	-.02	-.07	-.08	-.04	-.01	-.16	-.10	-.02	-.03	-.01	-.01	-.02	.02									
15. Regular exercise	.00	-.01	-.02	-.01	.00	.17	-.02	.00	-.01	.00	-.22	.00	.00	.20								
16. Second. School	-.03	-.09	-.24	-.07	-.31	-.15	-.23	-.16	-.04	-.15	-.04	-.03	.02	.43	.09							
17, Religiousness	-.02	-.08	-.02	-.02	-.02	-.05	-.09	-.02	-.04	-.01	-.01	-.29	.02	.01	.00	.01						
18. Well-being	-.01	-.16	-.16	-.24	-.11	.02	-.22	-.03	-.27	-.13	-.52	-.04	.46	.02	.16	.04	.01					
19. Urbanization	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	-.06	-.01	-.03	.21	.00				
20. Sex	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.23	.00	.00	.00	.00	.00	.00	.00	.00			
21. Alcohol preg.	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.36	.07	.15	.00	.00	-.16	.00		
22. Smoking preg.	.06	.19	.25	.11	.04	.04	.29	.04	.09	.02	.04	.06	-.05	-.34	-.07	-.16	-.02	-.06	.00	.00	.00	
Alcohol Initiation	.61	.27	.09	.29	.05	.30	.67	.54	.04	.01	.02	.19	-.13	-.05	-.01	-.15	-.05	-.10	.00	.00	.00	.16

Note. For correlations where one or both variables were continuous, covariance was standardized with estimated variance(s). Gen. = genetic; alc. = alcohol; init. = initiation; external. = externalizing; childh. = childhood; adol. = adolescent; att. = attention; prob. = problems; social. = socializing; w/ = with; internal. = internalizing; SES = socioeconomic status; second. = secondary; lvl. = level.

alcohol initiation. During adolescence, they were associated with internalizing and externalizing problems and socializing with friends. These variables were related to poor family functioning, well-being, and secondary school level, which in turn were indirectly related to increased risk of alcohol initiation (Figure 1).

Discussion

A developmental model was constructed in a Dutch adolescent sample (ages 13–15 years) to predict early initiation of alcohol use. A comprehensive set of risk and protective factors, prospectively measured throughout childhood, was evaluated. Direct and indirect associations with alcohol initiation were examined by simultaneously including all factors in the model.

The best model explained 66% of variance in alcohol initiation. Three predictors were directly related to early alcohol initiation: Adolescents who were at higher alcohol-specific genetic risk, who had friends who used alcohol, and who had started smoking were at increased risk of initiating alcohol use early. Adolescents with increased alcohol-specific genetic risk were likely to spend more time with friends, which in turn was directly related to higher levels of peer alcohol use and smoking initiation. The commonly observed association between early alcohol initiation and externalizing behavior was confirmed (r = .32), but in the prediction model this relationship was mediated through other variables. Considered separately, the influence of alcohol-specific genetic risk, peer characteristics, and adolescent smoking on alcohol initiation has previously been demonstrated (e.g. Anderson et al., 2011; Fisher et al., 2007; Geels et al., 2012).

We contribute to the knowledge on determinants of early alcohol initiation showing, in contrast to previous findings, that in a permissive environment such as The Netherlands, alcohol initiation is moderately and indirectly related to behavioral problems and only marginally and indirectly related to emotional (internalizing) problems. These differences are obvious when we relate our findings to those of Kendler et al. (2011b), who constructed a similar model predicting alcohol use (ages 15–17 years) and symptomsof alcohol use disorders in young adult American men. A genetic risk/externalizing pathway, social/familial pathway, and minor internalizing pathway were observed. One may hypothesize that the differences between these findings reflect an interaction between alcohol predictors and cultural attitudes toward early alcohol use. The Netherlands has permissive views on early alcohol use, whereas in the United States early alcohol use is considered a much greater social and behavioral problem. This is reflected in the minimum legal ages for buying alcohol: age 21 in the United States versus age 16 in The Netherlands (WHO,

2004). Kendler et al. (2011b) examined alcohol use and symptoms of alcohol use disorder, whereas the outcome in the present study was alcohol initiation. It is possible that the association with behavioral and emotional problems was weaker in this study because these factors may be more strongly related to more severe forms of alcohol use.

An alternative explanation is that the variables that were related to alcohol initiation in fact reflect an underlying risk factor for externalizing behavior. Genetic risk for alcohol initiation may capture not only alcohol-specific genetic risk but also risk for other aspects of externalizing behavior since it was strongly related to socializing with friends, peer alcohol use, and smoking initiation. Moreover, genetic risk was based on co-twin alcohol use, and adolescent alcohol use is influenced by a general externalizing factor (K. S. Kendler et al., 2011a). Socializing with friends and peer alcohol use may be expressions of the same underlying trait, because adolescents who are more genetically predisposed to drink alcohol tend to select friends who also drink alcohol (Agrawal et al., 2010a; Hill et al., 2008). Similarly, the association between cigarette and alcohol use is likely attributable to underlying risk for externalizing behavior (Little, 2000). Alcohol initiation may be related to less severe forms of externalizing behavior than those measured by the Youth Self-Report (Achenbach & Rescorla, 2001). More serious behavioral problems may be related to more advanced forms of adolescent alcohol use.

The simultaneous modeling of many predictors showed that previously observed associations with alcohol initiation may be mediated through other factors. For example, low school grades have been related to early alcohol initiation (Donovan, 2004), but this study shows that the relationship between secondary school level and alcohol initiation was mediated through peer alcohol use and smoking initiation. Similarly, family functioning was not directly associated with alcohol initiation, as previously observed by Hung et al. (2009) and others, but mediated through smoking initiation. These mediation effects might be explained by interpreting peer alcohol use and smoking initiation as expressions of a general underlying externalizing trait that influences secondary school level, family functioning, and alcohol initiation. Genetic risk factors were significant predictors of early alcohol initiation. Estimating genetic risk requires data from biological relatives such as twins or parents, which raises questions regarding the predictive value of the model if genetic risk data are unavailable. In an additional analysis, the best fitting model was rerun excluding the genetic risk variables. The remaining factors explained 52.6% of variance in alcohol initiation, suggesting that alcohol initiation can still be predicted quite well when genetic risk data are unavailable (results available on request).

Because of the large number of factors included, only main effects were examined. Predictive factors likely do not influence alcohol initiation independently but also interact with each other. For example, Kendler et al. (2011a) observed that genetic risk for adolescent alcohol consumption was stronger in a less restricting environment. The predictors identified in this study can provide a starting point for investigating relevant interaction effects on alcohol initiation.

The family environment was indexed by family functioning, which was not significantly associated with early alcohol initiation in the developmental model, possibly because it did not include parenting strategies, which have been consistently related to early alcohol initiation (e.g. Donovan & Molina, 2011; Goldschmidt et al., 2011; review by Ryan et al., 2010). Similarly, parental alcohol use can provide additional information on alcohol views and availability in the family environment, which are also important predictors of early alcohol initiation (Donovan & Molina, 2011; Hung et al., 2009). Parental alcohol use also provides information on genetic risk for alcohol initiation, which was based solely on co-twin data in this study. This may have led to an underestimation of genetic risk because the co-twins were still in the period of alcohol initiation, and genetic risk may not have been entirely expressed yet. In addition, it cannot be ruled out that the co-twin data contained shared environmental effects as well as genetic risk and that this could explain part of the similarity in alcohol initiation between twins (e.g. Geels et al., 2012).

In summary, in a permissive environment genetic risk for alcohol initiation, peer alcohol use, and smoking initiation were directly associated with early alcohol initiation. Other factors, including behavioral and emotional problems, were only indirectly related to early alcohol initiation.

4

Vroege alcoholinitiatie en verhoogde alcoholconsumptie op volwassen leeftijd: Oorzaak of indicator?

This chapter is submitted as:

Geels, LM, Vink, JM, van Beek, JHDA, Willemsen, G, Bartels, M, Boomsma, DI. Vroege alcoholinitiatie en verhoogde alcoholconsumptie op volwassen leeftijd: Oorzaak of indicator? Tijdschrift voor Psychiatrie.

Samenvatting

Achtergrond Alcoholinitiatie op jonge leeftijd is geassocieerd met verhoogde alcoholconsumptie en -misbruik onder volwassenen. Het is onduidelijk welke mechanismen deze associatie verklaren. *Doel* Onderzoeken of er een oorzakelijk verband bestaat tussen vroege alcoholinitiatie en latere alcoholconsumptie. *Methode* Vragenlijstgegevens werden verzameld bij deelnemers (18-80 jaar) van het Nederlands Tweelingen Register (NTR). In een eerdere studie in deze onderzoeksgroep waren correlaties tussen leeftijd van alcoholinitiatie en later drinkgedrag bepaald. Deze associaties werden in deze studie verder onderzocht met een discordant tweelingdesign (co-twin control design). Binnen ééneiige tweelingparen werden tweelingen die vroeg waren begonnen met drinken vergeleken met hun broer of zus die later was begonnen m.b.t. frequentie van alcoholgebruik, aantal glazen alcohol, aantal alcoholintoxicaties, overmatig drinken, alcoholmisbruik/-afhankelijkheid, en schadelijk drinken. Door te vergelijken binnen ééneiige tweelingparen werd gecontroleerd voor effecten van genen en gedeelde omgeving op alcoholconsumptie. *Resultaten* In het gehele NTR sample was er een verband tussen vroege initiatie en latere uitkomstmaten, maar binnen ééneiige tweelingparen verschilden de vroege drinkers niet significant van hun broer/zus. *Conclusie* Vroege alcoholinitiatie lijkt geen causaal effect te hebben op latere alcoholconsumptie. Campagnes gericht op het verhogen van de minimumleeftijd voor alcoholgebruik hebben mogelijk slechts een beperkt effect op alcoholconsumptie op volwassen leeftijd.

Inleiding

De Nederlandse overheid heeft gedurende de afgelopen decennia uitgebreid campagne gevoerd om alcoholgebruik onder jongeren terug te dringen. Daarnaast zijn controles op naleving van de minimumleeftijd voor het kopen van alcohol (16 jaar voor zwak alcoholische dranken, 18 jaar voor sterk alcoholische dranken) aangescherpt (Ministerie van Volksgezondheid Welzijn en Sport, 2012b; Nederlands Instituut voor Alcoholbeleid (STAP), 2009). Per 1 januari 2013 gaat de nieuwe Drank- en Horecawet in waardoor alcoholbezit onder de 16 jaar strafbaar zal worden (Ministerie van Volksgezondheid Welzijn en Sport, 2012a). Recente cijfers laten tussen 2003 en 2011 een afname zien in alcoholgebruik onder jongeren van 12-15 jaar, maar niet onder jongeren van 16-18 jaar (Verdurmen et al., 2012). Meerdere regeringspartijen stellen dan ook voor de minimumleeftijd voor het kopen van zwak alcoholische dranken te verhogen van 16 naar 18 jaar (Nederlands Instituut voor Alcoholbeleid (STAP), 2012).

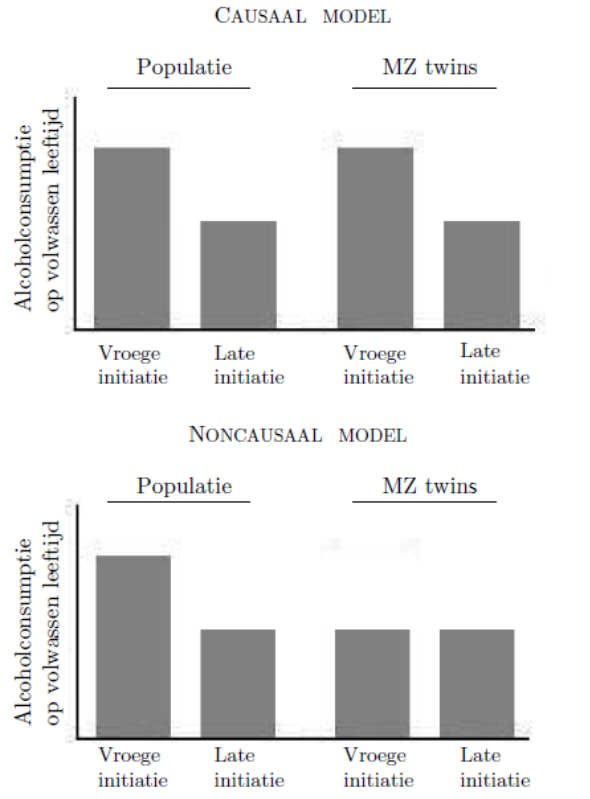
Een belangrijke reden om ernaar te streven dat jongeren op latere leeftijd beginnen met drinken is dat alcoholinitiatie op jonge leeftijd consistent is geassocieerd met verhoogde alcoholconsumptie, schadelijk alcoholgebruik, en alcoholafhankelijkheid in (jong) volwassenen (e.g. Agrawal et al., 2009; Guttmannova et al., 2011; Lee et al., 2012; Sartor et al., 2011). Een mogelijke verklaring voor deze associaties is dat alcohol veranderingen teweeg brengt in de hersenen, die tijdens de adolescentie extra kwetsbaar zouden kunnen zijn voor schadelijke invloeden. Deze veranderingen, vermoedelijk in dopamine en glutamaattransmissie en in epigenetische mechanismen, zouden verhoogd alcoholgebruik en –afhankelijkheid tot gevolg hebben (reviews door Bava & Tapert, 2010; Guerri & Pascual, 2010; Witt, 2010).

Een alternatieve verklaring voor de associatie tussen vroege alcoholinitiatie en latere alcoholconsumptie is dat de relatie tussen vroege alcoholinitiatie en latere alcoholconsumptie of -afhankelijkheid niet causaal van aard is, maar dat beide worden veroorzaakt door een onderliggende aanleg voor alcoholgebruik, die bepaald wordt door genen en/of gedeelde familieomgeving. De mate waarin deze factoren de samenhang tussen vroege alcoholinitiatie en latere alcoholconsumptie verklaren kan worden bepaald door gegevens over alcoholinitiatie en –gebruik van meerdere familieleden, met name van tweelingen, te analyseren (Neale et al., 2006). Tweelingstudies hebben vrijwel unaniem geconstateerd dat tenminste een substantieel deel van de associatie tussen vroeg alcoholgebruik en latere alcoholconsumptie of -afhankelijkheid wordt verklaard door genen en gedeelde omgevingsfactoren (e.g. McGue et al., 2001b; Sartor et al., 2009), maar zijn niet eenduidig over of vroege

alcoholinitiatie additionele causale effecten heeft. Verschillende tweelingstudies vonden geen aanwijzingen voor causale effecten: Prescott & Kendler (1999) zagen dat de associatie tussen de leeftijd waarop alcoholgebruik werd geïnitieerd en alcoholafhankelijkheid in volwassenen vrijwel geheel verklaard werd door genen en gedeelde omgeving. Vergelijkbare resultaten zijn gevonden voor de associatie tussen vroege alcoholinitiatie (≤14 jaar) en alcoholgebruik als copingsmechanisme; ook deze werd geheel verklaard door genen (Young-Wolff et al., 2012). King & Chassin (2007) corrigeerden op een andere manier voor de invloed van genen en gedeelde omgeving; zij onderzochten de associatie tussen vroege alcohol initiatie (≤13 jaar) en latere alcoholafhankelijkheid in een groep kinderen van alcoholverslaafden en een controlegroep, en vonden dat de associatie niet significant was wanneer gecorrigeerd werd voor alcoholisme van de ouders.

In een aantal tweeling- en familiestudies daarentegen, was de associatie tussen vroege alcoholinitiatie en latere alcoholconsumptie significant na corrigeren voor invloeden van genen en gedeelde omgeving, wat suggereert dat vroege blootstelling aan alcohol causale effecten heeft. In een longitudinale studie van Buchmann e.a. (2009) was de leeftijd waarop alcoholgebruik werd geïnitieerd gerelateerd aan zwaar drinken in jongvolwassenen, na correctie voor genen, externaliserend gedrag, en gedeelde omgeving. Agrawal e.a. (2009) onderzochten de associatie tussen vroege alcoholinitiatie (≤12 jaar) en alcoholafhankelijkheid met een tweelingdesign, en vonden een interactie-effect tussen vroege alcoholinitiatie en genetische invloeden, zodanig dat de invloed van genen groter was in tweelingen die eerder waren begonnen met drinken.

Samengevat: vroege initiatie van alcoholgebruik is sterk gerelateerd aan verhoogde alcoholconsumptie en alcoholafhankelijkheid op volwassen leeftijd, maar het is onduidelijk of deze associatie verklaard wordt door causale effecten van vroege blootstelling aan alcohol of door een onderliggende aanleg voor alcoholgebruik. Een sterk onderzoeksdesign dat het mogelijk maakt te controleren voor effecten van genen en gedeelde omgeving is het co-twin control design. Dit design maakt gebruik van monozygote (MZ) tweelingparen, die discordant zijn voor vroege alcoholinitiatie (de één is vroeg begonnen met het drinken van alcohol, en zijn/haar co-twin is laat begonnen). Binnen tweelingparen worden tweelingen die vroeg zijn begonnen vergeleken met hun broer of zus die later is begonnen m.b.t. alcoholconsumptie op volwassen leeftijd. Figuur 1 laat zien dat vroege initiatie op populatieniveau is geassocieerd met latere alcoholconsumptie. Als de associatie tussen vroege initiatie en latere alcoholconsumptie wordt veroorzaakt door een causaal effect van vroege alcoholinitiatie, of door omgevingsfactoren die de leden van een tweelingpaar



FIGUUR 1 Verwachte alcoholconsumptie in de populatie en in MZ twins onder causaal/noncausaal model

verschillend maken, zal ook binnen MZ tweelingparen vroege initiatie geassocieerd zijn met verhoogde alcoholconsumptie in de volwassenheid. Als daarentegen de associatie het resultaat is van een onderliggende aanleg tot alcoholgebruik (non-causaal model), zal vroege initiatie op populatieniveau geassocieerd zijn met latere alcoholconsumptie, maar binnen MZ tweelingparen zullen de vroege drinkers niet verschillen in latere alcoholconsumptie van hun co-twin die later is begonnen. Monozygote tweelingen delen immers 100% van hun genetisch materiaal en hun gehele gedeelde (familie) omgeving, en zijn dus onder het non-causale model in dezelfde mate blootgesteld aan risicofactoren voor verhoogde alcoholconsumptie (e.g. Kendler et al., 1993; Ligthart & Boomsma, 2012; Lynskey et al., 2003; Vink et al., 2007). Grant e.a. (2006) onderzochten met dit design de associatie tussen regelmatig drinken en op jonge leeftijd (<16 jaar) en latere alcoholafhankelijkheid binnen 622 mannelijke tweelingparen. De tweelingen die op jonge leeftijd regelmatig dronken hadden

een hoger risico op alcoholafhankelijkheid dan de co-twins die later regelmatig waren gaan drinken, wat een causaal effect van herhaaldelijke blootstelling aan alcohol suggereert.

In een eerder onderzoek, gebaseerd op vragenlijstgegevens van volwassen deelnemers van het Nederlands Tweelingen Register (NTR; n=16,587) was de leeftijd waarop alcoholgebruik was geïnitieerd significant gerelateerd aan alcoholconsumptie op volwassen leeftijd (resultaten beschikbaar op aanvraag). In deze studie wordt het co-twin control design toegepast op discordante MZ tweelingen binnen diezelfde onderzoeksgroep, om de associatie tussen vroege alcoholinitiatie en latere alcoholconsumptie verder te onderzoeken. Monozygote tweelingen die vroeg zijn begonnen met drinken, worden vergeleken met hun co-twin, die laat is begonnen met drinken, op frequentie van alcoholgebruik, wekelijks aantal glazen, aantal alcoholintoxicaties, overmatig drinken, alcoholmisbruik/-afhankelijkheid en schadelijk drinken. In lijn met de huidige minimumleeftijd voor het kopen van alcohol (16 jaar) wordt onderzocht of er een causaal verband bestaat tussen alcoholinitiatie op 15-jarige leeftijd of jonger en latere alcoholconsumptie. Gezien de huidige plannen binnen de regering om de minimumleeftijd voor het kopen van zwak alcoholische dranken te verhogen naar 18 jaar, zal daarnaast worden getoetst of er effecten zijn van alcoholinitiatie op 16-jarige leeftijd of jonger ten opzichte van initiatie op 18-jarige leeftijd of ouder, op latere alcoholconsumptie.

Methode

Steekproef

Respondenten namen deel aan longitudinaal vragenlijstonderzoek van het Nederlands Tweelingen Register (NTR), dat in 1987 werd opgericht aan de Vrije Universiteit Amsterdam. Meerlingen en hun familieleden werden ongeveer iedere 3 jaar uitgenodigd om een survey in te vullen over gezondheid, leefgewoonten en persoonlijkheid (Boomsma et al., 2006). Deze studie is gebaseerd op de data van de achtste survey, die verzameld werd tussen 2009-2012 (een gedetailleerde beschrijving van de dataverzameling is beschikbaar op aanvraag). Vragenlijstgegevens waren beschikbaar voor 16.587 deelnemers uit 7.308 families. Van de deelnemers waren 8.283 personen meerlingen, waarvan 5.392 personen lid waren van een compleet tweelingpaar, trio (drielingen), of compleet paar binnen een trio, d.w.z. dat beide of alledrie de leden van het tweelingpaar/trio de vragenlijst hadden ingevuld. Binnen de complete paren/trio's waren 1.432 paren monozygoot (MZ; 2.864 individuen). Zygositeit was voor 55,3% van de MZ paren bepaald op basis van DNA-polymorfismen, en voor de overige paren op basis van longitudinaal verzamelde vragen over de

gelijkenis tussen de leden van een paar. In eerder onderzoek binnen het NTR kwamen zygositeitsbepalingen gebaseerd op deze vragen voor 97% overeen met bepalingen gebaseerd op DNA-polymorfismen (Willemsen et al., 2005).

Deelnemers werd gevraagd of ze ooit alcohol hadden gedronken en als ze dit bevestigden werden vervolgvragen over alcoholgebruik gesteld. Tweelingparen waarbinnen tenminste één van beiden nooit was begonnen met drinken werden geëxcludeerd (101 paren) en 82 paren werden geëxcludeerd omdat één van beiden geen leeftijd had ingevuld waarop hij/zij was begonnen met drinken. Gegevens over de leeftijd waarop alcoholgebruik was geïnitieerd waren beschikbaar voor 1.249 complete monozygote paren (2.498 individuen). Binnen deze groep was de gemiddelde leeftijd van alcoholinitiatie 15,7 jaar (SD 3,1). Twee definities van vroege versus late alcoholinitiatie werden getest: initiatie op 15-jarige leeftijd of jonger versus op 17-jarige leeftijd of ouder, en op 16-jarige leeftijd of jonger versus op 18-jarige leeftijd of ouder. Tabel 1 laat de aantallen concordante en discordante paren zien voor beide definities, onderverdeeld naar sekse. Voor de co-twin control analyses werden de zeer discordante paren geselecteerd: bij de eerste definitie van vroege/late initiatie (≤15 vs. ≥17) waren dit 35 mannelijke en 75 vrouwelijke paren, met gemiddelde leeftijd 41,8 bij het invullen van de vragenlijst (SD 15,1; leeftijdsrange 18-80).

Tabel 1 Aantal concordante en discordante MZ paren, naar definitie van vroege alcoholinitiatie en sekse

	Initiatie ≤15 vs. ≥17 jaar		Initiatie ≤16 vs. ≥18 jaar	
	mannen	vrouwen	mannen	vrouwen
Concordant vroeg	118	344	206	587
Concordant laat	47	173	26	115
Matig discordant[1]	123	334	50	121
Zeer discordant	**35**	**75**	**41**	**103**

[1] Tenminste één lid van het paar op 16/17-jarige leeftijd begonnen met drinken

Tweelingparen waarvan tenminste één van beide leden op 16-jarige leeftijd alcoholgebruik had geïnitieerd (matig discordant) werden geëxcludeerd (468 paren), om het contrast tussen vroege en late initiatie te verscherpen. Voor de tweede definitie (≤16 vs. ≥18), waarbij tweelingparen waarvan tenminste één van beiden op 17-jarige leeftijd was begonnen met drinken werden geëxcludeerd (171 paren), waren er 41 mannelijke en 103 vrouwelijke discordante paren, die gemiddeld 42.8 jaar oud waren bij het invullen van de vragenlijst (SD 13.8; leeftijdsrange 18-80).

Afhankelijke variabelen

Frequentie van alcoholgebruik in het afgelopen jaar werd uitgevraagd in zes categorieën ('niet', 'maandelijks of minder', '2-4 keer per maand', '2-3 keer per week', '4-5 keer per week', '6 keer per week of dagelijks'). Deelnemers rapporteerden daarnaast hoeveel glazen bier, wijn, en sterke drank ze per dag dronken in een normale week over het afgelopen jaar. Dit werd opgeteld tot een continue maat voor het aantal glazen per week. Deze variabele was sterk scheef verdeeld en werd daarom in categorieën ingedeeld ('≤3 glazen', '4-7 glazen', '8-14 glazen', '15-21 glazen', en '>21 glazen'), gebaseerd op het maximale aantal glazen per week aanbevolen door de Gezondheidsraad (7 voor vrouwen, 14 voor mannen), en de criteria voor overmatig alcoholgebruik (>14 glazen per week voor vrouwen, >21 glazen per week voor mannen) (Gezondheidsraad, 2006). Aanvullend werd aantal glazen per week gedichotomiseerd naar een specifieke maat voor overmatig drinken, apart voor mannen en vrouwen (1 voor mannen die meer dan 21 glazen per week dronken en voor vrouwen die meer dan 14 glazen per week dronken, anders 0). Verder werd gevraagd hoe vaak respondenten dronken of erg aangeschoten waren geweest (aantal alcoholintoxicaties). Vanwege een sterk scheve verdeling werd deze variabele in categorieën ingedeeld, gebaseerd op de steekproefverdeling ('0 intoxicaties', '1-2 intoxicaties', '3-5 intoxicaties', '6-10 intoxicaties', '11-25 intoxicaties', en '>25 intoxicaties'). Alcoholmisbruik en –afhankelijkheid ooit in het leven werd gemeten met de CAGE-vragenlijst. De CAGE bestaat uit 4 vragen naar symptomen van alcoholmisbruik en –afhankelijkheid (Ewing, 1984) De antwoordcategorieën 'nee', 'ja, niet in het afgelopen jaar' en 'ja, in het afgelopen jaar' werden samengevoegd naar 'nee'/'ja'. Item scores werden opgeteld en gedichotomiseerd (0 versus 1-4 symptomen), resulterend in een maat voor alcoholmisbruik en –afhankelijkheid ooit in het leven (van Beek et al., 2012). Schadelijk drinken werd gemeten door de 10 items van de Alcohol Use Disorders Identification Test (AUDIT) te sommeren, en te dichotomiseren (Babor et al., 2001). Hierbij werd voor mannen tot 65 jaar de cutoff score ≥7 gebruikt en voor vrouwen en mannen boven de 65 jaar de cutoff score van ≥6. In een Nederlandse steekproef had de AUDIT bij deze cutoffs voldoende tot goede sensitiviteit en specificiteit (tussen .57-1.00) om alcoholmisbruik en –afhankelijkheid te detecteren (Boschloo et al., 2010).

Analyses

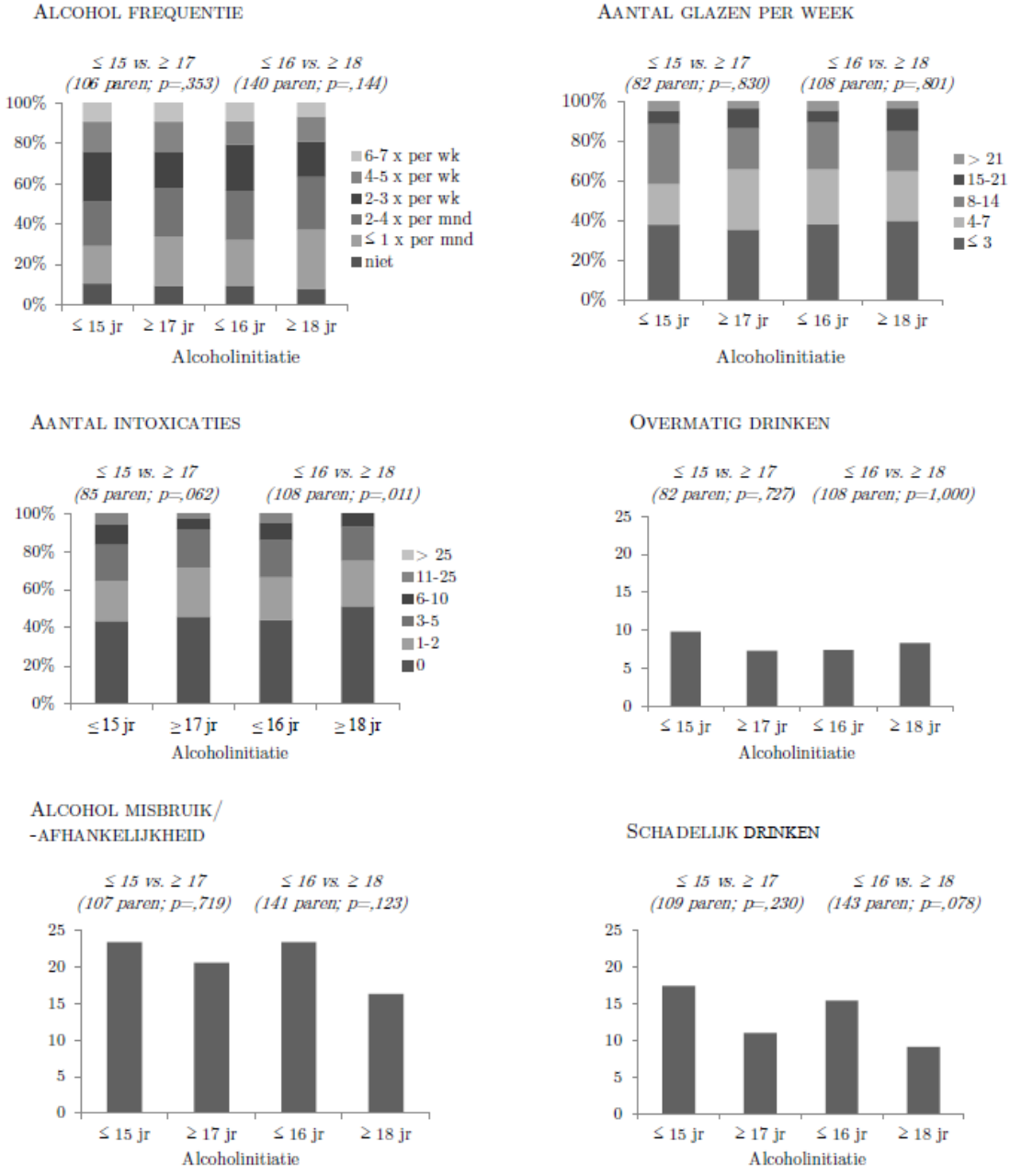
Correlaties tussen leeftijd waarop alcoholgebruik werd geïnitieerd en frequentie van alcoholgebruik, aantal glazen per week, aantal intoxicaties, alcoholmisbruik/-afhankelijkheid en schadelijk drinken waren in een eerdere

studie bepaald in de totale steekproef (N=16,587; resultaten beschikbaar op aanvraag). Verschillen tussen tweelingen die vroeg waren begonnen en de co-twins die laat waren begonnen op de ordinale alcoholmaten (frequentie van alcoholgebruik, hoeveelheid alcohol, aantal alcohol intoxicaties) werden getoetst met de Wilcoxon test voor gepaarde steekproeven. Verschillen op de dichotome alcoholmaten (overmatig drinken, symptomen van alcoholmisbruik/-afhankelijkheid en schadelijk drinken) tussen tweelingen die vroeg versus laat alcoholgebruik hadden geïnitieerd werden getoetst met de McNemar test voor gepaarde steekproeven (Van den Brink & Koele, 2002). Daar de uitkomstvariabelen onderling gecorreleerd waren, werd met behulp van spectrale decompositie van de correlatiematrix geschat voor hoeveel onafhankelijke toetsen het significantieniveau (α) moest worden gecorrigeerd (Nyholt, 2004). Dit werd geschat op 5,2146, derhalve werd voor alle tests een significantieniveau van ,05/5,2146≈,01 gehanteerd. De co-twin control analyses werden uitgevoerd voor mannen en vrouwen tegelijk, in SPSS 20 (IBM, 2011).

Resultaten

In een eerdere studie in de totale steekproef (N=16.587) was vroege leeftijd waarop alcoholgebruik was geïnitieerd significant geassocieerd (α=,01) met hogere frequentie van alcoholgebruik (r=-,05), hoger aantal glazen per week (r=-,14), groter aantal intoxicaties (r=-,25) en hogere prevalentie van alcoholmisbruik/afhankelijkheid (r=-,13) en schadelijk drinken (r=-,11; resultaten beschikbaar op aanvraag)

Figuur 2 toont de verdeling of prevalentie van de verschillende alcoholvariabelen, voor de tweelingen die vroeg waren begonnen en de co-twins die laat waren begonnen, onder de verschillende definities van vroege versus late alcoholinitiatie (exacte verdelingen beschikbaar op aanvraag). Tweelingen die waren begonnen met drinken op 15-jarige leeftijd of jonger, scoorden enigszins hoger op de uitkomstmaten dan de co-twins die voor het eerst hadden gedronken op 17-jarige leeftijd of later, maar de verschillen waren niet significant (p-waarden varieerden tussen ,062-,830). De alternatieve definitie van vroege/late alcoholinitiatie (voor het eerst drinken op 16-jarige leeftijd of jonger versus op 18-jarige leeftijd of later) leverde vergelijkbare resultaten op. De verschillen tussen de tweelingen die vroeg waren begonnen met drinken en hun co-twins die later waren begonnen waren niet significant (p-waarden tussen ,096-,801), met uitzondering van het aantal intoxicaties, dat de grens van significantie bereikte (p=,011).



FIGUUR 2 Verdeling van alcoholconsumptie in tweelingen discordant voor vroege alcoholinitiatie.

Discussie

In een eerdere studie in deze steekproef van NTR deelnemers uit de algemene Nederlandse populatie was alcoholinitiatie op vroege leeftijd significant gerelateerd aan hogere frequentie van alcoholgebruik, hogere wekelijkse alcoholconsumptie, groter aantal alcoholintoxicaties, en hogere prevalentie van alcoholmisbruik/-afhankelijkheid en van schadelijk drinken. Echter na correctie voor de invloed van genen en gedeelde familieomgeving, door alcoholconsumptie op volwassen leeftijd te vergelijken binnen ééneiige tweelingen, waren geen van de associaties tussen vroege initiatie en latere alcoholconsumptie significant, één uitzondering daargelaten.Deze bevindingen suggereren dat de associaties tussen vroege alcoholinitiatie en alcoholconsumptie, alcoholmisbruik/-afhankelijkheid, en schadelijk drinken op volwassen leeftijd, verklaard worden door de invloed van genen en van gedeelde familieomgeving. Ondanks dat de vroege drinkers op de meeste alcoholconsumptiematen iets hoger scoorden dan degenen die later waren begonnen, zijn er vrijwel geen aanwijzingen gevonden voor significante causale effecten van vroege blootstelling aan alcohol op latere verhoogde alcoholconsumptie en alcoholmisbruik/-afhankelijkheid. Voor iedere analyse is post hoc statistische power geschat (met G*Power; Faul et al., 2009). Hieruit bleek dat voor vrijwel alle analyses (met uitzondering van overmatig drinken) het aantal observaties groot genoeg was om kleine tot middelgrote effecten van vroege alcoholinitiatie te detecteren. De geobserveerde effecten van vroege alcoholinitiatie waren triviaal tot klein (resultaten beschikbaar op aanvraag). Met betrekking tot alcoholbeleid in Nederland is de implicatie van deze bevindingen dat campagnes die gericht zijn op het verhogen van de minimumleeftijd voor alcoholinitiatie, mogelijk slechts een beperkt effect zullen hebben op het terugdringen van alcoholconsumptie en alcoholmisbruik/-afhankelijkheid onder volwassenen. Interventies die op een later moment in het verloop van alcoholgebruik naar misbruik of afhankelijkheid ingrijpen zullen daarin mogelijk effectiever zijn (Prescott & Kendler, 1999).

In een eerdere studie in Nederlandse adolescenten, werd alcoholinitiatie/ geheelonthouding beïnvloed door andere mechanismen dan alcoholconsumptie (Geels e.a., 2012). De personen die nooit zijn begonnen met drinken zijn daarom buiten de co-twin control analyses gehouden. Als daarentegen aangenomen zou worden dat levenslange geheelonthouding en alcoholmisbruik/-afhankelijkheid uiteinden van hetzelfde continuüm zijn, zal uitsluiten van geheelonthouders de associatie tussen alcohol initiatie en latere alcoholconsumptie onterecht reduceren (Prescott & Kendler, 1999). In aanvullende analyses, waarbij de geheelonthouders bij de groep die laat was begonnen werden gevoegd, waren de verschillen tussen de tweelingen die vroeg waren begonnen en de co-twins die

later waren begonnen eveneens niet significant, met uitzondering van verschillen in frequentie van alcoholgebruik bij vroege initiatie gedefinieerd als ≤16 vs. ≥18 jaar (p=.005; resultaten beschikbaar op aanvraag).

Leeftijd waarop alcoholgebruik was geïnitieerd is retrospectief uitgevraagd. Gezien de grote leeftijdsrange (18-80 jaar) heeft dit mogelijk geresulteerd in onnauwkeurigheden in de rapportage, met name onder de oudere deelnemers (Schwarz, 2007), omdat oudere respondenten geneigd zijn een hogere leeftijd waarop alcoholgebruik is geïnitieerd te rapporteren. Hierdoor zou de associatie tussen vroege alcoholinitiatie en latere alcoholconsumptie onderschat worden ('forward telescoping bias'; Johnson & Schultz, 2005). Echter, een studie die dit specifiek heeft onderzocht voor alcoholgebruik heeft laten zien dat bias in retrospectieve rapportage leidt tot overschatting van de associatie tussen vroege initiatie en later gebruik, doordat zware drinkers alcoholinitiatie op jongere leeftijd rapporteren dan gematigde drinkers (Sartor et al., 2011). Ter controle zijn de co-twin control analyses nogmaals uitgevoerd, waarbij deelnemers ouder dan 45 jaar werden geëxcludeerd. Dit leverde nagenoeg dezelfde resultaten op (resultaten beschikbaar op aanvraag).

De co-twin control analyses zijn uitgevoerd op alle tweelingparen tegelijk, om zoveel mogelijk tweelingparen en statistische power te behouden. De associatie tussen vroege alcohol initiatie en alcoholafhankelijkheid is even sterk voor mannen en vrouwen (Sartor et al., 2009), maar in de vroege adolescentie beginnen jongens eerder met drinken dan meisjes, met name in oudere cohorten (Geels e.a. 2012; van Laar et al., 2011b). Daarnaast bestaan er gedurende de adolescentie sekseverschillen in tempo van hersenontwikkeling, en het is hierdoor niet uit te sluiten dat vroege blootstelling aan alcohol verschillende effecten heeft op jongens en meisjes (Dawson et al., 2008). De verschillen in alcoholconsumptie zijn aanvullend onderzocht in mannen en vrouwen apart, wat vergelijkbare resultaten opleverde (resultaten beschikbaar op aanvraag).

Samengevat: associaties tussen vroege alcoholinitiatie en alcoholconsumptie onder volwassenen worden niet verklaard door significante causale effecten van vroege blootstelling aan alcohol. De associaties worden vrijwel geheel verklaard door de invloed van genen en gedeelde omgeving. Deze bevindingen impliceren dat campagnes om de leeftijd waarop adolescenten alcoholgebruik initiëren te verhogen, mogelijk slechts beperkt effect hebben op alcoholconsumptie en alcoholmisbruik en -afhankelijkheid in de volwassen populatie.

Early alcohol initiation and increased adult alcohol consumption: cause or indicator? Geels LM, Vink JM, Beek van JHDA, Bartels M, Willemsen G, & Boomsma DI

Summary

Background Early alcohol initiation is strongly associated with increased alcohol consumption and alcohol abuse/dependence in adulthood. The mechanisms that underlie this association are unclear. *Aim* Examine whether the association between early alcohol initiation and later alcohol consumption is causal. *Method* Survey data were collected in participants (age range 18-80) of the Netherlands Twin Register (NTR). In a previous study in this sample, correlations between age at alcohol initiation and adult alcohol consumption had been computed. A discordant twin design (co-twin control design) was used to further examine these associations. Within monozygotic pairs, twins who initiated alcohol use early were compared to their brother/sister who started drinking later, on alcohol frequency, weekly alcohol consumption, number of alcohol intoxications, excessive drinking, alcohol abuse/-dependence, and hazardous drinking. By comparing within monozygotic twin pairs, effects of genes/shared environment are controlled for. *Results* In the total NTR sample, early alcohol initiation was associated with later alcohol consumption, but within monozygotic twin pairs, twins who had initiated early did not differ significantly from their brother/sister. *Conclusion* Early alcohol initiation did not have significant causal effects on adult alcohol consumption. Campaigns aimed at increasing age at alcohol initiation will possibly have limited effect on adult alcohol consumption.

Supplement to chapter 4

Suppl. Tabel 1 Verdeling van alcoholconsumptie in MZ tweelingen discordant voor vroege alcoholinitiatie (bij Figuur 2)

	Initiatie ≤ 15 jaar vs. ≥17 jaar				*Initiatie ≤ 16 jaar vs. ≥18 jaar*			
	N complete paren	≤ 15 jaar	≥ 17 jaar	p-waarde	N complete paren	≤ 16 jaar	≥ 18 jaar	p-waarde
Alcohol frequentie		%	%			%	%	
Niet	106	10,4	9,4	,353	140	9,3	7,9	,144
maandelijks of minder		18,9	24,5			22,9	29,3	
2-4 keer per maand		21,7	23,6			24,3	26,4	
2-3 keer per week		24,5	17,9			22,9	17,1	
4-5 keer per week		15,1	15,1			11,4	12,1	
6 keer per week of dagelijks		9,4	9,4			9,3	7,1	
Aantal glazen alcohol per week								
3 glazen of minder	82	37,8	35,4	,830	108	38,0	39,8	,801
4-7 glazen		20,7	30,5			27,8	25,0	
8-14 glazen		30,5	20,7			24,1	20,4	
15-21 glazen		6,1	9,8			5,6	11,1	
meer dan 21 glazen		4,9	3,7			4,6	3,7	
Aantal intoxicaties								
0 intoxicaties	85	43,5	45,9	,062	113	44,2	51,3	,011
1-2 intoxicaties		21,2	25,9			22,1	23,9	
3-5 intoxicaties		18,8	20,0			19,5	17,7	
6-10 intoxicaties		10,6	5,9			8,8	7,1	
11-25 intoxicaties		5,9	2,4			5,3	0	
> 25 intoxicaties		0	0			0	0	
Overmatig drinken	82	9,8	7,3	,727	108	7,4	8,3	1,000
Alcoholmisbruik/afhankelijkheid	107	23,4	20,6	,719	141	23,4	16,3	,123
Schadelijk drinken	109	17,4	11,0	,230	143	15,4	9,1	,078

De co-twin control analyses zijn aanvullend uitgevoerd met levenslange geheelonthouders (n=124) in de late-initiatie groep. Suppl. Tabel 2 toont de aantallen concordante en discordante paren voor deze analyses. Onder de tweelingparen waarvan de ene tweeling was begonnen met drinken op ≤ 15 jaar en de andere tweeling op ≥17 jaar was de gemiddelde leeftijd 40,4 (SD 15,3; leeftijdsrange 18-80). Onder de tweede definitie van vroege versus late alcoholinitiatie (≤ 16 vs. ≥18 jaar), was de gemiddelde leeftijd 40,3 jaar (SD 14,7; leeftijdsrange 18-80).

Suppl. Tabel 2 Aantal concordante en discordante MZ paren, naar definitie van vroege alcoholinitiatie en sekse

	Initiatie ≤15 vs. ≥17 jaar/niet		*Initiatie ≤16 vs. ≥18 jaar/niet*	
	mannen	vrouwen	mannen	vrouwen
Concordant vroeg	118	344	206	587
Concordant laat	58	232	35	165
Matig discordant[1]	124	344	52	130
Zeer discordant	**39**	**82**	**46**	**120**

[1] Tenminste één lid van het paar op 16/17-jarige leeftijd begonnen met drinken

Suppl. Tabel 3 Verdeling van alcoholconsumptie in MZ tweelingen discordant voor vroege versus late alcohol initiatie, met levenslange geheelonthouders bij late initiatie groep

	Initiatie ≤15 vs. ≥17 jaar/niet				*Initiatie ≤16 vs. ≥18 jaar/niet*			
	N complete paren	≤ 15 jaar	≥ 17 jaar	p-waarde	N complete paren	≤ 16 jaar	≥ 18 jaar	p-waarde
Alcohol frequentie		%	%			%	%	
Niet	116	12,1	17,2	,096	161	11,2	19,9	,005
maandelijks of minder		20,7	22,4			25,5	25,5	
2-4 keer per maand		21,6	21,6			24,2	23,0	
2-3 keer per week		22,4	16,4			20,5	14,9	
4-5 keer per week		13,8	13,8			9,9	10,6	
6 keer per week/dagelijks		9,5	8,6			8,7	6,2	
Aantal glazen alcohol per week								
3 glazen of minder	88	39,8	39,8	,589	119	40,3	45,4	,683
4-7 glazen		20,5	28,4			26,1	22,7	
8-14 glazen		28,4	19,3			23,5	18,5	
15-21 glazen		5,7	9,1			5,0	10,1	
meer dan 21 glazen		5,7	3,4			5,0	3,4	
Aantal intoxicaties								
0 intoxicaties	86	44,2	46,5	,062	114	44,7	51,8	,011
1-2 intoxicaties		20,9	25,6			21,9	23,7	
3-5 intoxicaties		18,6	19,8			19,3	17,5	
6-10 intoxicaties		10,5	5,8			8,8	7,0	
11-25 intoxicaties		5,8	2,3			5,3	0	
> 25 intoxicaties		0	0			0	0	
Overmatig drinken	88	10,2	6,8	,508	119	7,6	7,6	1,000

Noot. Alcoholmisbruik/afhankelijkheid en schadelijk alcoholgebruik ontbreken in de tabel omdat scores op deze variabelen niet beschikbaar waren voor levenslange geheelonthouders

Post hoc poweranalyse

Voor iedere analyse is post hoc power geschat met G*Power 3.1.4 (Faul et al., 2009; Faul et al., 2007), aan de hand van geobserveerde effectgrootte, significantieniveau (α), en totaal aantal observaties (N). Voor de ordinale variabelen (alcoholfrequentie, aantal glazen per week, aantal intoxicaties) is effectgrootte *(r)* bepaald met de gemiddelden, standaarddeviaties, en de correlatie tussen de vroege en late initiatiegroep. Voor de dichotome alcoholvariabelen (overmatig drinken, alcoholmisbruik/-afhankelijkheid en schadelijk drinken) is effectgrootte gedefinieerd als de odds ratio (OR). Tabel S4 laat zien dat de effecten van vroege alcoholinitiatie, ervan uitgaand dat de observeerde effectgroottes vergelijkbaar zijn met de effecten in de populatie, op latere alcoholfrequentie, aantal glazen per week, overmatig drinken, en alcoholmisbruik/-afhankelijkheid triviaal tot klein zijn. Effecten op het aantal intoxicaties en schadelijk drinken zijn klein tot middelgroot, met name voor de definitie van initiatie ≤ 16 vs. ≥18 jaar. Daarna is onderzocht welke minimale effectgrootte zou kunnen worden gedetecteerd (power 80%), gegeven het aantal observaties en significantieniveau. Voor alle analyses, met uitzondering van overmatig drinken, was de steekproef voldoende groot om bij een significantieniveau van α=,01 kleine tot middelgrote effecten van vroege alcoholinitiatie te detecteren (Suppl. Tabel 4).

Ter conclusie, de geobserveerde effecten van vroege alcoholinitiatie, gedefinieerd als initiatie ≤ 15 vs. ≥17 jaar en initiatie ≤ 16 vs. ≥18 jaar, op latere alcoholconsumptie, zijn triviaal tot klein. Één uitzondering daargelaten (overmatig drinken), was het aantal observaties groot genoeg om kleine tot middelgrote effecten te detecteren.

Suppl. Tabel 4 Statistische power voor co-twin control analyses

Alcohol initiatie ≤ 15 vs. ≥17 jaar	*Effectgrootte r/OR*	α	*N*	*Power*
Alcoholfrequentie				
Post hoc	,076	,01	212	,105
Min. effectgrootte voor power 80%	,225	,01	212	,803
Aantal glazen per week				
Post hoc	,033	,01	164	,028
Min. effectgrootte voor power 80%	,255	,01	164	,799
Aantal intoxicaties				
Post hoc	,171	,01	170	,651
Min. effectgrootte voor power 80%	,251	,01	170	,801
Overmatig drinken				
Post hoc	1,649	,01	164	,067
Min. effectgrootte voor power 80%	6,300	,01	164	,804
Alcoholmisbruik/-afhankelijkheid				
Post hoc	1,214	,01	214	,038
Min. effectgrootte voor power 80%	2,410	,01	214	,800
Schadelijk drinken				
Post hoc	1,770	,01	218	,292
Min. effectgrootte voor power 80%	2,700	,01	218	,806
Alcohol initiatie ≤ 16 vs. ≥18 jaar				
Alcoholfrequentie				
Post hoc	,105	,01	280	,269
Min. effectgrootte voor power 80%	,195	,01	280	,801
Aantal glazen per week				
Post hoc	,025	,01	216	,025
Min. effectgrootte voor power 80%	,222	,01	216	,800
Aantal intoxicaties				
Post hoc	,220	,01	226	,812
Min. effectgrootte voor power 80%	-	-	-	-

Overmatig drinken				
Post hoc	,804	,01	216	,013
Min. effectgrootte voor power 80%	6,670	,01	216	,800
Alcoholmisbruik/-afhankelijkheid				
Post hoc	1,835	,01	282	,456
Min. effectgrootte voor power 80%	2,345	,01	282	,801
Schadelijk drinken				
Post hoc	2,500	,01	286	,666
Min. effectgrootte voor power 80%	2,870	,01	286	,800

Noot. Ordinale variabelen: r=,01: klein effect; r=,03: middelgroot effect; r=,05: groot effect. Voor dichotome variabelen: OR=1,68: klein effect; OR=3,47: middelgroot effect; OR=6.71: groot effect (Chen et al., 2010; Field, 2009)

TABEL S5 Co-twin control analyses in mannen en vrouwen apart en in deelnemers ≤ 45 jaar

ALCOHOL INITIATIE ≤ 15 VS. ≥17 JAAR (ZONDER ABSTAINERS)	*N paren*	*p-waarde*
Alcohol frequentie		
Mannen	33	,335
Vrouwen	73	,631
≤ 45 jaar	61	,729
Aantal glazen per week		
Mannen	30	,544
Vrouwen	52	,496
≤ 45 jaar	45	,947
Aantal intoxicaties		
Mannen	22	,046
Vrouwen	63	,309
≤ 45 jaar	55	,215
Overmatig drinken		
Mannen	30	,500
Vrouwen	52	1,000
≤ 45 jaar	43	1,000
Alcoholmisbruik/-afhankelijkheid		
Mannen	34	,754
Vrouwen	73	1,000
≤ 45 jaar	62	1,000
Schadelijk drinken		
Mannen	35	,070
Vrouwen	74	1,000
≤ 45 jaar	63	,581
ALCOHOL INITIATIE ≤ 16 VS. ≥18 JAAR (ZONDER ABSTAINERS)		
Alcohol frequentie		
Mannen	40	,309
Vrouwen	100	,278
≤ 45 jaar	88	,712
Aantal glazen per week		
Mannen	36	,954
Vrouwen	72	,921
≤ 45 jaar	64	,766
Aantal intoxicaties		
Mannen	25	,028
Vrouwen	88	,107
≤ 45 jaar	74	,387

Overmatig drinken		
Mannen	36	,625
Vrouwen	72	,375
≤ 45 jaar	64	,625
Alcoholmisbruik/-afhankelijkheid		
Mannen	40	,791
Vrouwen	101	,115
≤ 45 jaar	88	,815
Schadelijk drinken		
Mannen	41	,289
Vrouwen	102	,267
≤ 45 jaar	89	1,000
ALCOHOL INITIATIE ≤ 15 VS. ≥17 JAAR/NIET	*N paren*	*p-waarde*
Alcohol frequentie		
Mannen	37	,280
Vrouwen	79	,200
≤ 45 jaar	70	,231
Aantal glazen per week		
Mannen	30	,544
Vrouwen	58	,286
≤ 45 jaar	49	,596
Aantal intoxicaties		
Mannen	22	,046
Vrouwen	64	,309
≤ 45 jaar	56	,215
Overmatig drinken		
Mannen	30	,500
Vrouwen	58	1,000
≤ 45 jaar	49	1,000
ALCOHOL INITIATIE ≤ 16 VS. ≥18 JAAR/NIET		
Alcohol frequentie		
Mannen	45	,204
Vrouwen	116	**,010**
≤ 45 jaar	108	,139
Aantal glazen per week		
Mannen	37	,735
Vrouwen	82	,606
≤ 45 jaar	75	,269

Aantal intoxicaties		
Mannen	25	,028
Vrouwen	89	,107
≤ 45 jaar	75	,387
Overmatig drinken		
Mannen	37	,625
Vrouwen	82	,688
≤ 45 jaar	75	1,000

5

Increases in alcohol consumption in women and elderly groups: Evidence from an epidemiological study

This chapter is submitted as:

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Abstract

Background In most Western countries, alcohol consumption continues to increase, specifically among women and the elderly and insight into these trends may aid intervention strategies. From a large (N > 16,000), population-based Dutch sample, ascertained based on the presence of twins in the family, data on alcohol consumption by age and sex are presented, as well as associations between alcohol use and demographic/lifestyle traits. *Methods* A set of 16 indicators of normative and problematic alcohol use was assessed in participants of the Netherlands Twin Register between 2009-2012 (ages 18-97; 6,052 men; 10,535 women). Alcohol consumption and demographic/lifestyle traits, including educational attainment, work-related/financial stress, urbanization, religiousness, smoking/cannabis initiation, and BMI were described by age and sex and associations were examined by regressing aspects of alcohol use on age, sex, their interaction, and demographic/lifestyle variables. *Results* Frequency of alcohol use was lowest between 18-25 years, with 3.2% of men and .6% of women drinking 6-7 times/week, and highest above age 65 years, with 30.6-32.7% of men and 20.2-22.0% of women drinking 6-7 times/week. Women consumed the lowest quantities of alcohol between 25-45 years, with a 5.7-5.9% prevalence of excessive drinking (>14 glasses/week), and the largest quantities between 55-65 years (15.5% excessive drinkers). Age at alcohol initiation, at onset of regular drinking, and at first alcohol intoxication were lowest between ages 18-25 years and highest above age 65 years. Among older participants, men initiated alcohol use and regular drinking earlier, and had lower age at first intoxication than women, but among young adults, no sex differences were observed. Age, sex, and initiation of cigarette and cannabis use were the most important predictors of alcohol use. *Conclusions* As previously observed, alcohol consumption was high in the elderly Dutch population, especially among women. Alcohol initiation, onset of regular drinking, and first alcohol intoxication occur at increasingly younger ages, and the previous gap between men and women in age at alcohol initiation, at onset of regular drinking, and at first alcohol intoxication has closed almost entirely. Heavy alcohol use was most strongly predicted by older age, sex (male), and initiation of smoking and cannabis use.

Introduction

Alcohol use is widespread in the Netherlands, with as much as 88% of the adult population having consumed alcohol in the past year (European Commission, 2010). The drinking pattern of the Dutch population is characterized by frequent but moderate alcohol consumption (Anderson et al., 2012). The Dutch drink on average 9.3 liters of alcohol per person each year, which is highly similar to the average in the USA (9.4 liters) and below that observed across Europe (10.5 liters) (WHO-HFA, 2012; WHO, 2011). However, the proportion of recent drinkers (in the last month) is higher in the Netherlands (76%) than across European countries (67%) and the USA (between 28-64%) (European Commission, 2010; Substance Abuse and Mental Health Services Administration, 2011; van Rooij et al., 2011). Despite the general pattern of moderate drinking, the prevalence of heavy episodic drinking is 8.4% in Dutch adults (16% in men, 5% in women), which is somewhat higher than in the UK, Spain, Slovenia, Estonia, Portugal, or the USA (Nazareth et al., 2011; WHO, 2011). The proportion of problem drinkers is 9.4%, and the prevalence of alcohol use disorders is 5% in men and 1% in women, comparable with other western European countries (Ouwehand et al., 2011; World Health Organization, 2011). Alcohol use contributes 4.5% to the total burden of disease in the Netherlands (van Laar et al., 2010), as it increases risk of alcohol abuse and dependency and, when used excessively, contributes to numerous types of disease such as cancer, cardiovascular disease, and liver cirrhosis, as well as to alcohol-related injuries, e.g. traffic accidents (WHO, 2009).

Alcohol use typically starts in adolescence, and drinking patterns vary across the lifespan. Before age 16, 72-85% of Dutch adolescents has used alcohol, and 37% drank alcohol in the past month (Geels et al., 2012; Van Laar et al., 2011). The prevalence of heavy drinking ($>$ 5 glasses on at least one occasion weekly) is highest between ages 18-24 (Van Laar et al., 2011), and young adults in this age group are at additional risk for alcohol abuse and alcohol-related problems in the college environment, especially when they are members of a fraternity or sorority (Netherlands Institute on Mental Health and Addiction, 2009). Overall, the prevalence of binge drinking ($>$ 5 glasses per occasion) declines between ages 15 and 64 years (from 37% to 27%), while the prevalence of recent drinking (in the past month) increases slightly from 72% to 79% (Van Laar et al., 2011). Above age 65 years, the number of drinkers is relatively low (Zantinge & van Laar, 2011). Across all age groups, the prevalence of alcohol use is higher among men than women, and men have higher incidence of recent drinking and binge drinking (Van Laar et al., 2011). Despite the relatively low

number of drinkers above age 65 years, alcohol use disorders in the elderly population seem to be a growing problem. Between 1998-2008, the proportion of people above age 55 years who sought treatment for alcohol use disorders almost doubled (corrected for the aging population). Elderly women are particularly vulnerable; women than in young, and the number of elderly women who seek treatment for alcohol use disorders has increased more rapidly than the number of men (Weingart, 2009). The gap between men and women is similarly narrowing among young adults; between 2008-2009, the proportion of male heavy drinkers between ages 18-24 decreased substantially (from 37-30%), while remaining stable among women at 12% (Van Laar et al., 2011).

As well as age and sex, socioeconomic circumstances and lifestyle factors are related to specific patterns of alcohol use. High educational attainment is associated with higher prevalence of alcohol use, but with lower levels of heavy alcohol use (Savelkoul et al., 2011). High levels of work-related stress have been related to increased quantity of alcohol consumed (Maas van der, 2006; Schutten et al., 2003). Financial stress may also be related to alcohol use, as observed in an elderly American sample (Moos et al., 2006), and in the Netherlands, alcohol dependence is more prevalent among the unemployed and those incapable to work (van Laar et al., 2010). Alcohol use and binge drinking occur more frequently in rural than in urban areas (Donath et al., 2011; Van Laar et al., 2011), in contrast with the globally observed association of higher alcohol use in urban areas (Lawrence & Fudge, 2009). Religiousness and higher frequency of church attendance are related to lower prevalence of heavy drinking (Aldridge-Gerry et al., 2011; Drerup et al., 2011; Statistics Netherlands, 1999). Smoking cigarettes and cannabis use often co-occur with alcohol use (van Laar et al., 2010; van Laar et al., 2011; Willemsen et al., 2011). The Netherlands have a unique 'policy of tolerance' towards cannabis, meaning that the substance itself is illegal, but possession of limited amounts of cannabis and selling by licensed establishments is not prosecuted (2012).

Body mass index (BMI) may be related to alcohol use through more than one route: in several European countries and the USA, low BMI is related to frequent consumption of small quantities of alcohol and to the preference of wine over beer or strong liquor, whereas high BMI is related to infrequently drinking large quantities of alcohol and preferring strong liquor (Breslow & Smothers, 2005; Sayon-Orea et al., 2011).

A detailed insight in the latest trends in alcohol use in a large population-based sample is valuable to inform intervention strategies for groups who are at relatively high risk for alcohol use disorders and to identify such groups. In the present study, an analysis of alcohol use and risk factors for alcohol use and

abuse, including demographic/lifestyle traits, is presented. Data were collected between 2009-11 in a large, population-based adult sample of twins (50%) and non-twins from the Netherlands Twin Register (NTR; N=16,587). Various aspects of alcohol use were examined as a function of sex and age-group, namely initiation and frequency of alcohol use, quantity of alcohol consumed, age at initiation and at onset of regular drinking, preferred beverage, situation-specific urges to drink, and more severe aspects of alcohol use: number of alcohol intoxications, age at first alcohol intoxication, lifetime alcohol abuse disorder (AAD) symptoms, and hazardous drinking. Associations between alcohol use and age/sex were assessed by regression analysis, with demographic and lifestyle traits: educational attainment, work-related stress, financial stress, degree of urbanization, religiousness, smoking initiation, cannabis initiation, and body mass index (BMI) as covariates.

Methods

Sample

Participants were registered with the Netherlands Twin Register (NTR), which was established in 1987 at the VU University in Amsterdam. Longitudinal survey collection in adult twins and their family members started in 1991 and has been ongoing since. Participants were invited about every 3 years to complete a survey containing questions about health, lifestyle, personality and psychopathology (Boomsma et al., 2006). The present study is based on data from the 8th wave of survey collection, that was carried out between 2009 and 2011. After obtaining approval from the Medical Ethics Committee of the VU University Medical Center Amsterdam, all NTR participants aged 18 years and older, who were registered at a valid address and were willing to participate in survey studies, were invited to complete the survey (N=47,151). Participants first received a written invitation including a link to the webpage where they could log on to a web-based version of the survey with a unique, personal login name and password. If subjects did not access the web-based survey in the 6 weeks after the invitation, they received a paper version of the survey. Between 3-9 months after the paper versions of the survey were sent, subjects who had not responded received a reminder card by post, or a reminder by email (if an email address was available). Additionally, several groups of non-responders of particular interest (e.g. twins from incomplete twin pairs; subjects who took part in biobank studies) were reminded in a phone call. Data were available for 16,607 subjects (35% response rate). From these, 20 subjects were removed because they had only completed a small part of the survey. This resulted in a

Table 1 Family roles of participants, stratified by complete and incomplete twin pairs/triplet trios and parental/spouse pairs

	N_{ind}	% women	Mean age (sd)	no. complete pairs/trio's
Twins (N=8,093)				
Families with one twin pair (N=7,958)				
Incomplete twin pair	2,760	67.5	32.4 (12.2)	-
Complete twin pair	5,198	69.5	34.1 (15.5)	2,599
Families with two twin pairs (N=133)				
One incomplete twin pair participating	27	77.8	27.5 (10.3)	-
Two incomplete twin pairs participating	20	80.0	32.1 (11.6)	-
One complete twin pair participating	36	63.9	26.0 (13.4)	18
One complete, one incomplete twin pair	30	73.3	37.4 (12.7)	10
Two complete twin pairs	20	55.0	35.6 (16.2)	10
Families with three twin pairs (N=2)				
One incomplete twin pair	2	100	38.7 (-)	-
Triplets (N=154)				
One triplet	36	72.2	27.4 (12.3)	-
Two triplets	58	65.5	25.6 (12.5)	29
Complete trios	60	75.0	25.3 (11.2)	20
Quadruplets (N=1)				
1 quadruplet (individual)	1	0	18.7 (-)	-
Single Multiples[1] (N=35)	35	54.3	44.7 (10.9)	-
	Total no. of complete twin pairs/trios			2,686
Parents (N=5,198)				
Incomplete parental pair	2,048	73.2	55.0 (8.9)	-
Complete parental pair	3,150	50.0	56.5 (7.5)	1,575
Spouses (N=875)				
Spouse of non-participating multiple	161	47.8	42.9 (9.5)	-
Spouse of participating multiple	714	36.7	44.7 (12.3)	714
	Total no. of complete spouse pairs			2,289
Non-twin siblings[2] (N=1,898)				
8 siblings	8	62.5	58.5 (8.0)	-
7 siblings	7	42.9	65.3 (4.6)	-
6 siblings	6	33.3	59.2 (3.3)	-
5 siblings	15	33.3	48.5 (13.8)	-
4 siblings	48	56.3	47.0 (13.1)	-
3 siblings	138	59.4	48.8 (14.7)	-
2 siblings	370	61.1	42.1 (13.0)	-
1 sibling	1,306	65.4	35.4 (12.8)	-
Other[3] (N=333)	333	65.5	32.7 (9.3)	-
Total	16,587	63.5	41.6 (16.0)	

[1]multiples without information on co-multiples; [2]number of participating siblings within families, not including multiples; [3]other includes participants registered without any family members (N=16), spouses of siblings of twins (N=28), children of twins/siblings (N=288), and a spouse of a child of a twin (N=1).

sample of 16,587 subjects from 7,308 families. The average age was 41.6 (SD=16.0; range 18 -97), and 64% of the participants were women. Participants and their parents were mostly born in the Netherlands (92.7%). First and second generation immigrants from western countries other than the Netherlands formed 5.6% of the sample, and 1.7% were first or second generation immigrants from non-western countries. Table 1 describes the different roles within families. Twins and higher-order multiples formed the largest group (N=8,248; 68.8-70.3% women), followed by parents (N=5,198; 59.2% women), non-twin siblings (N=1,898; 63.4% women), and spouses (N=875; 38.7% women) of multiples. Twins and higher-order multiples ranged in age between 18-97 years, parents between 30-94 years, non-twin siblings between 18-88 years, and spouses between 25-87 years.

Measures

Demographic and lifestyle variables. Educational attainment was available in 3 categories (primary school/lower vocational schooling', intermediate vocational/upper secondary school', and 'upper vocational/university'; cf. Statistics Netherlands) (2012a) for 14,799 subjects. Student status at the time of data collection was based on a single item that asked if subjects were in college or high school (0 'no', 1 'yes'). The frequency of work-related stress in the previous year was assessed ('never', 'occasionally', 'regularly', 'constantly', 'not applicable'), as well as the degree of financial stress ('little/none', 'moderate', 'severe'). Respondents who answered 'not applicable' to the question about work-related stress were included in the category 'never' (85% of these respondents did not have a job).

Degree of urbanization was based on address density in the residential area and measured on a scale of 1 to 5 (very high, high, moderate, low, very low) (Willemsen et al., 2005). Following Statistics Netherlands, degree of urbanization was summarized in two categories (very low - moderate, and heavy - very heavy) (Statistics Netherlands, 2008). Participants were asked if they were religious, to which they could answer 'no', 'yes, not an active member of church/religious society', or 'yes, active member of church/religious society'.

Subjects were asked if they had ever smoked ('no', 'yes, a few times to try', and 'yes'). The latter two categories were collapsed, creating a binary variable that indexed ever having smoked. Participants were also asked if they had ever used cannabis (0 'no', 1 'yes'). BMI was calculated as $weight_{kg}$ /$height_{m}^{2}$ and categorized into 'underweight' (BMI <18.5), 'normal weight'

(BMI ≥18.5 & <25), and 'overweight' (BMI ≥25) (2010b). BMI was not computed for women who were pregnant when they completed the survey.

Alcohol use. Participants were asked if they had ever used alcohol ('no', 'a few times to try', and 'yes'). If subjects stated having ever used alcohol, they were asked at what age they had their first drink and at what age they had started drinking regularly (if at all). Subjects were asked how often they had used alcohol in the previous year ('no', 'monthly or less', 'two to four times a month', 'two or three times a week', 'four or five times a week', and 'six times a week or daily'). Quantity of alcohol consumed in the previous year was measured as the number of glasses of beer, wine, and strong liquor, consumed on each day of a normal week. The reported number of drinks was summed into a continuous score for quantity of alcohol consumed per week. This variable was highly positively skewed and therefore categorized into '3 glasses or less', '4-7 glasses', '8-14 glasses', '15-21 glasses', and 'more than 21 glasses'. The categories were based on the maximum weekly number of drinks recommended by the Health Council of the Netherlands (7 for women, 14 for men), and criteria for excessive alcohol use (over 14 drinks per week for women, over 21 drinks per week for men) (Health Council of the Netherlands, 2006; 2002).

Respondents were asked which alcoholic beverage they preferred ('wine', 'beer', 'strong liquor', 'none'). Urges to drink alcohol in several situations (social situations, during/after dinner, after work, when relaxing, when concentrating, when under stress/pressure) were assessed, based on items about situation-specific urges to smoke (West & Russell, 1985). Answer options for each situation were 'not at all', 'mild', and 'strong'. If participants stated they had ever been intoxicated, they were asked at what age they had been intoxicated for the first time, and the number of times they had been intoxicated. Number of intoxications was severely skewed, and was therefore categorized into 'once or twice', '3-5 times', '6-10 times', '11-25 times', and '>25 times', based on the distribution in the sample. Lifetime prevalence of alcohol abuse and dependence (AAD) symptoms were assessed with the CAGE questionnaire, which consists of 4 items indexing AAD symptoms, that can be answered with 'no', 'yes, not in the past year' or 'yes, in the past year' (Ewing, 1984). The last two categories were combined, resulting in four binary items for lifetime prevalence of each symptom. Item scores were summed and dichotomized into no (0) versus any (1-4) alcohol abuse and dependence symptoms (J. H. D. A. van Beek et al., 2012). Hazardous drinking was indexed by summing the 10 items of the Alcohol Use Disorders Identification Test (AUDIT), and dichotomizing at the cutoff

score of 8, with scores above indicating hazardous drinking (Babor et al., 2001).

Analyses

The sample (N=16,587) was stratified by sex and age (age groups: 18-25, 25-35, 35-45, 45-55, 55-65, ≥65 years). In each sex by age group, prevalence/distribution of demographic and lifestyle variables was computed. Subsequently, each case was assigned a proportional weight to correct for the most important deviations from the general population on demographic traits. Alcohol variables were described by sex and age while cases were weighted. For binary/categorical alcohol variables, prevalence/frequency distributions were computed using SPSS 18 (SPSS Inc, 2009). For continuous alcohol variables, mean and standard deviation were computed in Mplus 5 while correcting for familial clustering, since standard deviations are underestimated if subjects are not independent (Muthén & Muthén, 2010; Rebollo et al., 2006). Correlations between alcohol use indicators were also corrected for familial clustering in Mplus 5, as were regressions of alcohol use on demographic/lifestyle variables. Data on all predictor variables were available for 12,222 subjects. From this group, 75% (N=9,103) was randomly selected as the main dataset in which the regression analyses were carried out. The remainder of the sample (N=3,119) was used to cross-validate the results of the regression analyses in. Age, sex (0 'male', 1 'female'), an age*sex interaction term, and all other demographic/lifestyle variables were used to predict each aspect of alcohol use. The continuous alcohol measures (age at alcohol initiation, age at onset regular drinking, age at first alcohol intoxication) were predicted with linear regressions, and binary and ordered categorical alcohol measures (frequency of alcohol use, quantity of alcohol consumed, situation-specific urges to drink, number of alcohol intoxications, AAD symptoms, hazardous drinking) were predicted with logistic regressions. Alcohol initiation and preferred beverage were predicted with multinomial regressions, in which the reference categories were 'no' for alcohol initiation and 'no preference' for preferred beverage.

Results

Demographic and lifestyle variables. Table 2 shows prevalence/distribution of demographic and lifestyle factors, stratified by age-group and sex. The largest proportion of participants aged 25 years or older had completed upper vocational or university education (between 36.8 - 60.4%), with the

exception of women above age 55 years. Among participants between 18-25 years old, a large proportion (68%) were students and had not yet completed upper vocational school or university. Participants were more often highly educated than the general population, mainly in the younger age groups (differences ranged between 1-25%) (2012a), and between ages 18-25 years, the proportion of students was larger than in the general population (68% versus 40%) (2012).Work-related stress (in the previous year) was most prevalent between ages 25-35 (51.0% in men; 57.0% in women) and least prevalent above age 65 years (28.9% in men, 21.7% in women). The majority of the participants had experienced little or no financial stress in the previous year (64.6-84.6%). Women reported somewhat more financial stress than men, especially at younger ages. The proportion of participants who lived in densely populated (urban) areas ranged between 27.6–48.4% and was about 7-16% lower than in the general population (Statistics Netherlands, 2008). Between ages 18-25, about 40% of participants was religious (either non-actively; about 28%, or actively; about 11%). Religiousness was most prevalent above age 65 years (68.1% in men, 78.8% in women). Across age groups, religiousness was less prevalent than in the general population (about 1-10% difference) (Arts, 2009). Between ages 18-25, about half of the participants had initiated smoking cigarettes. Among men, this proportion was 83.3% above age 65 years. Among women, the prevalence of smoking initiation was 76.5% at ages 55-65, but 57.0% above age 65 years. Overall, the prevalence of smoking initiation was 63.2%, slightly higher than the prevalence in the general population (60.0%) (Statistics Netherlands, 2007). Cannabis initiation was most prevalent between ages 25-35 years (47.9% in men, 31.2% in women), and least prevalent above age 65 years (4.0% in men and 1.3% in women). The prevalence of cannabis initiation in women was highly similar to that in the general population, while in men, it was slightly lower (the largest difference was 9%) (2010a). BMI was higher in the older than in the younger age groups. Above age 45 years, more than half of the men were overweight (54.9-62.4%). Women were mostly classified in the normal weight range, except above age 55 years, where about 50% of women were overweight. Overweight was less prevalent than in the general population, especially in the younger age groups (about 0-12% difference) (Statistics Netherlands, 2010b).

Table 2 Distribution or prevalence of demographic and lifestyle variables, stratified by age and sex

	Age 18-25		*Age 25-35*		*Age 35-45*		*Age 45-55*		*Age 55-65*		*Age 65 or older*	
	Men	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*
	N=1,235	*N=2,499*	*N=839*	*N=1,769*	*N= 995*	*N= 1,891*	*N=1,192*	*N= 2,265*	*N= 1,255*	*N= 1,507*	*N= 536*	*N= 604*
Educational attainment (N=14,799)	%	%	%	%	%	%	%	%	%	%	%	%
Primary/lower vocat.	19.3	20.0	8.8	7.4	12.3	16.7	22.2	28.7	30.5	50.6	35.8	57.3
Intermediate vocat./ upper sec.	70.2	66.5	30.8	32.2	35.5	40.4	29.5	34.4	24.8	21.5	22.5	18.6
Upper vocat./university	10.5	13.5	60.4	60.4	52.2	42.9	48.3	36.8	44.7	27.9	41.6	24.1
Work-related stress (N=13,961)												
Never	61.0	53.8	49.0	43.0	51.0	46.7	48.6	48.6	58.2	60.9	71.1	78.3
Occasionally	32.7	36.8	35.6	36.8	33.1	38.0	38.8	36.1	30.9	27.7	22.1	15.8
Regularly	5.4	8.9	14.6	18.1	14.1	13.7	10.9	13.7	9.0	10.3	5.0	5.1
Constantly	.9	.5	.8	2.1	1.7	1.6	1.7	1.7	1.9	1.1	1.8	.8
Financial stress (N=14,990)												
None/little	73.8	64.6	71.7	65.5	71.7	66.8	71.2	67.2	78.5	77.4	84.6	84.4
Moderate	23.3	29.3	23.5	26.3	23.8	26.9	25.5	26.1	18.1	18.4	14.2	13.4
Severe	2.9	6.1	4.8	8.2	4.5	6.3	3.3	6.7	3.4	4.2	1.2	2.2
Degree of urbanization (N=16,201)												
Urban residential area	28.7	32.4	48.4	44.1	36.1	32.0	28.3	27.6	31.9	31.6	34.2	37.3
Religiousness (N=16,180)												
Not religious	62.0	59.8	61.9	57.2	55.9	47.6	47.3	39.2	41.2	31.9	31.9	21.2
Religious, not actively	26.7	28.9	26.2	29.1	29.6	37.4	35.1	41.7	40.1	44.9	39.1	37.8
Active church member	11.3	11.3	12.0	13.7	14.4	15.1	17.7	19.1	18.7	23.2	29.0	40.9
Smoking initiation (N=15,527)	52.6	47.0	62.1	55.9	60.7	55.4	68.9	72.7	84.3	76.5	83.3	57.0
Cannabis initiation (N=15,122)	41.6	29.5	47.9	31.2	30.6	20.6	16.6	10.9	11.9	7.3	4.0	1.3
BMI (N=15,889)												
Underweight	7.7	8.8	2.2	4.6	.2	2.0	.3	1.1	.2	1.3	.2	1.1
Normal weight	81.6	79.1	65.1	71.4	51.2	63.5	41.8	55.3	37.4	49.5	44.9	48.5
Overweight	10.7	12.1	32.7	24.0	48.6	34.5	57.8	43.5	62.4	49.2	54.9	50.4

Alcohol use Each case was assigned a proportional weight based educational attainment, since the sample distribution of this variable deviated most from the distribution in the general population. Table 3 shows prevalence/distribution or mean and standard deviation of alcohol use indicators, stratified by age and sex (see additional Table A1 for prevalence/means in the unweighted sample). Around 94-98% of the participants had ever used alcohol, except women aged 65 years or older (85.7%). Across all age groups, more men had initiated alcohol use than women. Frequency of alcohol use was lowest among young adults (age 18-25 years), with 3.2% of men and .6% of women drinking 6-7 times a week. Men above age 65 years drank most frequently (32.7% drank 6-7 times per week). The proportion of women who drank 6-7 times a week was highest above age 55 years (20.2-22.0%). Across all age groups, men drank more frequently than women. Quantity of alcohol showed somewhat different age patterns in men and women. In men, drinking more than the recommended 14 glasses per week occurred most often between ages 18-25 years (34.6%) and nearly as often between ages 55-65 years (32.4%). The proportion of men who drank more than 14 glasses a week was smallest between 25-35 years (20.8%). Excessive drinking (>21 glasses a week) occurred least often in men aged between 35-45 years (9.3%), and most often between ages 18-25 years (17.2%). Among women, quantity of alcohol consumed was lowest between ages 25-35 years, with 19.5% drinking more than the recommended 7 glasses per week, and 5.7% excessive drinkers (>14 glasses per week). Women drank the largest quantities above age 55 years, with about 40% drinking more than the recommended 7 glasses per week, and about 15% excessive drinkers (>14 glasses per week). Men drank larger quantities of alcohol than women across the entire age range. Wine was more popular above age 65 than in the youngest age group (46.3 versus 2.3% in men, 85.3 versus 37.3% in women). Among men, beer was the most popular beverage, while women mostly preferred wine, followed by strong liquor. The urge to drink in social situations was reported most often (64.1 – 87.6%), and the least often reported urge to drink was while concentrating (.9 - 6.5%). The prevalence of urges to drink in different situations was slightly higher at older ages, especially among women. Generally, men experienced more urges to drink alcohol than women.

The number of alcohol intoxications was highest for individuals between 25-35 years; 26.9% of men and 8.4% of women had been intoxicated more than 25 times in this age group, compared to 4.8% of men and .8% of women above age 65 years. Men reported more alcohol intoxications than women. In men,

Table 3 Prevalence/distribution or mean and standard deviation of alcohol use indicators, by age and sex (subjects weighted for educational attainment)

		Age 18-25		*Age 25-35*		*Age 35-45*		*Age 45-55*		*Age 55-65*		*Age 65 or older*	
		Men N=1,222	*Women N=2,493*	*Men N=829*	*Women N=1,754*	*Men N= 978*	*Women N= 1,874*	*Men N=1,201*	*Women N= 2,294*	*Men N= 1,264*	*Women N= 1,523*	*Men N= 543*	*Women N= 612*
Alcohol initiation (N=16,243*)*		%	%	%	%	%	%	%	%	%	%	%	%
No		3.0	4.1	1.7	5.0	2.1	5.1	2.8	4.7	2.1	5.9	2.9	14.3
A few times to try		5.4	7.7	3.2	7.2	4.9	9.2	1.8	7.1	1.7	5.8	2.5	8.7
Yes		91.6	88.2	95.1	87.7	93.0	85.7	95.4	88.2	96.1	88.4	94.7	77.0
Frequency of alcohol use (N=15,972*)*													
Never		7.4	10.7	6.2	18.8	7.3	16.6	6.8	13.7	5.1	14.5	9.6	23.8
Monthly or less		13.0	28.1	14.7	33.7	16.6	28.4	9.4	19.5	9.2	16.3	9.8	15.0
2-4 times a month		34.9	41.8	34.3	26.4	26.6	24.4	21.2	20.1	14.5	16.0	16.1	12.4
2-3 times a week		34.1	17.1	31.9	15.4	27.7	18.0	29.3	21.6	24.3	18.7	16.3	18.0
4-5 times a week		7.5	1.8	8.2	3.7	10.8	6.9	14.2	10.8	16.2	12.5	15.5	10.7
6-7 times a week		3.2	.6	4.8	2.0	11.0	5.6	19.1	14.3	30.6	22.0	32.7	20.2
Weekly alcohol quantity (N=12,828*)*													
3 glasses or less		22.0	39.5	23.9	52.1	24.6	46.4	20.5	35.6	14.6	29.3	17.5	30.7
4-7 glasses		20.1	29.8	27.0	28.4	27.6	30.3	24.0	31.8	23.2	29.4	28.5	29.6
8-14 glasses		23.4	19.1	28.2	13.8	25.7	17.4	31.5	22.3	29.9	25.8	26.4	25.4
15-21 glasses		17.4	6.3	10.5	3.0	12.8	3.4	13.6	7.8	18.6	10.5	15.1	11.3
More than 21 glasses		17.2	5.4	10.3	2.7	9.3	2.5	10.4	2.7	13.8	5.0	12.5	3.1
Preferred beverage (N=14,407*)*													
Wine		2.3	37.3	14.0	56.5	22.0	66.3	31.3	79.9	37.9	82.4	46.3	85.3
Beer		70.4	13.7	64.0	11.4	53.3	8.5	49.4	5.4	41.4	4.0	25.8	2.3
Strong drinks		13.0	29.2	8.5	19.8	8.6	14.0	7.1	5.2	8.3	5.1	12.7	4.5
No preference		14.3	19.7	13.5	12.3	16.2	11.2	12.1	9.5	12.4	8.4	15.2	7.9
Urges to drink alcohol													
Social situations	No	20.0	23.1	12.4	23.5	18.1	26.9	19.1	30.2	21.3	31.9	32.2	35.9
(N=15,052)	Mild	52.5	58.3	55.2	56.4	56.0	57.4	62.6	58.0	66.0	59.5	60.4	58.3
	Strong	27.5	18.5	32.4	20.1	25.9	15.7	18.3	11.8	12.7	8.6	7.5	5.8
At dinner	No	72.8	79.6	64.0	69.8	64.7	69.0	63.6	64.7	55.7	55.5	47.7	45.6
(N=14,968)	Mild	26.5	19.2	33.2	26.8	31.6	27.1	32.2	31.0	39.3	40.2	46.2	51.0
	Strong	.7	1.2	2.8	3.4	3.7	3.9	4.3	4.2	5.0	4.3	6.1	3.3
After work	No	66.1	90.5	75.4	90.5	79.7	91.2	81.3	87.7	73.9	81.3	78.0	82.2
(N=14,828)	Mild	26.9	8.6	21.2	7.9	17.8	7.0	15.2	9.6	22.2	15.4	18.5	16.4
	Strong	7.0	.9	3.4	1.6	2.5	1.8	3.4	2.7	3.9	3.3	3.5	1.4
Relaxing	No	36.9	53.3	39.6	54.2	39.6	51.9	28.7	41.7	25.8	40.5	36.8	38.1
(N=15,000)	Mild	51.5	41.7	51.7	41.8	48.8	42.6	62.1	52.5	67.3	53.9	59.5	58.6
	Strong	11.7	5.0	8.7	4.1	11.6	5.5	9.1	5.8	6.8	5.6	3.7	3.3

		Age 18-25		*Age 25-35*		*Age 35-45*		*Age 45-55*		*Age 55-65*		*Age 65 or older*	
		Men	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*
		N=1,222	*N=2,493*	*N=829*	*N=1,754*	*N= 978*	*N= 1,874*	*N=1,201*	*N= 2,294*	*N= 1,264*	*N= 1,523*	*N= 543*	*N= 612*
		%	%	%	%	%	%	%	%	%	%	%	%
Concentrating	No	97.3	98.3	98.0	99.0	97.4	99.1	97.6	99.0	96.3	96.5	93.5	96.3
(N=14,667)	Mild	2.6	1.6	1.7	.9	2.3	.8	2.3	1.0	3.4	3.3	6.3	3.7
	Strong	.1	.1	.3	.1	.3	.1	.1	.1	.3	.2	.2	0
Under stress	No	79.8	84.0	80.2	84.6	82.2	82.9	78.1	78.2	74.4	72.9	75.1	73.4
(N=14,705)	Mild	16.4	13.0	16.2	11.9	15.5	13.9	18.0	17.6	20.6	21.7	19.9	21.2
	Strong	3.8	3.0	3.6	3.5	2.3	3.2	3.9	4.2	5.0	5.4	5.0	5.4
No. of intoxications (N=8,885)													
Once or twice		29.3	39.6	14.7	32.1	18.6	37.9	22.3	44.5	25.6	50.9	36.3	55.3
3-5 times		26.4	32.2	21.0	30.9	21.7	34.3	32.8	35.8	33.5	29.7	31.8	28.5
6-10 times		18.3	13.7	23.2	17.1	19.9	16.2	23.9	13.1	22.0	12.1	18.0	10.6
11-25 times		13.6	7.8	14.2	11.4	16.0	7.1	10.5	3.6	10.5	4.4	9.0	4.9
More than 25 times		12.5	6.7	26.9	8.4	23.8	4.5	10.5	3.0	8.4	2.9	4.8	.8
Alcohol abuse disorder symptoms (N=15,227)		35.2	20.9	41.4	18.2	32.5	17.1	34.1	22.2	36.6	23.3	28.0	16.2
Hazardous drinking (N=15,467)		29.5	13.0	22.7	7.2	19.1	6.6	16.6	9.8	18.6	9.4	12.7	5.5
		mean (sd)	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*	*mean (sd)*
Age at alcohol initiation		14.5	14.6	15.0	15.5	15.6	16.5	15.5	16.6	16.3	17.9	18.1	20.3
(N=15,155)		(2.0)	(2.0)	(2.4)	(2.4)	(2.5)	(2.9)	(2.4)	(3.0)	(2.8)	(4.3)	(4.5)	(6.4)
Age at onset regular drinking		16.7	16.8	18.0	19.2	18.9	22.5	20.4	25.6	22.6	29.1	28.2	33.8
(N=8,483)		(1.2)	(1.6)	(2.9)	(4.5)	(4.0)	(6.2)	(5.7)	(9.0)	(8.3)	(11.0)	(12.6)	(13.3)
Age at first intoxication		16.5	16.6	17.4	18.8	18.2	20.9	18.7	21.9	20.5	25.1	24.2	31.6
(N=10,102)		(1.5)	(1.6)	(2.6)	(3.3)	(3.0)	(5.4)	(3.9)	(6.7)	(5.3)	(8.8)	(8.6)	(12.0)

lifetime AAD symptoms were most prevalent between ages 25-35 years (41.4%) and least prevalent above age 65 years (28.0%). Among women, lifetime AAD symptoms occurred most frequently between ages 55-65 years (23.3%), and least frequently above age 65 years (16.2%). Lifetime prevalence of AAD symptoms was higher in men than in women. Hazardous drinking occurred most frequently between ages 18-25 years (29.5% in men, 13.0% in women), and least often above age 65 years (12.7% in men, 5.5% in women). Hazardous drinking was more prevalent in men than in women. Between ages 18-25 years, age at alcohol initiation was lowest, and highly similar for men andwomen (14.5 in men, 14.6 in women). Among individuals aged 35 years and older, men initiated alcohol use at a younger age than women. Average age at initiation was highest for individuals aged 65 years or older (18.1 years in men and 20.3 in women). Similarly, average age at onset of regular drinking was lowest in the youngest age group (16.7 years in men, 16.8 in women) and highest above age 65 years (28.2 years in men, 33.8 in women). Average age at first intoxication showed the same pattern: it was lowest between ages 18-25 years (16.5 in men, 16.6 in women) and highest above age 65 years (24.2 in men, 31.6 in women). With the exception of the 18-25 age group, men were younger than women at first alcohol intoxication.

Table 4 shows the correlation coefficients between all indicators of alcohol use, except the nominal alcohol variables (initiation of alcohol use and preferred beverage). Correlations that were significant at α=.01 are shown in black font and non-significant correlations in grey font. Nearly all aspects of alcohol use were significantly inter-related. Age at onset of regular drinking and age at first alcohol intoxication were least strongly associated with other alcohol variables (absolute values of correlations ranged between .03 and .55). Frequency of alcohol use was most strongly related to other alcohol variables (absolute values of correlations ranged between .05 and .69).

Associations of alcohol use with demographic and lifestyle variables

Associations (standardized regression coefficients β) between each aspect of alcohol use and demographic and lifestyle variables are shown in Table 5. The regression (β) coefficients that were significant at α=.01 in both the main sample and the validation sample are shown in bold font. The regression coefficients that were significant in the main sample but not in the validation sample are shown in regular font, and the non-significant coefficients are shown in grey (see additional Table A2 for the cross-validation results). The regression analyses confirmed that age at alcohol initiation, age at onset of regular

Table 4 Correlations between alcohol use indicators (N=16,253)

	Alcohol frequency	Alcohol quantity	Social situations	At dinner	After work	When relaxing	Concentrating	Under stress	No. of intoxic.	AAD symptoms	Hazardous drinking	Age alc. initiation	Age onset reg. drink.
Alcohol frequency													
Alcohol quantity	.69 (.01)												
Social situations	.40 (.01)	.40 (.01)											
At dinner	.42 (.01)	.29 (.01)	.31 (.01)										
After work	.38 (.01)	.38 (.01)	.23 (.01)	.35 (.01)									
When relaxing	.42 (.01)	.39 (.01)	.37 (.01)	.24 (.01)	.28 (.01)								
Concentrating	.14 (.01)	.14 (.01)	.09 (.01)	.13 (.01)	.22 (.02)	.14 (.01)							
Under stress	.35 (.01)	.35 (.01)	.27 (.01)	.23 (.01)	.35 (.01)	.31 (.01)	.27 (.01)						
No. of intoxications	.19 (.01)	.33 (.01)	.29 (.01)	.11 (.01)	.15 (.01)	.14 (.01)	.04 (.01)	.13 (.01)					
AAD symptoms	.35 (.01)	.41 (.01)	.29 (.01)	.19 (.01)	.26 (.01)	.24 (.01)	.11 (.01)	.32 (.01)	.31 (.01)				
Hazard. drinking	.32 (.01)	.46 (.01)	.29 (.01)	.15 (.01)	.29 (.01)	.24 (.01)	.11 (.01)	.32 (.01)	.30 (.01)	.53 (.01)			
Age alc. initiation	-.05 (.01)	-.14 (.01)	-.20 (.01)	-.03 (.01)	-.07 (.01)	-.08 (.01)	.01 (.01)	-.05 (.01)	-.25 (.01)	-.13 (.01)	-.11 (.01)		
Age onset rg. drink.	.22 (.01)	.03 (.01)	-.10 (.01)	.12 (.01)	.03 (.01)	.03 (.01)	.04 (.01)	.06 (.01)	-.21 (.01)	-.01 (.01)	-.03 (.01)	.47 (.01)	
Age first intox.	.12 (.01)	-.03 (.01)	-.13 (.01)	.07 (.01)	-.01 (.01)	.00 (.01)	.03 (.01)	.02 (.01)	-.31 (.01)	-.05 (.01)	-.08 (.01)	.53 (.02)	.55 (.02)

Note. Correlations are standardized covariances. Standard errors are in parentheses. Black font: significant at α=.01; grey font: non-significant.

Table 5 Regression (β) of alcohol use on demographic and lifestyle variables

	age	*sex (0=male)*	*age*sex*	*educ. attainm.*	*student*	*work stress*	*fin. stress*	*urban.*	*relig*	*smoking init.*	*Cann. init.*	*bmi*	R^2
Alcohol inititation (N=9,092)													
A few times	-.70	.16	.21	**.27**	-.04	-.42	.26	-.05	-.01	**.67**	.23	.01	
	(.30)	(.16)	(.24)	**(.16)**	(.18)	(.17)	(.16)	(.15)	(.14)	**(.16)**	(.27)	(.16)	
Yes	.00	**-.22**	-.09	**.34**	.05	-.06	.02	.04	-.15	**.67**	.38	-.07	
	(.10)	**(.05)**	(.08)	**(.05)**	(.06)	(.05)	(.05)	(.05)	(.05)	**(.06)**	(.08)	(.05)	
Alcohol frequency	**.29**	**-.17**	.01	**.13**	**.10**	.00	.00	-.01	**-.03**	**.18**	**.07**	**-.09**	.17
(N=9,009)	**(.02)**	**(.01)**	(.02)	**(.01)**	**(.01)**	(.01)	(.01)	(.01)	**(.01)**	**(.01)**	**(.01)**	**(.01)**	
Alcohol quantity	**.10**	**-.25**	.05	-.01	**.10**	-.01	.04	-.03	**-.06**	**.19**	**.11**	-.03	.16
(N=7,521)	**(.02)**	**(.01)**	(.02)	(.01)	**(.01)**	(.01)	(.01)	(.01)	**(.01)**	**(.01)**	**(.01)**	(.01)	
Pref. beverage (N=8,285)													
Wine	**.58**	**.75**	.02	**.36**	-.02	.13	-.10	-.01	.07	.19	-.05	-.12	
	(.06)	**(.03)**	(.06)	**(.04)**	(.05)	(.05)	(.04)	(.04)	(.04)	(.04)	(.04)	(.04)	
Beer	**-.31**	**-.91**	.03	-.06	-.02	.07	-.05	-.10	-.05	**.34**	.07	-.04	
	(.07)	**(.02)**	(.08)	(.05)	(.05)	(.05)	(.06)	(.05)	(.05)	**(.05)**	(.05)	(.05)	
Strong liquor	-.12	.35	-.82	-.37	-.20	.19	.00	-.01	-.05	.33	-.15	.21	
	(.19)	(.11)	(.15)	(.09)	(.10)	(.12)	(.12)	(.10)	(.10)	(.10)	(.11)	(.10)	
Urge to drink alcohol													
Social situations	**-.14**	**-.10**	.03	**.14**	**.07**	.00	.02	.04	-.02	**.15**	**.12**	-.03	.11
(N=8,624)	**(.02)**	**(.01)**	.02)	**(.01)**	**(.01)**	(.01)	(.01)	(.01)	(.01)	**(.01)**	**(.01)**	(.01)	
At dinner (N=8,567)	**.19**	-.02	.06	**.20**	.05	-.02	.03	**.10**	.00	.05	**.08**	-.06	.10
	(.02)	(.01)	(.02)	**(.01)**	(.02)	(.01)	(.01)	**(.01)**	(.01)	(.01)	**(.01)**	(.01)	
After work (N=8,506)	.02	**-.19**	**.16**	.00	.06	.02	.03	.06	**-.05**	.14	**.13**	-.07	.13
	(.03)	**(.02)**	**(.02)**	(.02)	(.02)	(.02)	(.02)	(.02)	**(.02)**	(.02)	**(.02)**	(.02)	
When relaxing	.03	**-.14**	.04	-.01	.02	.03	.01	-.01	.04	**.13**	.04	-.01	.05
(N=8,597)	(.02)	**(.01)**	(.02)	(.01)	(.02)	(.01)	(.01)	(.01)	(.01)	**(.01)**	(.01)	(.01)	

	age	*sex (0=male)*	*age*sex*	*educ. attainm.*	*student*	*work stress*	*fin. stress*	*urban.*	*relig*	*smoking init.*	*Cann. init.*	*bmi*	R^2
When concentting	.20	**-.18**	-.08	-.08	.07	-.04	**.13**	.11	.01	.27	-.01	-.08	.17
(N=8,441)	(.07)	**(.04)**	(.07)	(.04)	(.05)	(.05)	**(.05)**	(.04)	(.04)	(.06)	(.05)	(.04)	
Under sttress	.16	.00	.03	.04	.05	.04	.09	.03	-.01	**.19**	.12	-.04	.11
(N=8,460)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	**(.02)**	(.02)	(.02)	
No. of intoxications	-.08	**-.24**	-.02	**.09**	-.03	**-.05**	**.05**	.04	**-.09**	**.09**	**.22**	.03	.17
(N=5,216)	(.02)	**(.01)**	(.02)	**(.01)**	(.02)	**(.02)**	**(.02)**	(.01)	**(.01)**	**(.01)**	**(.02)**	(.01)	
AAD symptoms	.02	**-.15**	.05	**.09**	.06	.04	.04	.02	**-.05**	**.18**	**.16**	-.03	.13
(N=8,733)	(.02)	**(.01)**	(.02)	**(.01)**	(.02)	(.02)	(.02)	(.01)	**(.01)**	**(.02)**	**(.01)**	(.01)	
Hazardous drinking	-.09	**-.19**	.09	.05	**.08**	.03	.06	.03	-.03	**.24**	**.12**	-.01	.15
(N=8,788)	(.03)	**(.02)**	(.03)	(.02)	**(.02)**	(.02)	(.02)	(.02)	(.02)	**(.02)**	**(.02)**	(.02)	
Age at alc. initiation	**.25**	**.11**	**.13**	-.05	-.01	-.03	.01	.04	.01	**-.15**	**-.09**	-.01	.17
(N=8,633)	**(.02)**	**(.01)**	**(.02)**	(.01)	(.01)	(.01)	(.01)	(.01)	(.01)	**(.01)**	**(.01)**	(.01)	
Age onset reg. drink.	**.34**	**.19**	**.19**	.03	.02	**-.09**	.05	.02	.03	-.02	-.06	-.05	.27
(N=4,880)	**(.02)**	**(.01)**	**(.02)**	(.01)	(.01)	**(.01)**	(.01)	(.01)	(.01)	(.01)	(.01)	(.01)	
Age 1st intoxication	**.31**	**.22**	**.21**	-.01	.04	.00	-.02	.05	.06	-.09	**-.09**	-.01	.25
(N=5,897)	**(.02)**	**(.01)**	**(.02)**	(.01)	(.01)	(.01)	(.01)	(.01)	(.01)	(.01)	**(.01)**	(.01)	

Note. β (beta): standardized regression coefficients, with standard errors in parentheses. Betas in bold font were significant at α=.01 in the main sample**and** in the validation sample. Betas in regular black font were significant (at α=.01) in the main sample but **not** in the validation sample. Betas in grey were not significant (at α=.01).

drinking, and age at first alcohol intoxication were lower in younger, than in older participants (betas between .25 and .34). Older participants drank more frequently and larger quantities of alcohol use, and more often preferred wine, whereas young participants more often drank beer (absolute beta values ranged between .10 and .58). The urge to drink alcohol at dinner was more prevalent among older participants (β=.19). In contrast, the urge to drink in social situations was more prevalent in younger participants (β= -.14). The urge to drink after work was more prevalent in older than in younger women, but in men, no association with age was observed (β=.16). Men scored higher than women on nearly all aspects of alcohol use (betas between -.10 and -.25). Men were also younger at alcohol initiation, onset of regular use, and first intoxication (betas between .11 and .22). This sex difference was significantly smaller in the young, than in the older participants (betas ranged between .13 and .21). Women mostly preferred wine (β=.75), and among men, beer was the most popular beverage (β= -.91). High educational attainment, being a student, experiencing financial stress, and high degree of urbanization were associated with higher levels of alcohol use (betas between .05 and .34), while work-related stress, religiousness, and high BMI were related to lower levels of alcohol use (betas between -.05 and -.09). Initiation of smoking and cannabis were strongly, positively related to many aspects of alcohol use (betas between .07-.67), as well as to early age at alcohol initiation and age at first intoxication (betas between -.09 and -.15).

The proportion of variance explained by the demographic and lifestyle variables ranged between 5% (urge to drink while relaxing) and 27% (age at onset regular drinking). Pseudo R^2-values are not provided for alcohol initiation and preferred beverage, since these are not available in Mplus for multinomial regressions.

Discussion

A detailed analysis of multiple alcohol consumption variables in a large, population-based sample of adults, ranging in age between 18 and 97 years, has yielded a wealth of information on drinking patterns in the Dutch population. Frequency and quantity of alcohol use, urges to drink, and indicators of more severe alcohol use are strongly associated with each other, but less strong with age at initiation of alcohol use, at onset of regular drinking, and age at first alcohol intoxication. We highlight several important observations that were confirmed by regression analyses, including a large set of demographic and lifestyle variables.

First, men and women (above age 55 years) drink more frequently than young adults, and among women, excessive drinking (>14 glasses of alcohol per week) is substantially more prevalent above age 55 years than in the young adult age group. The prevalence of excessive drinking is lowest in women between ages 25-35 years, when they typically have children, and the high proportion of excessive drinking in elderly women may be explained by them having more opportunity to drink when their children have grown up. The high levels of alcohol consumption in men and women above age 55 years are consistent with previously observed increases in alcohol use disorders among the elderly Dutch population (Weingart, 2009), and suggest that this trend is continuing. These increases may be due to the growing number of healthy life years, in combination with a higher average income, which has increased substantially over the past years in the elderly Dutch population (Otten et al., 2006; van den Berg Jeths et al., 2004).

Secondly, in the young adult group, women initiate alcohol use, start drinking regularly, and report first alcohol intoxication at the same ages as men. These findings corroborate the trend that in young adults, the gap between male and female drinking is narrowing (Grucza et al., 2009; 2004; Van Laar et al., 2011).

The declining sex differences are mainly caused by increases in alcohol use in women, which may result from women having more freedom and financial independence. Drinking among women has also become more socially accepted than several decades ago, and young women nowadays may have fewer family responsibilities (Smith & Foxcroft, 2009).

Additionally, since the 1940s-50s, when the individuals in the highest age category were adolescents, alcohol initiation, onset of regular drinking, and alcohol first intoxication have been occurring at increasingly younger ages. This is in line with the increase in alcohol consumption in the Dutch population observed between 1950 and 1980, specifically of beer and imported wine (Dutch Institute for Alcohol Policy (STAP), 2009; Karlsson & Osterberg, 2006). The increase was likely due to strong economic growth and increases in international commerce during this period (Smits, 1999). It should be noted that the differences in age at alcohol initiation between young and old participants may be slightly overestimated due to retrospective reporting bias (Johnson & Schultz, 2005; Sartor et al., 2011).

Because of the large number of predictor variables, no other interaction effects were examined than between age and sex, while these may be relevant in the prediction of alcohol consumption. For example, those above age 55 years

who seek treatment for alcohol use disorders are more often highly educated than treatment seekers below age 55 years (Weingart, 2009). Similarly, alcohol dependence is related to high education in women, but not in men (van Laar et al., 2010).

We observed that elderly Dutch men and women continue to drink alcohol more frequently than young adults, and excessive drinking is substantially more prevalent in elderly women than in young adult women. Until now, alcohol prevention campaigns have predominantly targeted adolescents and young adults, but the high levels of alcohol consumption among the elderly warrant prevention and intervention campaigns aimed specifically at this age group (Netherlands Institute of Mental Health and Addiction, 2011). Initiation of alcohol use, onset of regular drinking, and first alcohol intoxication occur at increasingly younger ages, and the gap that previously existed between men and women in age at alcohol initiation, age at onset of regular drinking, and age at first alcohol intoxication continues to close. This trend has important consequences for public health, since women seem more susceptible to the harmful effects of alcohol, such as liver and heart disease, than men (National Institute on Alcohol Abuse and Alcoholism, 2011).

Conclusions

In the Netherlands, men and women above 55 years drink alcohol more frequently than young adults, and excessive drinking is substantially more prevalent in these women than in young adult women. Alcohol initiation, onset of regular drinking, and first alcohol intoxication occur at increasingly younger ages, and the gap that previously existed between men and women in age at alcohol initiation, age at onset of regular drinking, and age at first alcohol intoxication, continues to close.

Supplement to chapter 5

Suppl. Table 1 Prevalence/distribution or mean and standard deviation of alcohol use indicators, by age and sex, in unweighted subjects

		Age 18-25		*Age 25-35*		*Age 35-45*		*Age 45-55*		*Age 55-65*		*Age 65 or older*	
		Men	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*
		N=1,235	*N=2,499*	*N=839*	*N=1,769*	*N= 995*	*N= 1,891*	*N=1,192*	*N= 2,265*	*N= 1,255*	*N= 1,507*	*N= 536*	*N= 604*
Alcohol initiation (N=16,239)		%	%	%	%	%	%	%	%	%	%	%	%
No		2.6	3.6	1.6	3.8	2.2	4.8	2.8	4.5	2.0	6.0	2.9	14.3
A few times to try		4.4	7.0	3.1	7.0	4.2	8.5	1.9	6.8	1.6	5.7	2.5	8.7
Yes		93.0	89.4	95.4	89.2	93.6	86.7	95.3	88.7	96.3	88.3	94.7	77
Alcohol frequency (N=15,321)													
Never		3.5	6.0	3.9	12.9	4.3	11.4	3.9	9.0	2.9	9.0	6.9	10.0
Monthly or less		12.2	28.0	13.2	33.4	15.9	29.2	9.2	19.5	8.7	17.0	10.1	17.7
2-4 times a month		36.1	42.9	35.2	29.3	27.7	26.2	21.4	20.8	14.8	17.2	16.6	14.6
2-3 times a week		36.2	20.1	34.2	17.5	29.8	19.5	29.8	23.3	24.5	19.9	16.8	21.2
4-5 times a week		7.8	2.4	8.4	4.6	11.3	7.8	15.6	11.7	17.1	13.5	16.0	12.6
6-7 times a week		4.2	.6	5.0	2.4	11.0	6.0	20.1	15.7	32.1	23.4	33.7	23.9
Weekly alc. quantity (N=13,026)													
3 glasses or less		21.5	39.3	23.3	51.8	25.3	46.5	20.1	34.8	14.3	28.9	17.5	30.7
4-7 glasses		18.6	29.2	28.6	28.7	29.2	30.0	24.6	32.1	23.1	29.4	28.5	29.6
8-14 glasses		25.0	19.9	28.4	13.9	26.0	17.7	31.6	22.7	29.5	26.2	26.4	25.4
15-21 glasses		16.7	6.4	10.1	3.0	11.9	3.4	13.8	7.7	18.9	10.6	15.1	11.3
More than 21 glasses		18.2	5.2	9.7	2.6	7.7	2.5	9.9	2.7	14.3	4.9	12.5	3.1
Preferred beverage (N=14,482)													
Wine		3.2	39.9	16.7	60.4	26.1	68.1	34.3	80.7	39.4	82.6	46.3	85.3
Beer		72.3	14.4	62.9	10.9	50.7	8.4	46.9	5.3	40.6	3.9	25.8	2.3
Strong drinks		11.5	27.4	7.1	16.9	7.1	12.7	6.8	5.0	8.2	5.1	12.7	4.5
No preference		13.1	18.3	13.3	11.8	16.2	10.8	12.1	9.0	11.8	8.4	15.2	7.9
Urges to drink alcohol													
Social sit.	No	17.4	20.7	10.7	20.9	16.5	25.4	18.1	29.3	20.3	32.0	32.2	35.9
(N=15,115)	Mild	53.3	59.3	55.2	57.5	57.0	57.8	63.4	58.4	66.4	59.1	60.4	58.3
	Strong	29.3	20.1	34.1	21.6	26.5	16.8	18.5	12.3	13.2	8.9	7.5	5.8
At dinner	No	70.6	77.5	60.0	65.5	61.7	66.2	61.8	63.2	53.9	54.8	47.7	45.6
(N=15,038)	Mild	28.3	21.1	36.6	30.6	34.1	29.2	33.6	32.2	40.3	40.6	46.2	51.0
	Strong	1.0	1.5	3.4	3.9	4.2	4.5	4.6	4.6	5.8	4.6	6.1	3.3

		Age 18-25		*Age 25-35*		*Age 35-45*		*Age 45-55*		*Age 55-65*		*Age 65 or older*	
		Men N=1,235	*Women N=2,499*	*Men N=839*	*Women N=1,769*	*Men N= 995*	*Women N= 1,891*	*Men N=1,192*	*Women N= 2,265*	*Men N= 1,255*	*Women N= 1,507*	*Men N= 536*	*Women N= 604*
		%	%	%	%	%	%	%	%	%	%	%	%
After work	No	66.3	90.8	77.1	89.3	81.0	90.5	81.3	87.1	72.8	80.9	78.0	82.2
(N=14,892)	Mild	26.8	8.4	20.1	9.0	16.7	7.6	15.3	10.1	22.8	15.6	18.5	16.4
	Strong	6.9	.8	2.8	1.7	2.3	1.9	3.5	2.9	4.4	3.5	3.5	1.4
Relaxing	No	36.4	52.8	40.2	52.7	40.5	51.7	29.5	41.8	25.9	40.6	36.8	38.1
(N=15,056)	Mild	51.9	42.2	51.6	43.1	48.9	42.5	61.9	52.3	66.7	53.6	59.5	58.6
	Strong	11.7	4.9	8.2	4.2	10.6	5.8	8.6	5.8	7.3	5.8	3.7	3.3
Concentrating	No	97.0	98.5	98.1	99.0	97.6	99.1	97.5	99.0	96.2	96.5	93.5	96.3
(N=14,735)	Mild	2.8	1.5	1.8	.9	2.2	.8	2.3	.9	3.5	3.3	6.3	3.7
	Strong	.2	0	.1	.1	.2	.1	.2	.1	.3	.2	.2	0
Under stress	No	79.9	84.1	80.3	83.9	82.1	82.6	78.2	77.6	72.9	73.1	75.1	73.4
(N=14,775)	Mild	16.2	13.3	16.1	12.8	15.4	14.2	17.8	18.1	21.8	21.3	19.9	21.2
	Strong	4.0	2.6	3.6	3.3	2.5	3.2	4.0	4.3	5.3	5.6	5.0	5.4
No. of intoxications (N=9,056)													
Once or twice		25.8	36.7	13.4	30.1	18.4	36.7	22.3	44.1	26.2	50.7	36.3	55.3
3-5 times		26.5	32.3	20.8	30.5	21.3	34.4	33.3	36.0	32.8	29.7	31.8	28.5
6-10 times		18.1	15.5	23.2	17.3	19.5	16.4	23.6	13.1	22.3	12.3	18.0	10.6
11-25 times		14.2	8.6	14.0	12.4	15.7	7.5	10.4	3.9	10.4	4.5	9.0	4.9
More than 25 times		15.4	6.9	28.6	9.7	25.2	4.9	10.4	2.9	8.3	2.8	4.8	.8
Alcohol abuse disorder sympt. (N=15,288)		36.6	21.5	42.5	19.4	33.7	18.0	34.6	23.2	37.4	24.0	28.0	16.2
Hazard. drink. N=15,516)	29.8		13.4	23.3	7.7	18.6	6.7	16.7	10.3	19.3	9.9	12.7	5.5
Age at alcohol initiation		14.4	14.5	15.1	15.4	15.6	16.4	15.4	16.5	16.2	17.8	18.1	20.4
(N=15,202)		(1.9)	(1.7)	(2.2)	(2.4)	(2.5)	(2.8)	(2.3)	(3.0)	(2.6)	(4.5)	(4.1)	(6.7)
Age at onset regular drinking		16.7	16.9	18.2	19.2	19.2	22.4	20.6	25.6	22.7	29.6	27.8	33.3
(N=8,638)		(1.4)	(1.6)	(2.8)	(4.4)	(4.0)	(6.1)	(5.9)	(8.9)	(8.3)	(11.1)	(11.9)	(12.6)
Age at first intoxication		16.5	16.7	17.5	18.7	18.3	20.7 (5.1)	18.6 (3.7)	21.9 (6.9)	20.5 (5.2)	24.9 (8.2)	24.1	31.1
(N=10,253)		(1.5)	(1.5)	(2.7)	(3.3)	(3.1)						(8.2)	(11.0)

Suppl. Table 2 Cross-validation of regression (β) of alcohol use on demographic and lifestyle variables

	age	sex	*age*sex*	*educ. attainm.*	*student*	*wrk stress*	*fin. stress*	*urban.*	*relig*	*smoking initiation*	*cannabis initiation*	*bmi*	R^2
Alcohol initiation (N=3,118)													
A few times to try	-.57	.33	.18	.35	.34	-.01	-.01	.02	-.11	.44	.09	-.22	
	(.41)	(.22)	(.36)	(.23)	(.26)	(.25)	(.23)	(.19)	(.19)	(.21)	(.47)	(.20)	
Yes	.08	-.14	-.06	.32 (	**-.27**	.00	.01	-.01	-.18	.56	.49	-.15	
	(.14)	(.08)	(.13)	.09)	**(.11)**	(.10)	(.09)	(.07)	(.07)	(.10)	(.13)	(.08)	
Alcohol frequency (N=3,082)	.28	-.20	.04	.14	.11	-.03	-.01	.02	-.05	.19	.04	-.09	.19
	(.03)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
Alcohol quantity (N=2,592)	.14	-.24	.03	.00	.10	.01	.00	-.04	-.09	.17	.12	-.03	.15
	(.04)	(.02)	(.03)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
Preferred beverage (N=2,820)													
Wine	.57	.73	-.09	.38	-.15	.03	.00	**-.19**	-.08	.15	-.02	.09	
	(.12)	(.05)	(.12)	(.07)	(.09)	(.09)	(.08)	**(.07)**	(.08)	(.07)	(.08)	(.08)	
Beer	-.32	-.84	.03	.07	**-.06**	.05	.04	**-.36**	-.11	.19	.14	.02	
	(.12)	(.04)	(.12)	(.08)	**(.08)**	(.09)	(.09)	**(.07)**	(.07)	(.07)	(.08)	(.08)	
Strong liquor	-.55	.23	-.39	-.18	-.11	.15	-.08	-.34	-.14	.09	.02	**.59**	
	(.26)	(.15)	(.24)	(.14)	(.15)	(.17)	(.16)	(.14)	(.15)	(.14)	(.15)	**(.13)**	
Urge to drink alcohol													
Social situations	-.17	-.11	.05	.15	.06	.00	.05	.02	-.05	.14	.11	.14	.12
(N=2,951)	(.04)	(.02)	(.03)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
At dinner (N=2,934)	.19	-.03	.05	.17	.03	-.02	.00	.10	.01	.02	.05	.02	.09
	(.04)	(.02)	(.03)	(.02)	(.03)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	
After work	-.02	-.20	.14	.04	.03	.04	.02	.00	-.10	.17	.11	.17	.13
(N=2,921)	(.04)	(.03)	(.04)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	

	age	sex	*age*sex*	*educ. attainm.*	*student*	*wrk stress*	*fin. stress*	*urban.*	*relig*	*smoking initiation*	*cannabis initiation*	*bmi*	R^2
When relaxing	.03	-.15	.06	.02	.02	.00	.05	-.02	.01	.06	.04	.06	.04
(N=2,932)	(.04)	(.02)	(.03)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
Concentrating	.16	-.17	.05	-.08	.03	-.12	.21	-.03	-.06	.19	.01	.19	.15
(N=2,890)	(.11)	(.07)	(.11)	(.07)	(.10)	(.09)	(.07)	(.07)	(.08)	(.09)	(.07)	(.09)	
Under stress	.07	-.03	**.11**	.06	.04	.07	.00	.02	**-.08**	.18	.07	.18	.09
(N=2,896)	(.04)	(.03)	**(.04)**	(.03)	(.03)	(.03)	(.03)	(.03)	**(.03)**	(.03)	(.03)	(.03)	
No. of intoxications (N=1,815)	-.15	-.26	.01	.07	-.06	-.08	.09	.02	-.09	.10	.19	.10	.17
	(.04)	(.02)	(.03)	(.02)	(.03)	(.03)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	
AAD symptoms (N=2,986)	.06	-.18	.06	.10	.07	.03	.01	.01	-.07	.18	.12	.18	.13
	(.04)	(.02)	(.03)	(.02)	(.03)	(.03)	(.03)	(.02)	(.02)	(.03)	(.02)	(.03)	
Hazard. drinking (N=3,001)	-.02	-.20	.00	.03	.09	.07	.01	.01	-.05	.19	.16	.19	.15
	(.05)	(.03)	(.05)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	(.03)	
Age alc. initiation (N=2,948)	.25	.11	.12	-.05	**-.05**	-.02	-.02	.01	.01	-.17	-.09	.01	.19
	(.03)	(.02)	(.03)	(.02)	**(.02)**	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
Age onset reg. drink.	.38	.17	.16	.03	.03	-.08	.01	.03	.07	-.05	-.01	-.03	.27
(N=1,680)	(.03)	(.02)	(.03)	(.03)	(.01)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	
Age first intox. (N=2,008)	.33	.19	.16	.00	.02	.00	-.04	.03	.03	-.07	-.09	-.07	.23
	(.03)	(.02)	(.03)	(.02)	(.02)	(.03)	(.02)	(.02)	(.02)	(.02)	(.02)	(.02)	

Note. β (beta): standardized regression coefficients, with standard errors in parentheses. Betas in black font were significant at α=.01. Betas in grey were not significant (at α=.01). Betas in bold font were significant (at α=.01) in the validation sample but not observed in the main sample.

6

Prenatal smoking predicts offspring externalizing but not internalizing problems at age three

This chapter is submitted as:

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Abstract

Background Prenatal tobacco exposure is related to offspring externalizing and internalizing problems. The mechanisms that underlie these associations may be causal or may reflect genetic and environmental factors that are shared between parents and offspring. *Methods* Associations between parental prenatal smoking and offspring externalizing/internalizing problems at age three were examined in a population-based sample of Dutch children (N=9,982). Causal effects of prenatal tobacco exposure and effects of shared genes and environment were disentangled by comparing the associations of maternal and paternal smoking. Effects of prenatal tobacco exposure were further examined by selecting offspring of mothers who had ever smoked and comparing offspring of mothers who quit before pregnancy to mothers who continued smoking during pregnancy. Finally, effects of tobacco exposure in different pregnancy trimesters were investigated. *Results* Maternal and paternal prenatal smoking were both related to offspring externalizing problems. Maternal but not paternal smoking was related to offspring internalizing problems. For externalizing problems, maternal prenatal smoking was a stronger predictor than paternal smoking. Offspring of mothers who continued to smoke during pregnancy showed increased externalizing, but not internalizing problems at age three. No differential effects of maternal smoking only in the first or last trimester were observed. *Conclusions* Associations between maternal prenatal smoking and offspring externalizing problems mainly reflect shared genes and environment and a small additional causal effect. Shared genes and environment explain all the association between maternal prenatal smoking and offspring internalizing problems, without evidence for causality.

Introduction

A considerable percentage of Dutch women continue to smoke cigarettes during pregnancy. In a population-based sample (N=4,329), assessed between 2002 and 2006, 21.7% of women smoked while pregnant (Roza et al., 2008). This is in line with the prevalence of maternal prenatal smoking reported in several other European countries and the USA (range 15-30%) (Brion et al., 2010; Ekblad et al., 2010; Monshouwer et al., 2011; Tong, 2009). The proportion of Dutch mothers who quit smoking in the first trimester was 8.4%, and 13.3% continued smoking throughout the pregnancy. Within the group of mothers who smoked during pregnancy, 49.5% smoked less than 5 cigarettes a day, 27.1% smoked between 5-9 cigarettes a day, and 23.5% smoked at least 10 cigarettes a day (Roza et al., 2008).

Maternal prenatal smoking has been consistently linked to offspring externalizing problems, which include ADHD symptoms, delinquent behavior, aggressive behavior, overactivity, impulsivity, and criminal offending (Cornelius et al., 2011; Lavigne et al., 2011; Paradis et al., 2011). Whether prenatal maternal smoking is associated with offspring internalizing psychopathology is less extensively studied, and results are conflicting. Associations between prenatal smoking and offspring anxiety, depression, and emotional problems have been observed, but other studies reported no significant relationships between prenatal smoking and internalizing psychopathology (Ashford et al., 2008; Ekblad et al., 2010; Indredavik et al., 2007; Lavigne et al., 2011; Monshouwer et al., 2011; Orlebeke et al., 1999; Rückinger et al., 2009).

The association between prenatal maternal smoking and offspring externalizing/internalizing problems may be explained by genetic or environmental factors that influence both maternal smoking and offspring externalizing/internalizing problems, or by causal effects of prenatal tobacco exposure. The teratogenic effects of prenatal tobacco exposure and its compounds, most importantly nicotine, include disruption of neural development, fetal hypoxia, and exposure to many other toxic chemicals, including several carcinogens. (2009; Ernst et al., 2001; Hellström-Lindahl, 2000; Rogers, 2009). Such causal effects of prenatal maternal smoking on offspring externalizing and internalizing problems may be mediated through birthweight, since prenatal tobacco exposure has a well-established decreasing effect on birthweight, and lower birthweight has in turn been associated with delays in neurobehavioral development in childhood, independently of prenatal smoking (Cnattingius, 2004; Ernst et al., 2001; Hayes & Sharif, 2009).

Detecting direct, causal effects of prenatal tobacco exposure is complicated

because exposure is not random, but related to maternal characteristics. Women who smoke during pregnancy tend to have lower education and socioeconomic status, be younger, single, and have more psychopathology (Rogers, 2009; Roza et al., 2008; Tong, 2009). If smoking and aspects of externalizing and internalizing problems, e.g. disinhibitory psychopathology, are influenced by the same genes or by the same environmental risk factors, such factors confound the association between prenatal smoking and offspring externalizing/internalizing problems. Maternal smoking is related to paternal smoking, and mothers who smoke during pregnancy tend to have a partner who smokes as well (Boomsma et al., 1994; Homish et al., 2012). Paternal smoking during pregnancy, although less extensively studied, has been similarly related to adverse circumstances, specifically to lower educational attainment and hazardous drinking (Everett et al., 2007). As environmental or genetic factors that influence both smoking and psychopathology can equally be transmitted by the father, comparing effects of maternal versus paternal prenatal smoking on offspring problems can help to estimate the extent to which the association is direct causal versus due to confounding factors. In a large Dutch sample, Roza et al. (2008) compared effects of maternal and paternal smoking and found no significant effects of prenatal tobacco smoking on offspring externalizing problems at the age of 18 months (Roza et al., 2008). However, in samples from the UK and Brasil, causal effects of prenatal smoking on offspring conduct and externalizing problems at age four were detected using this approach (Brion et al., 2010).

Several other approaches have been used to disentangle confounding influences. One involves adjusting the association between prenatal maternal smoking and offspring outcomes for related characteristics of mothers and fathers. In a large population-based cohort of Dutch adolescents, Monshouwer et al. (2011) adjusted for maternal age at birth, maternal alcohol use during pregnancy, maternal or paternal daily smoking, maternal or paternal history of internalizing and externalizing problems, family socioeconomic status, problems during pregnancy or childbirth, and birthweight. After adjusting for these factors, associations between prenatal maternal smoking and adolescent externalizing problems and substance use disappeared, indicating that they were due to confounding influences and not to causal effects of prenatal smoking (Monshouwer et al., 2011). This finding was replicated in another large, prospective cohort study in Dutch children and in a sample of American children (Lavigne et al., 2011; Roza et al., 2008). In contrast, in a large Finnish sample (N=175,869), Ekblad et al. (2010) observed that prenatal maternal smoking was still significantly associated with a wide range of offspring psychiatric disorders, including externalizing problems and internalizing

psychopathology, after adjusting for maternal age, parity, and psychiatric morbidity before birth of the child, and child's sex, gestational age, birthweight, and 5-minutes Apgar score (Ekblad et al., 2010). This was corroborated in samples of varying size (N=330 to 3,766), some of which adjusted only for maternal psychopathology, while others adjusted for psychopathology in both parents and still observed significant associations (Boutwell et al., 2011; Cornelius et al., 2011; Nomura et al., 2011; Paradis et al., 2011).

Family-based studies offer the possibility to examine how much of the association between prenatal smoking and offspring psychopathology is due to shared genetic and environmental factors by contrasting the similarity between family members on smoking and psychopathology to their genetic relatedness (Plomin, 2008). In a review of 60 studies, Knopik (2009) concluded that the effect of prenatal tobacco exposure was heavily confounded by genetic and environmental factors, but after these factors were taken into account, prenatal smoking was still significantly associated with offspring externalizing/internalizing problems.

However, not all family-based studies agree with this conclusion. Silberg et al. (2003) fitted a series of structural equation models in a sample of twin boys and their mothers, and demonstrated that the association between maternal prenatal smoking and boys' conduct disturbance between age 12-17 years was explained by familial transmission of risk factors for conduct disorder and not by a causal effect of prenatal tobacco exposure. D'Onofrio et al. (2008) examined whether the transmission of externalizing problems occurred via genetic or environmental pathways in a sample of children of twins, siblings, and cousins. When offspring who had been exposed to prenatal maternal smoking were compared to their non-exposed siblings, no differences in externalizing problems were observed, indicating that the association was due to confounding by shared genetic or environmental factors. In a group of mothers who were related as twins, siblings, or cousins, the association between maternal smoking behavior and offspring externalizing problems was explained by shared environmental variables. That is, as yet unidentified environmental factors accounted for the association between maternal smoking and offspring externalizing problems, not teratogenic effects of maternal smoking.

In an elegant design, Thapar et al. (2009) examined ADHD in offspring of mothers who had become pregnant through assisted reproductive technologies. Mothers were either genetically related, or unrelated to their offspring (some mothers were surrogate mothers, and the remaining mothers had gotten pregnant through oocyte or embryo donations). If prenatal maternal smoking

has a teratogenic effect on offspring ADHD, the association would be observed both in genetically related and unrelated mother-child pairs. However, the association was only observed in related pairs, indicating the role of genetic factors. It should be noted that the prevalence of smoking was low (6%), and that the genetically related group, in which the association was found, was substantially larger (N=555) than the unrelated group, in which no association was detected (N=221).

In summary, the evidence favors a small direct effect of prenatal smoking on offspring externalizing/internalizing problems, additional to substantial effects of genetic and environmental factors, although not all family-based studies support this conclusion.

To add to this literature, we examine three different aspects of prenatal smoking and their associations with offspring externalizing/internalizing problems in a large population-based sample of children (N=9,982), enrolled in the Netherlands Twin Register (NTR). We expect that maternal prenatal smoking has a direct effect on offspring externalizing/internalizing, and examine this direct effect by first comparing the associations of prenatal smoking of fathers and mothers to offspring externalizing/internalizing problems. Mothers and fathers both share genetic and environmental factors with their offspring, but mothers also provide the prenatal tobacco exposure to the child (the putative causal effect). Paternal prenatal smoking may have a teratogenic effect on the fetus as well, but this effect is likely substantially smaller than the effect of maternal prenatal smoking. Salmasi et al. (2010) concluded from a meta-analysis that children of mothers who were exposed to secondhand smoke while they were pregnant and did not smoke themselves were on average 60 grams lighter at birth than children of non-exposed mothers. Children of mothers who actively smoking during pregnancy were on average 200 grams lighter than those of non-smoking, non-exposed mothers (Salmasi et al., 2010). If a stronger association is observed between maternal smoking and offspring externalizing/internalizing problems than between paternal smoking and offspring problems, it suggests a causal effect of prenatal tobacco exposure, although it cannot be ruled out that maternal factors, like diet or stress during the pregnancy, confound the effect (Smith, 2008; Brion et al., 2010; Huizink, 2009; D. Smith, 2008). If the associations are equally strong, this suggests that there are genetic and environmental factors that influence smoking behavior in parents as well as externalizing/internalizing problems in children.

Secondly, the offspring of mothers who continued to smoke during pregnancy are compared to offspring of mothers who gave up smoking before they became pregnant. By limiting the analyses to mothers who all had smoked

during their life, this design controls for differences between smoking and non-smoking mothers in genetic risk for smoking and comorbid externalizing problems, and thus provides additional support for, or against, a direct effect of prenatal tobacco exposure (Piper et al., 2012; Robinson et al., 2010). It should be noted that mothers who are unwilling or unable to quit smoking before pregnancy may be genetically different from mothers who quit smoking (Freathy et al., 2009).

Thirdly, we look at the group of mothers who continued smoking during pregnancy. In this group, effects of tobacco exposure in the first or last trimester of the pregnancy, versus exposure during the entire pregnancy are examined. Tobacco exposure in the first trimester, versus exposure during the entire pregnancy, is related to lower prevalence of preterm birth, easier infant temperament, fewer externalizing problems, and higher academic achievement (Baba et al., 2012; Pickett et al., 2008; Piper et al., 2012). Thus, under a causal model, we expect children who have been exposed only during the first trimester, to have fewer externalizing/internalizing problems at age three than children who were exposed during the entire pregnancy.

Methods

Sample

The Netherlands Twin Register was established in 1987 at the VU University in Amsterdam, the Netherlands (Boomsma et al., 2006). Young twins are recruited after birth and data are collected by longitudinal surveys starting with maternal reports on pregnancy, health, and temperament of the twins during their first two years of life. Parental reports on externalizing problems and internalizing psychopathology, health, school performance and socioeconomic status are collected starting at age three years. Data collection and participation rates have been described by Bartels et al. (2007b). For birth cohorts 1986-2003, the attrition rate between the survey collected before age one (survey 1) and at age three (survey 3) was 32.7%. A non-response analysis showed that in the families who dropped out, more mothers and fathers smoked during pregnancy (4.9% and 4.4% difference, respectively), more mothers and fathers were born in a country other than the Netherlands (about 4.0% difference), and the children were on average about 32 grams lighter at birth. It should be noted that 39% of this group again participated in longitudinal surveys when their children were 5, 7, 10, or 12 years old.

Maternal reports collected before age one and at age three were available for 19,843 children (9,861 complete and 121 incomplete twin pairs). From each

complete twin pair, one child was randomly selected, resulting in a sample of 9,982 children, 51.2% of whom were girls (no differences were observed between the selected twins and the excluded co-twins). About 95% of the parents were born in the Netherlands, 2.5% in another western country, and about 3% in a non-western country. A total of 99.4% of the children were born in the Netherlands.

Measures

Externalizing problems and internalizing psychopathology at age three were indexed with maternal reports on the Dutch version of the Child Behavior Checklist/2-3 (CBCL/2-3) (Achenbach & Rescorla, 2001). Externalizing problems were assessed with the oppositional, aggressive, and overactive subscales. The sum of all items that constitute these scales forms the broadband scale externalizing problems. Internalizing psychopathology was assessed with the withdrawn and anxious/depressed subscales. The sum of the items that make up these scales forms the broadband scale internalizing problems (Verhulst et al., 1997). Missing value percentages on the CBCL subscales ranged between .2% (overactive) and 1.1% (externalizing). Subjects who did not have a score on one of the CBCL subscales were excluded from the regression analyses for that particular scale.

Socioeconomic status (SES) at age three was scored according to the Standard Classification of Occupations (Statistics Netherlands, 2001). If this information was not available (3.7%), SES was scored according to the EPG occupational classes combined with parental level of education.(Erikson et al., 1979) SES consisted of three categories (low, middle, and high).

Maternal reports on parental smoking during the pregnancy were obtained shortly after the twins were born (on average 8.4 months). Mothers were asked whether they and the father had smoked during the pregnancy (no=0, yes=1). For the group of smoking mothers during pregnancy (N = 2,261), data were available on the trimester of the pregnancy in which the mother and father had smoked. In an early version of the survey, the answer categories on this question were ‘irregularly’, or ‘throughout the entire pregnancy’. In later versions of the survey, mothers were more specifically asked about smoking in the first and last trimester of the pregnancy. Data on whether the mother had ever smoked, maternal age at birth, offspring sex, and birthweight were obtained from the same survey.

Analyses

CBCL t-scores were positively skewed, therefore a transformation into normal

scores was done in Prelis (Jöreskog & Sörbom, 1993). All further data structuring and analyses were done in SPSS 18 (SPSS Inc, 2009). Analyses of variance (ANOVA) were carried out to examine if there were interaction effects (at α=.01) between maternal and paternal smoking and additional predictors (offspring sex, birthweight, SES, and maternal age at birth) on the CBCL scales. Since no significant interaction effects were observed, interaction terms were not included in further regression analyses. Whether offspring sex, birthweight, paternal smoking during pregnancy, SES, and maternal age at birth differed significantly between smoking and non-smoking mothers was examined with chi-squared tests (offspring sex, paternal smoking during pregnancy), a Mann-Whitney U test (SES), and ANOVA (birthweight, maternal age at birth).

The main analyses consisted of three sets of linear regression analyses (see Figure 1). The first set of analyses used the complete sample (N=9,982) and examined if maternal and paternal smoking during pregnancy predicted externalizing, oppositional, aggressive, overactive, internalizing, withdrawn, and anxious/depressed problems in offspring at age three. Conclusions regarding the significance of differences between maternal and paternal smoking behavior as predictors (included together in each analysis) were based on 95% confidence intervals of the regression coefficients.

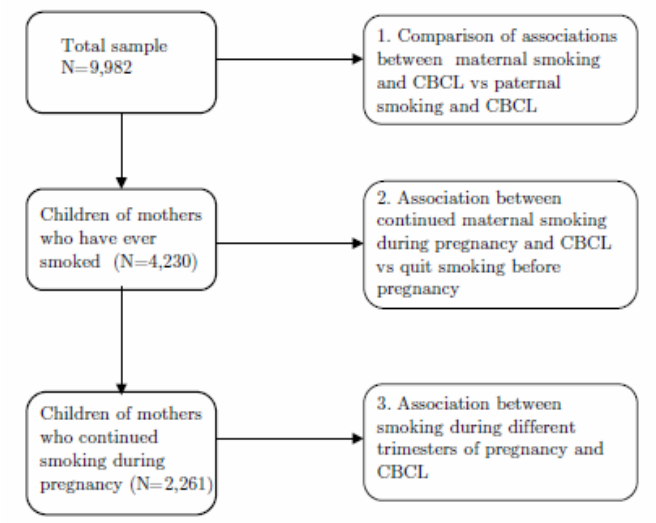

Figure 1 Sample and subsamples in which associations between different aspects of prenatal smoking and offspring externalizing and internalizing problems at age three were examined.

The second set was performed in the subsample of mothers who had ever smoked during their lifetime and excluded non-smoking mothers (N=4,230). Externalizing and internalizing problems of children of mothers who continued to smoke during pregnancy were compared to those of mothers who quit smoking before they became pregnant. These analyses included paternal smoking during pregnancy as an additional predictor, since continued maternal smoking was related to paternal smoking during pregnancy.

The third set was carried out in the subsample of mothers who smoked during pregnancy (N=2,261). The effect of smoking during the first or last trimester of the pregnancy versus smoking during the entire pregnancy was investigated. Mothers were classified into 3 groups: only smoking in the first trimester of the pregnancy, only in the last trimester, or throughout the entire pregnancy. Mothers who had stated they had smoked irregularly were excluded from these analyses. The categories were summarized by two dummy variables. The first dummy variable (D_1) contrasted maternal smoking in the first trimester of the pregnancy to maternal smoking during the entire pregnancy. The second dummy variable (D_2) contrasted maternal smoking in the last trimester of the pregnancy to smoking during the entire pregnancy. These analyses also included paternal smoking during pregnancy as a predictor.

All regression analyses included offspring sex, offspring birthweight, SES, and maternal age at birth as additional predictors.

Results

Sample Characteristics

The prevalence of maternal smoking during pregnancy was 16.0%, in line with the prevalence reported in the general Dutch population (Lanting et al., 2007). Offspring sex did not differ between non-smoking and smoking mothers (Table 1). Paternal smoking during pregnancy was more prevalent in families with a smoking mother than with a non-smoking mother (Table 1). Overall, paternal prenatal smoking was somewhat more prevalent in the sample (34.8%) than in the general population (28%) (Lanting et al., 2007). Parental concordance for prenatal smoking was 70.7%.

Low socioeconomic status was more prevalent in families in which the mother smoked during pregnancy (38.0%) than in families with a mother who did not smoke while pregnant (20.8%). The prevalence of middle SES was similar for smoking and non-smoking mothers. The distribution of SES across smoking and non-smoking mothers was consistent with that in the general population, although in the sample somewhat less families were classified in the low SES category, and more in the middle category

Table 1 Characteristics of families of mothers who smoked and who did not smoke during pregnancy

	Non-smoking mothers	*Smoking mothers*	*Test of differences*
Number of girls	4,277 (51.0%)	829 (51.9%)	$\chi^2(1)^a = .475$; p=.495
Paternal prenatal smoking	2,403 (28.7%)	1,071 (67.1%)	$\chi^2(1)^a = 873.633$; p<.001
SES			
Low	1,744 (20.8%)	606 (38.0%)	$U^b = -16.545$; p<.001
Medium	3,836 (45.7%)	714 (44.7%)	
High	2,806 (33.5%)	276 (17.3%)	
Mean birthweight (SD)	2,534.3 (546.8)	2,379.5 (523.0)	$F(1, 9980)^c = 108.861$; p<.001
Mean maternal age at birth (SD)	30.8 (3.8)	30.5 (3.9)	$F(1, 9980)^c = 9.816$; p=.002

Note. [a]χ^2: chi-squared test statistic; [b]standardized Mann-Whitney U test statistic, [c]ANOVA F test statistic

(Statistics Netherlands, 2012b). Smoking mothers had offspring with significantly lower birthweight than non-smoking mothers (2,534 grams versus 2,379 grams). Mothers who did not smoke during pregnancy were significantly older than mothers who did (Table 1), although the difference was small: mean age was 30.8 versus 30.5 years. Mean maternal age at birth across both groups was consistent with the average age at which Dutch women have children (Statistics Netherlands, 2012c).

Associations between maternal and paternal smoking during pregnancy with externalizing problems and internalizing psychopathology

Figure 2 shows average CBCL scale scores, stratified by maternal prenatal smoking. Children who had been exposed to prenatal smoking scored higher on all dimensions of with externalizing problems and internalizing psychopathology, and had slightly larger standard deviations than those who had not been exposed. The associations (regression coefficients) for maternal and paternal smoking during pregnancy with each of the outcome variables are presented in Table 2. Externalizing problems and its subscales oppositional, overactive, and aggressive problems, were significantly predicted by both maternal and paternal smoking, without and with covariates offspring gender, birthweight, SES, and

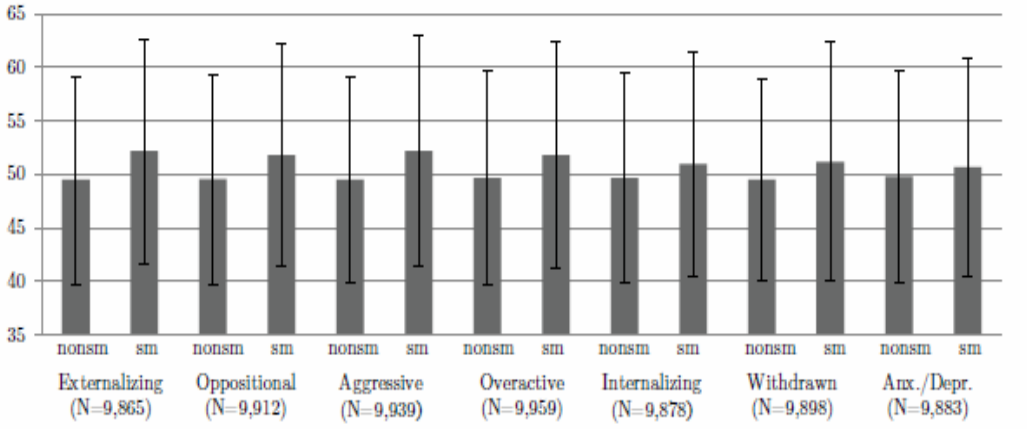

FIGURE 2 Mean CBCL t-scores (error bars show standard deviations), for non-smoking mothers (ns) and mothers who smoked during pregnancy (s)

maternal age at birth included. Including the covariates caused small decreases in all regression coefficients. The associations between maternal smoking and externalizing and aggressive behavior were stronger than the associations between paternal smoking and these behaviors and the confidence intervals of the regression coefficients did not overlap. This is consistent with causal effects of prenatal maternal smoking onoffspring externalizing problems and its subscale aggressive behavior, additional to genetic and environmental influences. The regression models explained between 2.8% and 8.5% of variance of the externalizing subscales. Maternal prenatal smoking independently explained between .1% and .5% of variance of these subscales and paternal prenatal smoking between .2% and .3% of variance. Maternal smoking was significantly related to internalizing psychopathology and its subscale withdrawn behavior, without and with covariates included. In contrast, paternal smoking was not significantly associated with internalizing psychopathology, except with withdrawn behavior, but only when no covariates were included. This is consistent with a weak influence of genetic and environmental factors, and additional effects of prenatal maternal smoking on internalizing psychopathology. The proportion of variance explained by the regression models ranged between .9% and 2.1% of variance of the internalizing subscales and maternal smoking explained between 0-.1% of variance of these subscales.

In the group of mothers who had ever smoked (N=4,230), 53.5% continued to smoke during pregnancy. Figure 3 shows mean CBCL scale scores for their offspring, stratified by maternal prenatal smoking. Offspring who had been

Table 2 Regression (β) of externalizing/internalizing problems on maternal and paternal smoking, without covariates (M1) and with covariates included (M2)

		Maternal prenatal smoking		*Paternal prenatal smoking*		
	Model	β_m [se][a]	B_m [95% CI][b]	β_f [se][c]	B_f [95% CI][d]	R^{2e}
Externalizing (N=9,865)	M1	.083** [.011]	2.24 [1.68, 2.79]	.056** [.010]	1.18 [.75, 1.60]	.013
	M2	.065** [.010]	1.75 [1.19, 2.30]	.037** [.011]	.76 [.34, 1.19]	.050
Oppositional (N=9,912)	M1	.069** [.010]	1.88 [1.32, 2.43]	.047** [.011]	.97 [.54, 1.40]	.009
	M2	.055** [.011]	1.48 [.93, 2.04]	.031** [.010]	.65 [.22, 1.08]	.028
Aggressive (N=9,939)	M1	.085** [.010]	2.28 [1.73, 2.83]	.054** [.011]	1.11 [.69, 1.53]	.013
	M2	.071** [.010]	1.92 [1.39, 2.46]	.035* * [.010]	.73 [.32, 1.14]	.085
Overactive (N=9,959)	M1	.059** [.010]	1.62 [1.06, 2.18]	.055** [.010]	1.15 [.72, 1.58]	.008
	M2	.039** [.010]	1.06 [.50, 1.61]	.033** [.010]	.69 [.26, 1.12]	.053
		Maternal prenatal smoking		*Paternal prenatal smoking*		
	Model	β_m [se][a]	B_m [95% CI][b]	β_f [se][c]	B_f [95% CI][d]	R^{2e}
Internalizing (N=9,878)	M1	.045** [.010]	1.23 [.67, 1.78]	.018 [.011]	.36 [-.07, .79]	.003
	M2	.032** [.011]	.87 [.31, 1.43]	.002 [.010]	.05 [-.39, .48]	.016
Withdrawn (N=9,898)	M1	.053** [.011]	1.42 [.86, 1.97]	.027** [.011]	.56 [.13, .98]	.004
	M2	.036** [.010]	.98 [.43, 1.53]	.010 [.010]	.21 [-.22, .64]	.021
Anxious/Depressed (N=9,883)	M1	.033** [.011]	.89 [.33, 1.45]	.009 [.010]	.19 [-.24, .62]	.001
	M2	.023 [.010]	.64 [.07, 1.20]	-.002 [.010]	-.05 [-.48, .39]	.009

Note. [a]beta coefficient of maternal smoking during pregnancy [standard error]; ** significant at α=.01,[b]B_m unstandardized regression coefficient of maternal smoking during pregnancy [95% CI]; [c]β_f beta coefficient of paternal smoking during pregnancy [standard error]; [d]B_f unstandardized regression coefficient of paternal smoking during pregnancy; [e]R^2 proportion of variance explained by model. Covariates: sex, SES, maternal age at birth, and birthweight

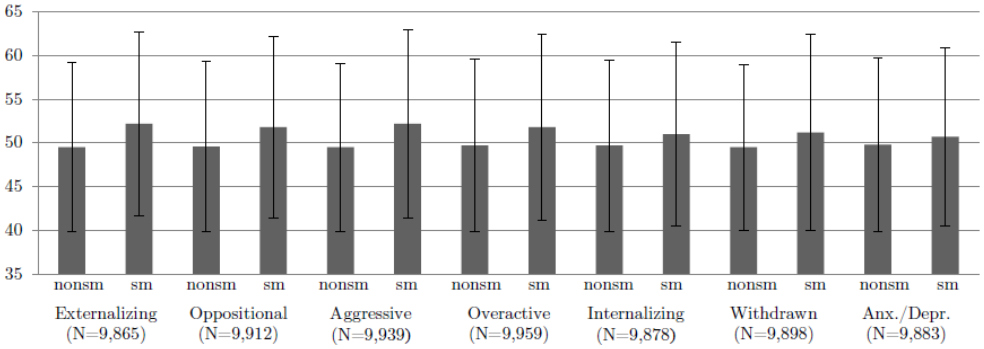

FIGURE 2 Mean CBCL t-scores (error bars show standard deviations), for non-smoking mothers (ns) and mothers who smoked during pregnancy (s)

exposed to prenatal smoking scored higher on all dimensions of externalizing/internalizing problems and had slightly larger standard deviations than those not exposed.

Table 3 shows that without including the covariates, continued smoking during pregnancy was positively associated with all dimensions of externalizing/internalizing problems, except for internalizing and anxious/depressed psychopathology. Including the covariates reduced all regression coefficients of continued maternal smoking to nonsignificant, except for aggressive behavior, again consistent with a causal effect of prenatal maternal smoking on this dimension of externalizing problems. The model predicting aggressive behavior explained the largest proportion of variance (8.2%), of which .3% was explained by maternal prenatal smoking. In the third subsample of mothers who smoked during pregnancy (N=2,261), 70.6% reported having smoked throughout the entire pregnancy. Another 11.1% had only smoked during the first trimester, 6.9% had only smoked in the last trimester, and 11.4% had smoked irregularly during the pregnancy (excluded from analysis). No effects of smoking in the first trimester versus smoking during the entire pregnancy, were observed.

We also examined whether tobacco exposure in the last trimester was related to fewer externalizing/internalizing problems than exposure throughout the entire pregnancy, but did not observe significant effects.

Table 3 Regression (β) of externalizing/internalizing problems on maternal prenatal smoking, without covariates (M1) and with covariates included (M2)

	Model	β_{sm} [se][a]	R^2
Externalizing (N=4,192)	M1	.103** [.015]	.011
	M2	.040 [.016]	.056
Oppositional (N=4,211)	M1	.081** [.015]	.007
	M2	.026 [.017]	.036
Aggressive (N=4,214)	M1	.102** [.015]	.010
	M2	.053** [.016]	.082
Overactive (N=4,222)	M1	.095** [.015]	.009
	M2	.030 [.016]	.054
Internalizing (N=4,196)	M1	.048 [.015]	.002
	M2	.011 [.017]	.019
Withdrawn (N=4,203)	M1	.054** [.015]	.003
	M2	.012 [.017]	.023
Anxious/Depressed (N=4,198)	M1	.035 [.015]	.001
	M2	.008 [.016]	.011

Note. [a]beta coefficient of maternal smoking during pregnancy [standard error]; ** significant at α=.01; Covariates: sex, SES, maternal age at birth, paternal prenatal smoking, and birthweight

Discussion

In this large, representative, sample of Dutch children, we found evidence consistent with direct effects of prenatal tobacco exposure on offspring externalizing problems at age three, by comparing effects of maternal smoking to paternal smoking. The support for direct effects was strengthened by comparing offspring of mothers who quit smoking before pregnancy to mothers who continued smoking while pregnant. Effects of prenatal smoking were additional to the influence of genetic and environmental factors. Associations of prenatal tobacco exposure with offspring internalizing psychopathology at age three were less strong, and no direct effects were indicated. Tobacco exposure in the first or last trimester, compared to exposure during the entire pregnancy, was not related to lower levels of offspring externalizing/internalizing problems.

The observed effects were weak and explained a small proportion of individual differences in externalizing problems, but nevertheless add evidence for causal, teratogenic effects of prenatal tobacco exposure that lead to increased externalizing problems in early childhood (Agrawal et al., 2010b; D'Onofrio et al., 2008; Ekblad et al., 2010; Knopik, 2009). Teratogenic effects of prenatal

tobacco exposure have often been demonstrated, but as noted by Knopik (2009), little is known about the magnitude of those effects on different aspects of offspring externalizing problems (e.g. ADHD, conduct problems) at different stages in childhood. We established that when offspring sex, birthweight, maternal age, paternal prenatal smoking, and socioeconomic status were accounted for, maternal prenatal smoking was significantly related to offspring aggressive and externalizing problems at age three (standardized regression coefficients were around .07), and that maternal prenatal smoking explained about .5% of individual differences in offspring aggressive and externalizing problems.

Another challenge lies in distinguishing whether the confounding factors that account for part of the association between prenatal smoking and offspring externalizing and internalizing problems, are due to shared genes or environment. Family-based designs indicate that both are important, but distinguishing between these influences is complex, because shared genetic and environmental factors transmitted by parents that influence offspring externalizing/internalizing problems, may not be independent (gene-environment correlation) (D'Onofrio et al., 2008; Thapar et al., 2009). Furthermore, prenatal tobacco exposure may interact with specific genotypes, leading to increased externalizing/internalizing problems (Knopik, 2009). Identifying the genetic and environmental pathways and how they interact with each other and with prenatal smoking, and to what extent this affects offspring externalizing and internalizing problems, remains a challenge.

Weaker associations between prenatal smoking and offspring internalizing psychopathology than with externalizing problems have previously been observed, but why these associations are less strong, is unclear (Lavigne et al., 2011; Monshouwer et al., 2011; Orlebeke et al., 1999). Underlying genetic factors may explain this difference. There is stronger evidence for underlying genes linking substance use to externalizing problems than there is for substance use and internalizing psychopathology (Edwards et al., 2011; Hicks et al., 2011; Kendler et al., 2003; Stephens et al., 2012). The difference between associations of prenatal smoking with offspring externalizing problems compared to internalizing psychopathology may be further amplified by interactions between offspring genes and prenatal tobacco exposure. Prenatal tobacco exposure interacts with fetal MAOA genotype and with several dopaminergic genes, leading to increased offspring externalizing problems in children who were already genetically susceptible (Brennan et al., 2011; Kahn et al., 2003; Langley et al., 2008; Neuman et al., 2007; Wakschlag et al., 2009). There is less evidence that genotype x prenatal tobacco exposure effects lead to more offspring

internalizing psychopathology, although Hsieh et al. (2010) observed an interaction between maternal prenatal passive smoking and a fetal metabolic gene (CYP1A1), which resulted in more offspring internalizing psychopathology at age two (Hsieh et al., 2010). Cents et al. (2012) examined effects of 5-HTTLPR genotype and prenatal tobacco exposure on offspring internalizing psychopathology at age three, and demonstrated that each separate predictor was not significantly associated, but that having the short allele of the 5-HTTLPR polymorphism in combination with prenatal tobacco exposure, predicted increased internalizing psychopathology at age three. Psychological mechanisms may also account for the stronger link between prenatal smoking and externalizing problems. Maternal smoking during pregnancy is related to maternal depression, which in turn predicts offspring aggressive behavior (Brook et al., 2006; Lancaster et al., 2010). Another possible mechanism is that young children suffering from depression may express this partly through indirect, 'masked' symptoms, like aggressive behavior and somatic complaints (Luby et al., 2003).

Children who were exposed to tobacco in the first trimester, did not have lower levels of externalizing/internalizing problems than children who were exposed during the entire pregnancy. The number of observations might have been too small to detect a difference (N=251 mothers smoked in the first trimester, N=1,596 mothers smoked during the entire pregnancy). The number of cigarettes smoked was not taken into account and may be significant, since dose-response effects of tobacco exposure on offspring externalizing problems have been observed (Ekblad et al., 2010; Liu et al., 2011; Pickett et al., 2008). However, we additionally examined dose-response effects, looking at number of cigarettes a day (0-5 cigarettes, 5-10 cigarettes, more than 10 cigarettes) in a subgroup of mothers (N=1,648), and did not detect significant effects on offspring externalizing/internalizing problems at age three (results available on request). The number of observations for smoking in the last trimester versus during the entire pregnancy was even smaller (N=156 versus N=1,596), and no differences in offspring externalizing/internalizing problems at age three were observed.

Some limitations of this study should be noted. No information on maternal psychopathology was included, and this may be an important confounder of effects of prenatal maternal smoking. In several studies, the association between prenatal maternal smoking disappeared after maternal psychopathology was included (Lavigne et al., 2011; Monshouwer et al., 2011; Roza et al., 2008). In others, the association remained significant, but was

attenuated (Boutwell et al., 2011; Cornelius et al., 2011; Ekblad et al., 2010; Nomura et al., 2011; Paradis et al., 2011). It cannot be ruled out that including maternal psychopathology would have rendered the association between maternal prenatal smoking and externalizing/internalizing problems non-significant. However, our conclusion rests on the comparison between maternal and paternal smoking, which showed that maternal smoking was more strongly associated with offspring externalizing problems. This conclusion probably is robust against effects of parental psychopathology, since maternal smoking often co-occurs with paternal smoking during pregnancy, and both are related to adverse circumstances (Everett et al., 2007; Rogers, 2009; Roza et al., 2008; Tong, 2009).

No information on postnatal parental smoking was included. Children whose mother smoked during pregnancy are more likely to also be exposed to second-hand smoke in childhood (Knopik, 2009). Environmental tobacco exposure has been linked to increased risk of hyperactive/inattention and externalizing problems in large samples of American and German children (Kabir et al., 2011; Tiesler et al., 2011). Including this information enables separating effects of prenatal tobacco exposure from passive smoking during childhood (Schlotz & Phillips, 2009; Thapar & Rutter, 2009).

Furthermore, using maternal reports on maternal and paternal smoking, as well as on offspring externalizing and internalizing problems, could introduce rater bias (e.g. projection bias) (Bartels et al., 2007a). Additional analyses of paternal ratings of offspring behavior (available for a subsample of 6,598-6,631 children) yielded the same pattern of results (data available on request). Retrospective self-reports on smoking during pregnancy may underestimate prenatal tobacco exposure, but a study comparing retrospective self-reports on prenatal smoking to prospective measurements and cotinine assessments, showed that generally, all types of measurements performed equally well (Pickett et al., 2009). In addition, reports of smoking among relatives are very highly correlated with those relatives' self-reports (Kendler et al., 2002). Moreover, information on parental smoking during pregnancy was gathered shortly after birth (on average 8.4 months), minimizing recall bias effects.

In summary, the associations between maternal smoking during pregnancy and offspring aggressive and externalizing problems at age three are consistent with there being shared genetic or environmental influences and a small additional effect of prenatal tobacco exposure. Associations between maternal prenatal smoking and offspring internalizing psychopathology are weaker and entirely explained by shared genetic or environmental factors.

7

Maternal prenatal smoking and offspring emotional problems: No moderating effect of maternal or child 5-HTTLPR genotype

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Introduction

In a recent paper, Cents and others (2012) demonstrated a novel interaction effect of a polymorphism in the serotonin transporter gene (5-HTTLPR) and maternal prenatal smoking on offspring emotional (internalizing) problems at age 3 in a sample of 1,529 Dutch mother-child dyads. The 5-HTTLPR polymorphism has previously been shown to moderate effects of stressful life-events and childhood maltreatment on depression (Karg and others, 2011). Cents and others (2012) extended those findings to the period of fetal life using maternal prenatal smoking as the environmental risk factor, and offspring emotional problems at age 3 as the outcome measure. Cents and others (2012) did not find a significant main effect of the 5-HTTLPR genotype or maternal prenatal smoking on offspring emotional problems, but detected an interaction effect. Having the short allele of the 5-HTTLPR polymorphism in combination with maternal smoking during pregnancy was associated with increased emotional problems at age 3 as rated by the mother. A similar interaction was observed for maternal 5-HTTLPR genotype and this effect was independent of the child's genotype, suggesting that maternal serotonin levels influence fetal development. The interactions remained significant after correction for maternal educational level, maternal psychopathology, and age and sex of the child. Maternal rater bias was controlled for by alternatively using paternal ratings of offspring emotional problems which showed comparable results. Moreover, when Cents and others (2012) repeated the analyses using paternal prenatal smoking as a predictor instead of maternal prenatal smoking, no interaction with 5-HTTLPR was observed, providing additional support for a direct effect of prenatal tobacco exposure as opposed to confounding effects.

Replication of this novel genotype x environment interaction effect is important, since the 5-HTTLPR x stress interaction effect on depression has been observed in some, but not all studies (Karg et al., 2011; Risch et al., 2009). Therefore, we repeat the analyses of Cents and others (2012) in an effort to replicate the interaction of 5-HTTLPR genotype and maternal prenatal smoking on emotional problems in another population-based sample of Dutch children (n=1,865).

The Netherlands Twin Register (NTR) was established in 1987 at the VU University in Amsterdam (D. I. Boomsma et al., 2006). Data on young twins are collected by longitudinal surveys, starting with maternal reports on the pregnancy shortly after the twins are born. Parental reports on emotional problems are collected starting at age 3 years. Data collection and participation rates have been described in detail by Bartels and others (2007b). Child 5-HTTLPR genotype and maternal reports on prenatal smoking and emotional

problems at age 3 were available for 1,988 children born between 1986-2004. Ethnic outliers were identified based on genome-wide genotype data and excluded from the analyses (n=72). From the 17.1% of the children without genome-wide genotype data, 11 children were additionally excluded since one or both of their parents were born in a non-western country. This resulted in a sample of 1,905 children (53.0% girls). Data on prenatal maternal smoking were available for 1,865 of these children and maternal 5-HTTLPR genotype data for 612 children.

Maternal and child DNA were derived from blood and buccal samples. In line with Cents and others (2012), maternal/child 5-HTTLPR was included in the analysis as an additive effect (ll=0, ls=1, ss=2). Hardy-Weinberg equilibrium (HWE) was tested using Sib-pair, as this program allows for a test of HWE in related individuals (Duffy, 2012).

Maternal reports on prenatal smoking were obtained on average 8.4 months after the children were born. Maternal prenatal smoking was coded as 'non-smoking' and 'smoking during pregnancy'. Following Cents and others (2012), mothers who reported having only smoked in the first trimester were excluded (n=33). Offspring emotional problems at age 3 were assessed with maternal reports on the internalizing subscale of the Child Behavior Checklist (CBCL/2-3; Achenbach & Rescorla, 2000), which were corrected for positive skewness with a square root transformation, in line with the original study. Cents and others (2012) included maternal educational attainment, maternal psychopathology, child age and child sex as covariates. In the NTR sample, maternal educational attainment was assessed simultaneously with offspring emotional problems, in 5 categories (ranging from 'primary school' to 'university'), which were dichotomized into the same categories as Cents and others (2012) used ('primary/secondary school' and 'higher education'). No data on maternal prenatal psychopathology were available. Child age was reported when emotional problems were measured and child sex was assessed at enrollment in the NTR.

Effects of prenatal maternal smoking and 5-HTTLPR genotype on offspring emotional problems were examined with linear mixed models in SPSS 20 (IBM, 2011). To account for familial clustering in the data, a random intercept was modeled over families, which was estimated separately for monozygotic and dizygotic twins. Main and interaction effects of prenatal maternal smoking and 5-HTTLPR genotype were tested without covariates first. Next, the covariates included by Cents and others (2012) were added (maternal educational attainment, child sex, child age). All tests were evaluated at α=.05.

The distribution of 5-HTTLPR genotype was in Hardy-Weinberg

Table 1 Main and interaction effects of maternal prenatal smoking and 5-HTTLPR genotype on offspring emotional problems (maternal reports) at age 3

	Without covariates (n=1,865)		*With covariates (n=1,794)*	
	B (95% CI)	p-value	B (95% CI)	p-value
Main effects				
Smoking during pregnancy	.24 (.02 – .47)	.033	.17 (-.07 – .40)	.175
Child 5-HTTLPR	.03 (-.05 – .12)	.439	.04 (-.05 – .13)	.389
Maternal 5-HTTLPR	.07 (-.10 – .24)	.408	.08 (-.09 – .25)	.359
Interaction effects				
Child 5-HTTLPR x Smoking during pregnancy	-.06 (-.26 – .13)	.524	-.05 (-.26 – .16)	.635
Maternal 5-HTTLPR x Smoking during pregnancy	-.17 (-.52 – .18)	.346	-.20 (-.56 – .17)	.288

Note. Covariates were offspring sex and age and maternal educational attainment. Main and interaction effects of maternal 5-HTTLPR genotype were assessed in separate analyses (n=608 without covariates; n=585 with covariates).

equilibrium (empirical p-value .32). The prevalence of prenatal maternal smoking was 18.9%. Mothers were on average 30.7 years old when they gave birth to the twins (sd 3.7) and the majority had completed higher education (68.9%). Children were on average 39.6 months old when emotional problems were assessed (sd 3.1). The average internalizing score was 1.9 (sd 1.0) and 8.6% of the children scored in the borderline/clinical range (defined as t-score ≥ 65).
The effects of prenatal maternal smoking and maternal/child 5-HTTLPR genotype are shown in Table 1. Child 5-HTTLPR genotype was not significantly associated with emotional problems at age 3, nor was maternal 5-HTTLPR genotype (p-values between .359 and .439). Prenatal maternal smoking was only significantly associated with offspring emotional problems before covariates were included in the model (p=.033 without covariates versus p=.175 with covariates). This attenuation was mainly due to the effect of maternal education. The interaction effect between child 5-HTTLPR genotype and maternal prenatal smoking is illustrated in Figure 1.

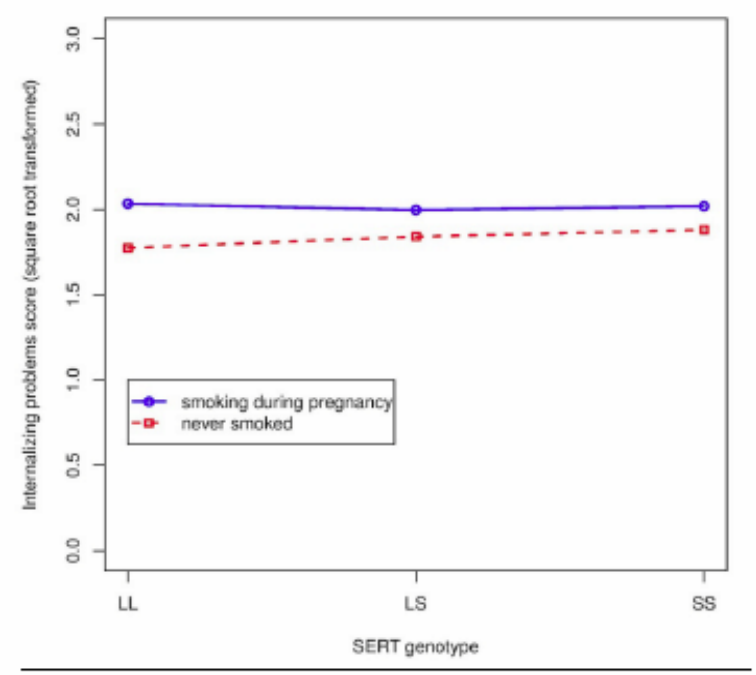


FIGURE 1 Mean internalizing scores by child 5-HTTLPR genotype and maternal prenatal smoking

The interaction effects between prenatal smoking and maternal/child 5-HTTLPR genotype were not significant, with or without covariates included (p-values between .288 and .635).

To summarize, the significant interaction between maternal/child 5-HTTLPR genotype and maternal smoking during pregnancy on emotional problems at age 3 was not replicated in this large, population-based sample of Dutch children. As previously noted, the study by Cents and others (2012) adds to a body of literature on gene x environment interactions at the serotonin transporter locus. Three meta-analyses have been performed on this topic, with inconsistent conclusions (Karg et al., 2011; Munafò et al., 2009; Risch et al., 2009). It has been argued that positive results may be spurious due to the large number of possible interaction models that can be tested and the generally low statistical power to detect interactions (Duncan & Keller, 2011; Hunter, 2005; Munafò et al., 2009). Munafò and others (2009) demonstrated that without a main effect of the genotype, an interaction effect is highly unlikely. As two meta-analyses have not confirmed a main effect of 5-HTTLPR on depression, one may question the existence of interaction effects at this locus (Munafò et al., 2009; Risch et al., 2009). However, others have argued that the contradictory findings are due to the varying quality of measurement instruments and differences in definitions of stressful life events (Caspi et al., 2010; Karg et al., 2011). To ensure that the current replication effort was not qualitatively different from the original study, we used the same instrument to measure emotional problems and similarly assessed and coded maternal prenatal smoking. The scoring method for the CBCL internalizing scale used in the NTR sample was slightly different from the original study (CBCL/2-3 versus CBCL/1,5-5), since more observations were available for the CBCL/2-3 scoring. However, the internalizing scores from both methods were highly correlated (r=.814) and when the analyses were repeated with the CBCL/1,5-5 scoring method, similar results were obtained. The NTR sample was quite similar to the original sample with respect to maternal prenatal smoking and educational attainment. Moreover, as both samples originate from the Netherlands and ethnic outliers were excluded, it is improbable that the lack of replication is due to genetic heterogeneity. Finally, statistical interactions can arise from anomalies in the data such as violations of distributional assumptions and heteroscedasticity. This problem, however, is not necessarily solved by replication as the replication dataset might suffer from the same distributional problems as the original report (Eaves, 2006; Kendler & Gardner, 2010).

In conclusion, a replication effort in a sample of Dutch children does not support a moderating effect of 5-HTTLPR genotype on the association between

prenatal maternal smoking and offspring emotional problems at age 3. Considering the ferocious debates surrounding 5-HTTLPR genotype by environment interactions, it is important that after the original first study, both replications and non-replications find their way into the literature.

8

Summary and Discussion

The aim of this thesis was to gain insight in the genetic and environmental determinants of, and their interactions on variation in alcohol use and comorbid traits throughout the life span, by analyzing longitudinal data from participants of the Netherlands Twin Register (NTR). In particular, alcohol initiation and drinking patterns in adolescence, multiple indicators of adult alcohol consumption and dependence, and effects of prenatal smoking on offspring externalizing and internalizing problems were examined. The causes of variation in adolescent alcohol use were inferred from patterns of resemblance between mono- and dizygotic twin pairs. I evaluated specific developmental predictors for early alcohol initiation (i.e. before age 16 years) and investigated causal effects of early alcohol initiation on adult alcohol consumption. The effects of prenatal tobacco exposure on offspring externalizing and internalizing problems at age three were evaluated, both as main effect and in interaction with serotonin transporter (5-HTTLPR) genotype.

Summary

In chapter 2, associations of age, sex, and birth cohort with adolescent alcohol use were investigated. Two cohorts of twins between ages 13-21 years, assessed in 1993 and in 2005-8, were compared on initiation and frequency of alcohol use and quantity of alcohol consumed. The prevalence of alcohol initiation was higher in the 2005-8 cohort than in the 1993 cohort and adolescents in the 2005-8 cohort also drank larger quantities of alcohol. In both cohorts, alcohol use increased with increasing age and from age 16 years onwards, boys drank more frequently and larger quantities than girls. Secondly, the data from these cohorts were analyzed from a gene x environment interaction perspective: the relative contributions of genetic and environmental factors on adolescent alcohol use were estimated and I examined whether these influences differed as a function of age, sex, or cohort. At age 13-15 years, individual differences in alcohol initiation and frequency were mainly explained by shared environmental factors (55% and 64%, respectively), while a minor proportion was explained by genetic factors (31% for initiation; 21% for frequency). As age increased, so did the importance of genetic factors, while the magnitude of shared environmental influences declined in parallel. No cohort differences were detected.

The specific factors that may constitute these genetic and environmental influences on adolescent alcohol use were examined in **chapter 3**. A prediction model was created for alcohol initiation at ages 13-15 years, in which 22 developmental predictors were evaluated. Predictors were identified based on the literature and included genetic risk for alcohol initiation and externalizing/internalizing problems (based on data of the co-twin on those

traits), prenatal substance exposure and childhood risk factors, e.g. childhood externalizing/internalizing problems and parental divorce, and adolescent risk factors, including externalizing/internalizing problems, lifestyle, and peer-related factors. Subjects at higher genetic risk for alcohol initiation, who had friends who drank alcohol, and who had started smoking at an early age, were at increased risk of initiating alcohol use before age 16 years. Externalizing problems were only moderately and indirectly associated with early alcohol initiation, and internalizing problems were marginally and indirectly associated.

Early alcohol initiation is consistently related to increased adult alcohol consumption and alcohol abuse. In **chapter 4**, a co-twin control design was applied to examine whether these associations are due to a general underlying vulnerability to alcohol consumption or to causal effects of early alcohol initiation. Within monozygotic twin pairs, twins who had started drinking early were compared to their co-twin, who had started later, on normative and problematic forms of alcohol use in adulthood. Early alcohol initiation was associated with adult alcohol consumption at the population level, but within MZ twin pairs, early drinkers did not differ significantly from their brother or sister, suggesting that early alcohol initiation does not lead to significant increases in adult alcohol consumption.

An epidemiological analysis of adult alcohol consumption in the adult Dutch population was described in **chapter 5.** Alcohol consumption and demographic/lifestyle traits were described by age and sex. Associations between alcohol consumption indicators and demographic/lifestyle traits were examined by regressing aspects of alcohol use on age, sex, their interaction, and demographic/lifestyle variables. The most striking age patterns were observed for frequency of alcohol use, which was lowest between 18-25 years and highest above age 65 years. Moreover, women consumed the lowest quantities of alcohol between 25-45 years and the largest quantities between 55-65 years. Participants in the younger age groups reported lower age at alcohol initiation, at onset of regular drinking, and at first alcohol intoxication than the older participants. Among older participants, men initiated alcohol use and regular drinking earlier, and had lower age at first intoxication than women, but among young adults, no sex differences were observed. Heavy alcohol use was most strongly predicted by older age, sex (male), and initiation of smoking and cannabis use, and to a lesser extent by high educational attainment, student status, and financial stress.

In **chapter 6**, I examined transmission of risk for externalizing and internalizing problems from parents to offspring. Causal effects of prenatal tobacco exposure and effects of shared genes and environment on offspring

externalizing and internalizing at age 3 were disentangled by comparing the associations of maternal and paternal smoking. Effects of prenatal tobacco exposure were further examined by selecting offspring of mothers who had ever smoked and comparing offspring of mothers who quit before pregnancy to mothers who continued smoking during pregnancy. Finally, effects of tobacco exposure in different pregnancy trimesters were investigated. Maternal prenatal smoking was more strongly related to offspring outcomes than paternal smoking, consitent with direct effects of prenatal tobacco exposure on offspring externalizing problems. Moreover, offspring of mothers who continued to smoke during pregnancy had more externalizing problems than offspring whose mothers quit before pregnancy, adding support for direct effects of prenatal tobacco exposure. Associations between prenatal smoking and internalizing problems were weaker and not consistent with causal effects. Tobacco exposure in the first or last trimester, compared to exposure during the entire pregnancy, was not related to lower levels of offspring externalizing/internalizing problems.

Chapter 7 elaborated on effects of prenatal maternal smoking by reporting a replication effort of an interaction between serotonin transporter genotype (5-HTTLPR) and prenatal maternal smoking on offspring internalizing problems, which was recently described in a Dutch population-based sample of children by Cents et al. (2012). In the original study, no main effects of serotonin transporter genotype or prenatal maternal smoking were observed, but children who carried the risk (s) allele on 5-HTTLPR and who were prenatally exposed to tobacco showed increased internalizing problems at age 3. The replication study revealed no significant main effects of maternal/child 5-HTTLPR genotype and prenatal maternal smoking on offspring internalizing problems, nor an interaction between these predictors.

General Discussion

Early alcohol use and comorbid traits

Timing of alcohol initiation is associated with multiple factors occurring throughout development that either increase risk of early initiation or protect against it (Kendler et al., 2011b; Zucker et al., 2008). I demonstrated that in Dutch adolescents, alcohol-specific genetic risk, smoking initiation, and peer alcohol use were more strongly related to early alcohol initiation than externalizing and internalizing problems, which were only indirectly associated (chapter 3). This is surprising, especially for externalizing problems, since a large body of literature supports strong associations between these problems and

alcohol initiation (e.g. Donovan, 2004; Hussong et al., 2011; Iacono et al., 2008). As discussed in chapter 3, possible explanations for these different findings involve age or severity of alcohol use indicator. Effects of age may be clarified by applying this model fitting approach to alcohol use in older adolescents. Whether severity of alcohol use indicator explained the different findings was addressed in an additional analysis. The same set of predictors and modeling procedure were applied to the same sample (N=1,563), but with weekly alcohol use (a dichotomous variable; drinking at least once a week/less often than once a week) as the outcome variable. In the 13-15 age group, this is a substantially more severe indicator of alcohol use than alcohol initiation. Standardized regression coefficients estimated under the best model are shown in Figure 1. Weekly alcohol use was directly predicted by genetic risk for weekly alcohol use and by peer alcohol use, and as with alcohol initiation, externalizing and internalizing problems were indirectly associated. These findings imply that in this young age group, severity of alcohol use indicator is not what determined the weak, indirect associations between alcohol initiation and externalizing/internalizing problems. Instead, age may be a more important factor in explaining this inconsistency with previous findings.

Correlated and intersecting pathways to adolescent alcohol use

Categorizing the predictors identified in chapter 3 (alcohol-specific genetic risk, smoking initiation, and peer alcohol use) as genetic, shared environmental, or nonshared environmental influences is complicated, since they do not necessarily reflect just one of these factors. Co-twin data were used to index genetic risk for alcohol initiation, but these data may include shared environmental effects as well. Peer-related processes mainly take place outside the family environment, and may therefore constitute nonshared environmental influences, but they may also reflect shared environmental effects, as twin pairs tend to have common friends (Loehlin, 2010). Peer alcohol use is often seen as an environmental factor, while this predictor likely also reflect genetic effects, since adolescents, particularly girls, who have a higher genetic liability to drink, tend to choose friends and romantic partners with similar drinking behavior (active gene-environment correlation or r_{GE}) (Agrawal et al., 2010a; Loehlin, 2010; van der Zwaluw et al., 2009).

Moreover, genetic and environmental factors do not affect alcohol initiation independently. If sensitivity to the environment differs between genotypes, this constitutes gene x environment interaction (Eaves, 1987). Several specific environmental factors interact with genetic factors, such as peer substance use (Agrawal et al., 2010a; Guo et al., 2009), religiosity

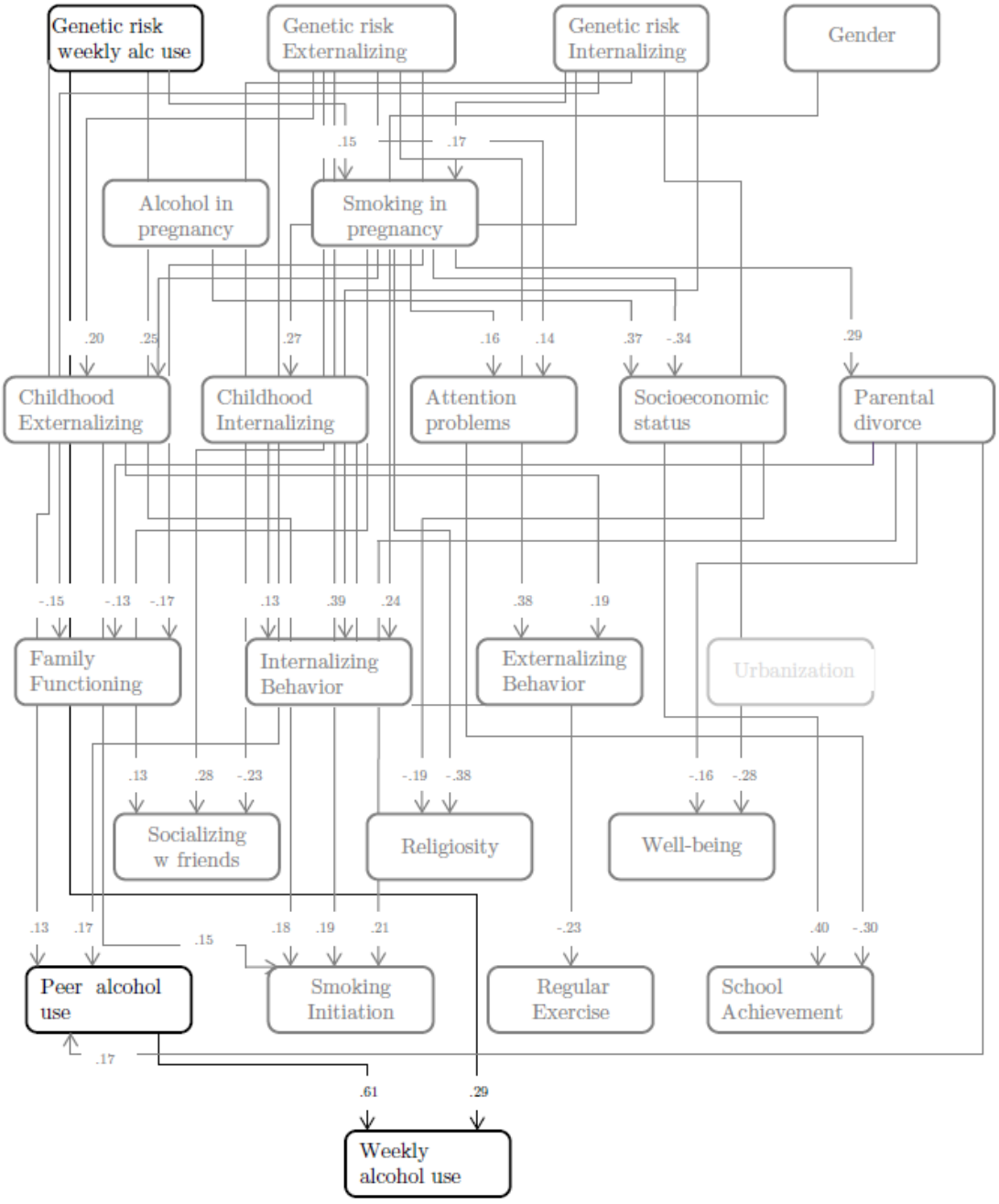


FIGURE 1 Standardized partial regression coefficients estimated under the best fitting model predicting weekly alcohol use. Variables are grouped by developmental timing (genetic risk, prenatal, childhood, and adolescence). Pathways directly related to alcohol initiation are depicted in black, the indirect pathways are shown in grey.

(Button et al., 2010; Koopmans et al., 1999b), socio-regional factors (Legrand et al., 2008; Rose et al., 2001a) and parental monitoring (Dick et al., 2007b). Generally, these studies suggest that genetic influences on adolescent alcohol use are stronger in more permissive environments. Environmental factors may also interact with specific genotypes, as has been observed, for example, for dopamine D2 receptor gene (DRD2) genotype and CHRM2 genotype (which encodes the muscarinic acetylcholine receptor M2). These genotypes have been found to modulate the protective effects of parental rule-setting and monitoring on adolescent alcohol use (van der Zwaluw et al., Dick et al., 2011; 2010a).

It should be noted that these gene-environment correlations and interaction effects on adolescent alcohol use may have consequences for the interpretation of the estimates of genetic and environmental influences as presented in chapter 2. When gene-environment correlations or interactions are present but not modeled, estimates of genetic and environmental influences may be biased (Eaves, 1984, 1987). If genetic and shared environmental factors are correlated, estimates of shared environmental influences will be inflated. Correlations between genetic and nonshared environment will result in overestimation of genetic influences. If genetic factors interact with the shared environment, genetic influences will be overestimated, while gene x nonshared environment interactions lead to overestimating nonshared environmental influences (Eaves, 1984; Purcell, 2002).

Early alcohol initiation and alcohol craving – cause or effect?

Chapter 4 demonstrated that the association between early alcohol initiation and adult alcohol consumption is entirely explained by an underlying vulnerability for alcohol use. Additionally, I applied the co-twin control design to examine if early alcohol initiation leads to increased alcohol craving in adulthood. Early alcohol initiation may affect brain reward systems (Witt, 2010), thereby resulting in increased alcohol craving (Ait-Daoud et al, 2009; Ait-Daoud et al., 2012), which is an important construct in the development, maintenance, and relapse of problem drinking (Kruse et al., 2012). Twins who had started drinking early were compared to their co-twins who had initiated alcohol use later on situation-specific urges to drink alcohol in adulthood, which were based on items about situation-specific urges to smoke (West & Russell, 1985). Results of these analyses are summarized in Figure 2 (see Supplemental Table 1 for the exact distributions). Figure 2 shows the distribution of the urge to drink in each situation for both definitions of early versus late alcohol initiation (initiation at age ≤15 vs. ≥17 years and at age ≤16 vs. ≥18 years), along with the p-value of the test of whether these distributions differed

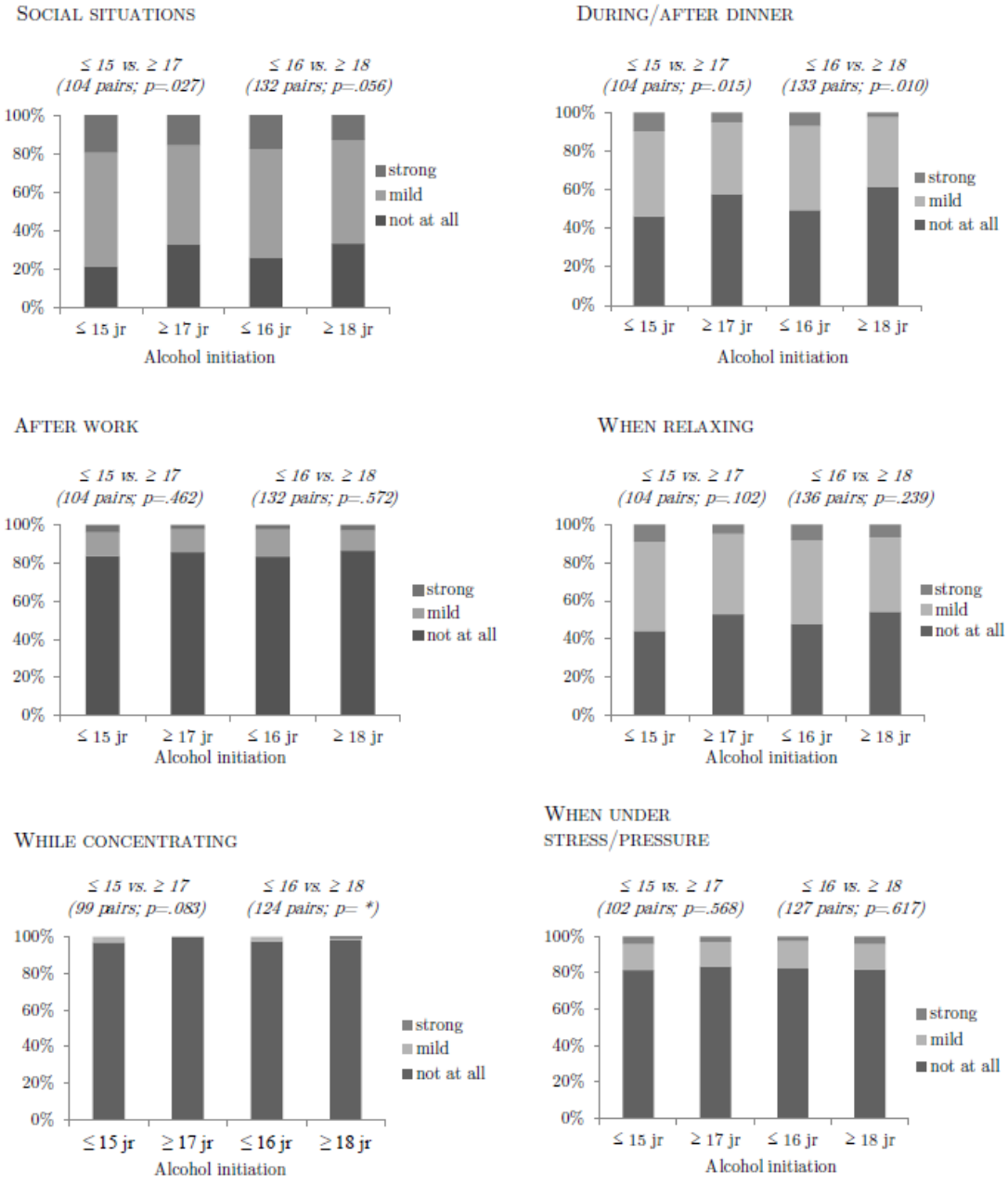


FIGURE 2 Situation-specific urges to drink in twins discordant for early alcohol initiation. Lifelong abstainers were excluded from the analyses. * p-value could not be computed due to empty cells.

between early and late initiators. Twins who had started drinking at age 16 years or younger more often experienced an urge to drink during or after dinner than their co-twins who had initiated alcohol use at age 18 years or older. Twins who started drinking at age 15 years or younger also more often reported the urge to drink at dinner than their co-twins who had started at age 17 years or older, but these differences did not reach significance (p=.015; 1st row, right figure). For the urge to drink in social situations, p-values were .027 (initiation at age ≤15 vs. ≥17 years) and .056 for initiation at age ≤16 vs. ≥18 years (1st row, left figure). When lifelong abstainers were included in the late-initiation group (not shown in Figure 2), twins who had started drinking at age 15 or earlier more often experienced the urge to drink alcohol in social situations than their co-twins who had started drinking at age 17 years or never. When early initiation was defined as initiation at age 16 or younger versus at age 18 years or older/never, early drinkers reported more urges to drink in social situations and at dinner than the co-twins who started drinking later or never (see Supplemental Table 1).

In summary, early alcohol initiation seems to increase adult alcohol cravings in social situations and at dinner, but not in other situations. This may be explained by considering that alcohol is widely used in social situations (Anderson et al., 2012), which may include dinners, and these situations may therefore provide many alcohol-related cues, which induce alcohol craving (Kruse et al., 2012). However, it cannot be ruled out that the early initiators already experienced stronger alcohol cravings when they initiated alcohol use, in which case early alcohol initiation is not the cause of these cravings but may be the result. Data on alcohol cravings at the time of alcohol initiation can help clarify the direction of causality in these relationships.

Twin discordance for early alcohol initiation

Mechanisms that influence early alcohol initiation are related to another interesting question, regarding which specific factors make monozygotic twins discordant for alcohol initiation. Monozygotic twins are assumed to share 100% of their genes and shared environment (e.g. Ligthart & Boomsma, 2012; Vink et al., 2007), so these factors are unlikely to have caused the discordance. As demonstrated in chapters 2 and 3, early alcohol initiation is to a large extent explained by genetic and shared environmental factors, e.g. alcohol-specific genetic risk and peer factors. Nevertheless, 14% of individual differences in alcohol initiation at age 13-15 were explained by nonshared environmental influences, and these likely contain the factors that made one twin start drinking early, and his or her co-twin later. Nonshared environmental influences

on adolescent drinking may involve romantic relationships. Among 18-20 year olds, changes in relationship status were related to increases and decreases in heavy drinking (Fleming et al., 2010). However, van der Zwaluw et al. (2009) observed that the alcohol use of a partner did not prospectively predict adolescent alcohol use. To the extent that friends are not shared by members of a twin pair and do not reflect genetic influences (by means of r_{GE}), they may be categorized as a nonshared environmental influence. This may apply in particular to dizygotic twins, who share fewer friends than monozygotic twins, and to boys, who share fewer friends than girls (Loehlin, 2010). Other predictors of alcohol initiation on which monozygotic twins may differ are school-related factors. Lower expectations for school achievement, lower levels of bonding to school, negative attitudes toward school, and lower grades are associated with early alcohol initiation (review by Donovan, 2004; Donovan and Molina, 2011). Twins may also differ in the extent to which they are exposed to alcohol advertising and promotion, which increase the risk for alcohol initiation and increased alcohol consumption during adolescence (review by Anderson et al., 2009). Media exposure, specifically alcohol consumption in movies is linked to increased prevalence of alcohol initiation, quantity of alcohol consumed, and binge drinking (reviews by Hanewinkel et al., 2012; Nunez-Smith et al., 2010). Finally, recent research has implicated epigenetic processes and copy number variations (CNVs) as possible contributors to monozygotic twin discordance, specifically in psychiatric disorders and attention problems (Ehli et al., 2012; Lin et al., 2012).

Genetic variants and biological pathways to alcohol use

Throughout this thesis, genetic influences were inferred from patterns of familial resemblance. This raises the question which specific genetic variants are related to various forms of alcohol use. Alcohol use is likely influenced by many genetic variants with small effects, which complicates the search for risk genes (Kendler et al., 2012). Nevertheless, several genes have been confirmed as contributing to risk for alcohol use or dependence. Those that most consistently have been associated with alcohol dependence are the genes in the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) clusters, and GABAergic genes (reviews by Agrawal et al., 2012; Kendler et al., 2012; Wang et al., 2012). Genes in the ADH and ALDH clusters are involved in alcohol metabolism. Carriers of specific variants experience facial flushing and unpleasant reactions to alcohol intake (flushing syndrome), which makes these variants protective for alcohol dependence (van Beek et al., 2010). Associations between GABAergic genes (GABRA2 and GABRB1) and alcohol use have been confirmed (Kendler et al.,

2012). It is known that gamma amino butyric acid (GABA) is an important inhibitory neurotransmitter which mediates pharmacological effects of alcohol in the brain, but the functional pathways through which GABA affects alcohol use are poorly understood at present (Kendler et al., 2012; Wang et al., 2012). In a recent review, Agrawal et al. (2012) additionally noted DRD2/ANKK1 genotype as confirmed risk factors for alcohol dependence. ANKK1 is a polymorphism in the dopamine D2 receptor (DRD2) gene, that is involved in dopamine synthesis in the brain (Neville et al., 2004). A meta-analysis has related autism susceptibility candidate 2 (AUTS2) to alcohol intake. AUTS2 is expressed in dopaminergic neurons involved in reward mechanisms, and in glutamatergic and GABAergic neurons that influence impulsivity and alcohol sensitivity (Schumann et al., 2011). Other genes that have been related to alcohol use are LTBP1, which encodes latent-transforming growth factor beta-binding protein 1 and is involved in alcohol metabolism; actin-filament binding protein frabin (FGD4), which is related to clustering and trafficking of $GABA_A$ receptors (Pei et al., 2012); serotonin transporter genotype (5-HTTLPR) (Agrawal et al., 2012); PECR, which is involved in fatty acid metabolism and mainly expressed in the liver; and KCNMA1, which is related to alcohol resistance (Kendler et al., 2012).

Alcohol use in adolescence may be influenced by the same genetic variants as adult alcohol use, since over the period from adolescence (age 15 years) to adulthood (age 32 years), lifetime prevalence of symptoms of alcohol abuse/dependence are influenced by a single, stable genetic factor (van Beek et al., 2012). In addition to the genotype x environment interaction effects described in the previous section (for DRD2 and CHRM2 genotype; Dick et al., 2011; van der Zwaluw et al., 2010a), several genes have been associated specifically to alcohol use and comorbid traits in children and adolescents. These include GABRA2, COMT (catechol-O-methyltransferase valine/methionine), C1QTNF7 (C1q and tumor necrosis factor-related protein 7) (Dick et al., 2010; Dick et al., 2006), ALDH2, (Irons et al., 2012), and 5-HTTLPR (van der Zwaluw et al., 2010b).

Implications for intervention strategies

Early patterns of alcohol use are mainly explained by shared environmental factors, and to a minor extent by genetic factors, as demonstrated in chapter 2. With increasing age, genetic factors gain in importance, while the influence of shared environment declines. This age pattern has consistently been observed across various countries (Bergen et al., 2007; Dick et al., 2007a; Hopfer et al., 2003; Kendler et al., 2008; Rose et al., 2001a). The strong influence of shared

environmental factors in early adolescence suggests that family-based prevention methods may be especially effective in delaying alcohol initiation and reducing alcohol consumption in that age group. A meta-analysis on family-based interventions in American samples indeed showed that such interventions significantly reduce the prevalence of alcohol initiation in adolescents under age 16 years and decrease frequency of alcohol use in that same age group. Universal family-based interventions (involving multiple families within a school) were most effective, presumably due to the additional influence of the school and peers (Smit et al., 2008).

As noted by Chun & Linakis (2012), a wide range of intervention programs reduce adolescent alcohol use, but it is unclear which programs are most efficacious. Oliva et al. (2012) published a commentary in reference to the study reported in chapter 2, in which they pointed out that the findings from that study imply that environmental interventions aimed at delaying alcohol initiation likely affect all adolescents similarly, while interventions aimed at reducing alcohol consumption in older adolescents may be most effective when targeting those at highest genetic risk for alcohol use. Such interventions to reduce alcohol consumption in older adolescents may include tailored programs such as motivational interviewing, cognitive behavioral therapy, and family therapy (review and meta-analysis by Tripodi et al., 2010).

With respect to adult alcohol consumption, the findings from chapter 4 imply that intervention methods aimed at reducing alcohol consumption in the adult population may be more effective when targeting groups at more immediate risk for problematic drinking, rather than by striving to increase age at alcohol initiation. The findings reported in chapter 5 can help identify these groups. In the Netherlands, the elderly population may be at risk for problematic drinking, which has been observed in previous years (Weingart, 2009). Currently, several programs exist to prevent depression in the elderly population (Netherlands Institute of Mental Health and Addiction, 2012), and our findings suggest it may be worthwhile to devote attention to alcohol use in this age group as well, especially among women. Other groups that may be at risk for excessive drinking are individuals who have initiated cannabis and cigarette use, the highly educated population, students, and individuals with increased financial stress. Comorbid initiation of cigarette and cannabis use suggests that genetic factors are important, since in American adults, a substantial part of this comorbidity was explained by common genetic influences (Kendler et al., 2008). High educational attainment has previously been associated with higher prevalence of alcohol use, but lower levels of heavy drinking (Savelkoul et al., 2011). However, the analyses in chapter 5 indicate

that high educational attainment is also associated with increased number of intoxications and lifetime prevalence of alcohol abuse and dependence symptoms. These associations were independent of student status, which is a well-established risk factor for alcohol abuse and alcohol-related problems (Netherlands Institute on Mental Health and Addiction, 2009). Financial stress was a less pronounced predictor of alcohol use, but nevertheless was associated with higher number of alcohol intoxications and may therefore be of importance for identifying risk groups.

Weak, causal effects of prenatal tobacco exposure on offspring externalizing problems were observed in chapter 6. The discussion on whether prenatal maternal smoking and offspring problem behavior are causally related is ongoing (e.g. Thapar & Rutter, 2009). However, the importance of correlated risk factors, such as maternal psychopathology or low socioeconomic status, is widely recognized. These risk factors can be genetic or environmental in origin and explain a substantial part, and in some studies all, of the association between prenatal maternal smoking and offspring problem behavior (review by Knopik, 2009). Consequently, intervention methods aimed at reducing negative outcomes of prenatal maternal smoking may be more effective if they address such correlated risk factors, e.g. maternal psychopathology, rather than focusing solely on prenatal smoking cessation. Current interventions for smoking cessation during pregnancy include cognitive behavioral therapy, feedback on fetal health status, measurement of tobacco byproducts in the mother, financial incentives or rewards and pharmacotherapy (e.g. nicotine patches). Overall, these interventions increase smoking cessation rates by 6%, although there is substantial variation between intervention methods (review and meta-analysis by Lumley et al., 2009). Addressing correlated risk factors in such interventions may also prevent smoking relapse after birth. As observed by Lauria et al. (2012), a substantial proportion of mothers starts smoking again after giving birth (up to 32.1% within 12 months after delivery). Exposure to secondhand smoke has adverse effects on children, such as increased risk of hyperactive/inattention and externalizing problems (Kabir et al., 2011; Tiesler et al., 2011). The efficacy of interventions may be additionally increased by involving partners of pregnant women. Partner support is an important predictor of smoking cessation in pregnancy and avoiding relapse after giving birth, yet at present, most programs focus only on the mother, and do not include their partners (review by Hemsing et al., 2012).

Considering the extensive literature on associations between childhood externalizing problems and later alcohol consumption (e.g. review by Meyers & Dick, 2010), it is important to provide adequate help for children with these

problems and their families. In their commentary to the study described in chapter 2, Oliva et al. (2012) refer to the 'Communities that Care' program, which is a community-based program targeting problem behavior in children and adolescents that has been implemented in several countries, including the Netherlands (Jonkman et al., 2009). Such interventions may not only benefit children with problem behavior, but may thereby also help prevent excessive drinking and alcohol-related problems in their adult life.

Overall conclusions

Considering the findings of this thesis, I come to the following overall conclusions:

1. The effectiveness of prevention and intervention campaigns for alcohol use in young adolescents in the Netherlands may be increased by taking into account that alcohol initiation and early use are predominantly related to shared environmental factors, which include family and peer-related factors, and alcohol-specific genetic risk (defined as alcohol initiation of the co-twin).
2. Alcohol consumption in older adolescents is more strongly influenced by genetic factors, therefore intervention programs tailored to those at highest genetic risk may be most efficacious in that age group.
3. Early alcohol initiation is associated with, but does not lead to significant increases in adult alcohol consumption, while it may result in increased alcohol craving in adulthood.
4. In the Netherlands, the population above age 65 may be at risk for problem drinking. Moreover, women consume the largest quantities of alcohol between age 55-65 years.
5. The most important risk factors for increased alcohol consumption in Dutch adults are high educational attainment, student status, cannabis and cigarette initiation, and to a lesser extent, financial stress. Comorbid initiation of cannabis and cigarette use indicates that genetic factors influence adult alcohol consumption.
6. Associations between maternal smoking during pregnancy and offspring aggressive and externalizing problems at age three are consistent with shared genetic or environmental influences and a small additional effect of prenatal tobacco exposure.
7. The effectiveness of intervention programs for prenatal smoking cessation may be increased by addressing correlated risk factors, and by involving partners, rather than focusing only on maternal smoking cessation during pregnancy.

Supplement to General Discussion

Table 1 Distributions (percentages) of situation-specific urges to drink in MZ twins discordant for early alcohol initiation

	Initiation age ≤ 15 years vs. ≥17 years				*Initiation age ≤ 16 years vs. ≥18 years*			
ABSTAINERS EXCLUDED	N pairs	Early	Late/never	p-value	N pairs	Early	Late/never	p-value
Social situations								
Not at all	104	21.2	32.7	.027	132	25.8	33.3	.056
Mild		59.6	51.9			56.8	53.8	
strong		19.2	15.4			17.4	12.9	
During/after dinner								
Not at all	104	46.2	57.7	.015	133	49.6	61.7	**.010**
Mild		44.2	37.5			43.6	36.1	
strong		9.6	4.8			6.8	2.3	
After work								
Not at all	104	83.7	85.6	.462	132	83.3	86.4	.572
Mild		12.5	12.5			14.4	10.6	
strong		3.8	1.9			2.3	3.0	
When relaxing								
Not at all	104	44.2	52.9	.102	136	47.8	54.4	.239
Mild		47.1	42.3			44.1	39.0	
strong		8.7	4.8			8.1	6.6	
While concentrating								
Not at all	99	97.0	100.0	.083	124	97.6	98.4	-
Mild		3.0	0			2.4	.8	
strong		0	0			0	.8	
When under stress/pressure								
Not at all	102	81.4	83.3	.568	127	82.7	81.9	.617
Mild		14.7	13.7			15.0	14.2	
strong		3.9	2.9			2.4	3.9	

ABSTAINERS INCLUDED IN LATE-INITIATION GROUP	*Initiation age ≤ 15 years vs. ≥17 years/never* N pairs	Early	Late	p-value	*Initiation age ≤ 16 years vs. ≥18 years/never* N pairs	Early	Late	p-value
Social situations								
Not at all	109	21.1	35.8	**.005**	142	26.1	38.0	**.006**
Mild		58.7	49.5			56.3	50.0	
strong		20.2	14.7			17.6	12.0	
During/after dinner								
Not at all	109	47.7	59.6	.011	143	52.4	64.3	**.007**
Mild		43.1	35.8			41.3	33.6	
strong		9.2	4.6			6.3	2.1	
After work								
Not at all	109	84.4	86.2	.462	142	84.5	87.3	.572
Mild		11.9	11.9			13.4	9.9	
strong		3.7	1.8			2.1	2.8	
When relaxing								
Not at all	109	45.0	55.0	.052	146	50.0	57.5	.144
Mild		45.9	40.4			41.8	36.3	
strong		9.2	4.6			8.2	6.2	
While concentrating								
Not at all	104	97.1	100.0	.083	134	97.8	98.5	-
Mild		2.9	0			2.2	.7	
Strong		0	0			0	.7	
When under stress/pressure								
Not at all	107	80.4	84.1	.228	137	82.5	83.2	.868
Mild		14.0	13.1			13.9	13.1	
strong		5.6	2.8			3.6	3.6	

Nederlandse samenvatting

Alcohol wordt veel gedronken in Nederland; 88% van de volwassen bevolking heeft in het afgelopen jaar tenminste één keer alcohol gebruikt (European Commission, 2010). Over het algemeen drinken Nederlanders frequent, maar gematigde hoeveelheden. Zoals in de meeste West-Europese landen is bier de meest geliefde drank, met name onder mannen (Anderson et al., 2012; Room, 1992). Ondanks dat het grootste deel van de Nederlandse bevolking gematigd drinkt, is problematisch alcoholgebruik niet ongewoon; ongeveer 9% van de volwassen bevolking is probleemdrinker en de prevalentie van alcoholmisbruik/-afhankelijkheid is 5% onder mannen en 1% onder vrouwen (Ouwehand et al., 2011; World Health Organization, 2011).

Patronen van alcoholgebruik variëren sterk over leeftijd. Het eerste glas alcohol wordt doorgaans tijdens de adolescentie gedronken; tussen 72-75% van de Nederlandse jongeren onder 16 jaar oud heeft ooit alcohol geprobeerd, en ongeveer 20% drinkt tenminste een aantal keer per maand. Schadelijk alcoholgebruik (AUDIT score $\geq$ 8; Babor et al., 2001) komt het meest voor onder jongvolwassenen (18-25 jaar), met een prevalentie van 30% onder mannen en 13% onder vrouwen. Tussen leeftijdsgroepen 18-25 jaar en 65 jaar of ouder neemt de prevalentie van schadelijk alcoholgebruik af tot 13% in mannen en 6% in vrouwen, terwijl de frequentie van (bijna) dagelijks alcoholgebruik toeneemt; van 3% tot 33% in mannen en van 1% tot 20% onder vrouwen.

Het doel van dit proefschrift was inzicht te verschaffen in de genetische en omgevingseffecten op alcoholgebruik en op comorbide internaliserende en externaliserende (gedrags)problemen, in verschillende ontwikkelingsstadia. Hiertoe werden gegevens geanalyseerd van Nederlandse tweelingen en hun familieleden, die hadden meegedaan aan vragenlijstonderzoek van het Nederlands Tweelingen Register (NTR). Binnen het NTR worden jonge tweelingen en hun broers/zussen vanaf de geboorte tot ongeveer hun 12^{e} jaar gevolgd door hun ouders en leerkrachten te vragen de kinderen te beoordelen op o.a. gezondheid, internaliserende/externaliserende problemen en schoolprestaties. Op 14- en 16-jarige leeftijd vullen de tweelingen vragenlijsten in over hun leefgewoonten, welzijn, school, en internaliserende/externaliserende (gedrags)problemen (Bartels et al., 2007b; Bartels et al., 2011). Als de jongeren ouder dan 12 zijn, wordt hun ouders om toestemming gevraagd ze te mogen benaderen en wordt de ouders ook gevraagd of er nog andere broers en zussen zijn, die benaderd mogen worden voor onderzoek.

Naast gegevens van jonge tweelingen worden binnen het NTR vragenlijstgegevens verzameld onder volwassen tweelingen en hun broers/zussen, ouders, partners, en kinderen (van 18 jaar of ouder) in langlopend onderzoek. Sinds 1991 vullen volwassenen ongeveer iedere 3 jaar een vragenlijst in over

gezondheid, persoonlijkheid, en leefgewoonten (D. I. Boomsma et al., 2006). Een deel van deze volwassenen is ingestroomd bij het NTR als adolescent, en voor deze groep zijn dus gegevens beschikbaar van toen ze jonger dan 18 waren.

In dit proefschrift werd specifiek onderzocht of genetische en omgevingsfactoren van invloed zijn op alcoholinitiatie en -consumptie in de adolescentie en verscheidene indicatoren van alcoholconsumptie en -afhankelijkheid onder volwassenen. Met name werd onderzocht wat het belang van deze factoren is in het verklaren van verschillen tussen mensen: waarom drinkt de een veel en de ander weinig? Een specifieke omgevingsfactor die werd onderzocht is prenataal roken. Voor externaliserende gedragsproblemen en internaliserende psychopathologie in kinderen is zowel een hoofdeffect van roken tijdens de zwangerschap getest, als een interactie met serotonine transporter (5-HTTLPR) genotype. Hieronder worden de bevindingen samengevat en besproken.

In **hoofdstuk 2** werd onderzocht of leeftijd, sekse en geboortecohort geassocieerd zijn met alcoholconsumptie onder jongeren. Twee cohorten van tweelingen tussen 13-21 jaar, die vragenlijsten hadden ingevuld in 1993 en 2005-8, werden vergeleken op prevalentie van alcoholinitiatie, frequentie van alcoholgebruik en wekelijks aantal glazen alcohol. De jongeren in het cohort 2005-8 hadden vaker alcoholgebruik geïnitieerd en dronken een groter aantal glazen alcohol per week dan in het cohort 1993. In beide cohorten nam alcoholgebruik toe naarmate leeftijd toenam en vanaf 16-jarige leeftijd dronken jongens vaker en grotere hoeveelheden dan meisjes. Vervolgens werden de relatieve bijdragen van genetische en omgevingsfactoren aan variatie in alcoholgebruik geschat in verschillende leeftijdsgroepen, en in beide sekses en cohorten, en werd getoetst of de erfelijkheid over deze groepen verschilde. Onder de jongste adolescenten (13-15 jaar) werden individuele verschillen in alcoholinitiatie en frequentie van alcoholgebruik voornamelijk verklaard door gedeelde omgevingsfactoren (55% en 64%, respectievelijk), en een kleiner deel door genetische factoren (31% voor initiatie; 21% voor frequentie). Naarmate leeftijd toenam, nam ook de invloed van genetische factoren toe, terwijl de rol van de gedeelde omgeving kleiner werd. Er werden geen verschillen tussen de cohorten gevonden in de relatieve bijdragen van genetische en omgevingsfactoren, dus een toename in alcoholgebruik tussen 1993 en 2005 leidde niet tot een verandering in de erfelijkheid.

In **hoofdstuk 3** werden deze genetische en omgevingsinvloeden verder onderzocht door alcoholinitiatie op 13-15-jarige leeftijd te voorspellen binnen een model, waarin 22 predictoren werden getest, gebaseerd op de literatuur. Dit waren genetische risicofactoren (afgeleid uit gegevens van de co-twin); en

omgevingsrisicofactoren: prenatale blootstelling aan alcohol en sigarettenrook; risicofactoren tijdens de jeugd, zoals attentieproblemen en scheiding van de ouders; en risicofactoren in de adolescentie, zoals externaliserende en internaliserende problemen, leefgewoonten, en alcoholgebruik van leeftijdsgenoten. De predictoren verklaarden tezamen 66% van de individuele verschillen in alcoholinitiatie. Deelnemers met hoger genetisch risico voor alcoholinitiatie, die op vroege leeftijd hadden geëxperimenteerd met roken en die vrienden hadden die alcohol dronken, liepen hoger risico op het initiëren van alcoholgebruik tussen 13 en 15-jarige leeftijd. Externaliserende gedragsproblemen waren zwak en indirect geassocieerd met vroege alcoholinitiatie, en dat gold ook voor internaliserende psychopathologie.

De grote invloed van gedeelde omgeving op alcoholgebruik in de vroege adolescentie suggereert dat preventieprogramma's die gericht zijn op de familieomgeving effectief kunnen zijn in het uitstellen van alcoholinitiatie en het terugdringen van alcoholgebruik in deze leeftijdsgroep. Een meta-analyse van Amerikaanse studies naar dergelijke interventies vond inderdaad dat deze de prevalentie van alcoholinitiatie en frequentie van alcoholgebruik onder 16-jarige leeftijd verlagen, met name wanneer de interventies werden toegepast op meerdere families binnen een school (Smit et al., 2008). Oliva et al. (2012) publiceerden een commentaar op de studie beschreven in hoofdstuk 2, waarin zij opmerkten dat de resultaten van die studie impliceren dat preventieprogramma's gericht op het terugdringen van alcoholinitiatie jonge adolescenten op vergelijkbare wijze beïnvloeden, terwijl interventies gericht op oudere adolescenten mogelijk het meest effectief zijn onder jongeren met een sterkere genetische aanleg voor alcoholgebruik.

Alcoholinitiatie op jonge leeftijd is consistent geassocieerd met verhoogde alcoholconsumptie en -misbruik onder volwassenen (e.g. Agrawal et al., 2009; Guttmannova et al., 2011; Lee et al., 2012; Sartor et al., 2011). In **hoofdstuk 4** werd een discordant tweelingdesign toegepast om te onderzoeken of dit verband causaal is, of het gevolg van een onderliggende aanleg voor alcoholgebruik. Binnen ééneiige tweelingparen werden tweelingen die vroeg waren begonnen met drinken vergeleken met hun broer of zus die later was begonnen op verschillende maten van alcoholconsumptie en –misbruik. In de gehele NTR steekproef was er een verband tussen vroege initiatie en latere uitkomstmaten, maar binnen ééneiige tweelingparen verschilden de vroege drinkers niet significant van hun broer/zus die later was begonnen met drinken. Deze bevindingen suggereren dat vroege alcoholinitiatie geen significant causaal effect heeft op alcoholconsumptie op volwassen leeftijd. Dit impliceert dat interventies met als doel het verhogen

van de leeftijd waarop jongeren voor het eerst alcohol drinken, slechts beperkt effect hebben op alcoholconsumptie onder volwassenen. Mogelijk kunnen programma's gericht op meer directe risicofactoren onder volwassenen daarin effectiever zijn.

In **hoofdstuk 5** werd een epidemiologische analyse van alcoholconsumptie in de Nederlandse populatie (leeftijd 18-97 jaar) beschreven. Verscheidene indicatoren van alcoholconsumptie en demografie/leefstijl werden gerapporteerd naar leeftijd en sekse en iedere indicator van alcoholconsumptie werd voorspeld met leeftijd, sekse, de interactie tussen leeftijd en sekse, en de overige demografische/leefstijlvariabelen. Frequentie van alcoholgebruik en aantal glazen per week vertoonden de meest opvallende samenhang met leeftijd. Jongvolwassenen (18-25 jaar) dronken het minst frequent, en deelnemers van 65 jaar of ouder het meest frequent. Daarnaast dronken vrouwen het kleinste aantal glazen alcohol tussen 25- en 45-jarige leeftijd en het hoogste aantal glazen tussen 55- en 65-jarige leeftijd. Deelnemers in de jonge leeftijdscategorieën rapporteerden een lagere leeftijd waarop ze voor het eerst alcohol hadden gedronken en waren begonnen met regelmatig drinken, en waarop ze voor het eerst aangeschoten of dronken waren geweest, dan oudere deelnemers. Onder de oudere deelnemers waren mannen op jongere leeftijd begonnen met (regelmatig) drinken en waren op jongere leeftijd voor het eerst aangeschoten/dronken geweest, dan vrouwen. Deze sekseverschillen werden niet gevonden onder de jongste deelnemers (18-25 jaar). De belangrijkste voorspellers van verhoogde alcoholconsumptie waren hogere leeftijd, sekse (man), en initiatie van roken en cannabisgebruik. Daarnaast was alcoholconsumptie hoger onder deelnemers met hoger opleidingsniveau, studenten, en deelnemers die financiële stress ervoeren.

Deze bevindingen kunnen helpen risicogroepen voor problematisch alcoholgebruik te identificeren onder de Nederlandse bevolking. De bevolking boven 65 jaar oud loopt mogelijk risico voor problematisch alcoholgebruik, gezien de hoge frequentie van alcoholgebruik in deze groep. Comorbide initiatie van sigaretten en cannabisgebruik suggereert dat genetische factoren een rol spelen, daar in een steekproef van Amerikaanse volwassenen een aanzienlijk deel van deze associatie verklaard werd door overlappende genetische factoren (Kendler et al., 2008). In eerder Nederlands onderzoek was de prevalentie van alcoholgebruik verhoogd onder deelnemers met hoog opleidingsniveau, maar zwaar drinken kwam minder vaak voor in deze groep (Savelkoul et al., 2011). De bevindingen in hoofdstuk 5 laten daarentegen zien dat hoog opleidingsniveau ook gerelateerd is aan verhoogd aantal alcoholintoxicaties en aan de prevalentie van alcoholmisbruik/-afhankelijkheid ooit in het leven. Deze associaties waren onafhankelijk van of deelnemers student waren, wat een welbekende risicofactor

is voor alcoholmisbruik en alcoholgerelateerde problemen (Netherlands Institute on Mental Health and Addiction, 2009). Financiële stress was een minder sterke predictor van alcoholgebruik, maar was desondanks geassocieerd met hoger aantal alcoholintoxicaties en zou daardoor een risicofactor kunnen zijn voor problematisch alcoholgebruik.

Risicofactoren voor middelengebruik en comorbide gedragsproblemen of psychopathologie kunnen worden doorgegeven van ouder op kind (e.g. Kendler et al., 2012; Meyers & Dick, 2010). In **hoofdstuk 6** heb ik specifiek onderzocht of de associatie tussen prenataal roken en externaliserende en internaliserende (gedrags)problemen in 3-jarige kinderen verklaard wordt door directe effecten van prenatale blootstelling aan sigarettenrook, of door effecten van genen en de omgeving die ouders met hun kinderen delen. Daarnaast werden effecten van roken tijdens verschillende trimesters van de zwangerschap onderzocht. Onder de gedeelde genen/omgeving-hypothese werden geen effecten van trimester-specifieke blootstelling op latere externaliserende/internaliserende problemen verwacht, terwijl onder de causale hypothese de mate van prenatale blootstelling hierop mogelijk wel effect heeft. Prenataal roken werd gerapporteerd door 16% van de moeders in de steekproef. Binnen deze groep had 70% gedurende de hele zwangerschap gerookt, 11% alleen in het 1^{e} trimester, 7% alleen in het 3^{e} trimester, en 11% had onregelmatig gerookt tijdens de zwangerschap. Het patroon van associaties was consistent met directe effecten van prenataal roken op externaliserend gedrag op 3-jarige leeftijd, naast de invloed van gedeelde genen en omgeving. De associaties tussen prenataal roken en internaliserende psychopathologie waren zwakker en niet consistent met directe effecten. Roken in het eerste of laatste trimester van de zwangerschap, ten opzichte van roken tijdens de gehele zwangerschap, was niet gerelateerd aan minder externaliserende of internaliserende (gedrags)problemen.

In **hoofdstuk 7** werd een replicatiestudie van het onderzoek van Cents et al. (2012) beschreven, naar een interactie-effect van prenataal roken van de moeder en serotonine transporter (5-HTTLPR) genotype van zowel moeder als kind op internaliserende psychopathologie in haar 3-jarige kinderen. Cents et al. (2012) vonden in een steekproef van Nederlandse kinderen uit de algemene bevolking geen hoofdeffect van serotonine transporter genotype, noch van prenataal roken, maar kinderen die een risico allel (s-allel van het short/long polymorfisme) droegen en waren blootgesteld aan prenataal roken, hadden verhoogde internaliserende psychopathologie op 3-jarige leeftijd. In mijn replicatiestudie werden geen significante hoofdeffecten van prenataal roken en serotonine transporter genotype gevonden, noch een significante interactie

tussen deze variabelen.

De bevindingen in hoofdstuk 6 impliceren dat er zwakke, directe effecten van prenatale blootstelling aan tabak zijn op externaliserend gedrag op 3-jarige leeftijd. Of de relatie tussen prenataal roken van de moeder en gedragsproblemen in kinderen causaal is, is een omstreden onderwerp in de literatuur (e.g. Thapar & Rutter, 2009). Echter, er bestaat consensus over de belangrijke rol van gecorreleerde risicofactoren, zoals psychopathologie van de moeder of lage socio-economische status. Deze risicofactoren kunnen door genen of de omgeving beïnvloed worden en verklaren een aanzienlijk deel van, en in sommige studies de hele associatie tussen prenataal roken van de moeder en gedragsproblemen in kinderen (review door Knopik, 2009). Derhalve kan de effectiviteit van interventies die tot doel hebben de negatieve consequenties van prenataal roken te verminderen mogelijk worden vergroot door zich ook op dergelijke risicofactoren te richten, in plaats van alleen op roken tijdens de zwangerschap. Dergelijke uitgebreidere interventies kunnen wellicht ook voorkomen dat het roken na de geboorte wordt hervat (Lauria et al., 2012). Meeroken heeft schadelijke effecten op kinderen, zoals verhoogd risico op hyperactiviteit en aandachtsproblemen, en op externaliserende gedragsproblemen (Kabir et al., 2011; Tiesler et al., 2011). Een mogelijke manier om de effectiviteit van dergelijke interventies verder te vergroten is door partners van zwangere vrouwen in het programma te betrekken. De steun van een partner voorspelt in belangrijke mate of moeders stoppen met roken tijdens de zwangerschap, en of ze na de geboorte weer beginnen met roken, maar de meeste interventies zijn alleen gericht op de moeder zelf, en niet op de partner (review door Hemsing et al., 2012).

Gezien de consistent gevonden associaties tussen externaliserende gedragsproblemen tijdens de jeugd en later alcoholgebruik (e.g. review door Meyers & Dick, 2010), is het belangrijk dat er adequate hulp beschikbaar is voor kinderen met dergelijke problematiek en hun families. In hun commentaar op de studie die in hoofdstuk 2 werd beschreven refereerden Oliva et al. (2012) aan het 'Communities that Care' programma, dat gericht is op gedragsproblemen in kinderen en adolescenten. Dit programma is geïmplementeerd in meerdere landen, waaronder Nederland (Jonkman et al., 2009). Dergelijke interventies kunnen niet alleen kinderen met gedragsproblemen tijdens hun jeugd helpen, maar verminderen mogelijk ook het risico op excessief alcoholgebruik in hun volwassen leven.

Conclusies

De effectiviteit van interventieprogramma's om alcoholgebruik onder Nederlandse jongeren terug te dringen kan worden vergroot door in ogenschouw te nemen dat variatie onder jongeren in vroege alcoholinitiatie en -gebruik voornamelijk wordt verklaard door gedeelde omgevingsfactoren, en door genetisch risico voor alcoholinitiatie. Alcoholconsumptie onder oudere adolescenten wordt sterker beïnvloed door genetische factoren, wat suggereert dat interventies gericht op jongeren die een sterkere genetische aanleg hebben, wellicht het meest effectief zijn in die leeftijdsgroep.

Het verband tussen vroege alcoholinitiatie en verhoogde alcoholconsumptie onder volwassenen is niet causaal van aard. De belangrijkste risicofactoren voor verhoogde alcoholconsumptie onder Nederlandse volwassenen zijn: hogere leeftijd, sekse (man), hoog opleidingsniveau, het studentenleven, initiatie van roken en cannabisgebruik, en in mindere mate, financiële stress.

Associaties tussen roken tijdens de zwangerschap en agressieve en externaliserende gedragsproblemen op 3-jarige leeftijd worden verklaard door gedeelde genetische of omgevingsinvloeden en een klein direct effect van prenatale blootstelling aan tabak. De effectiviteit van interventies om effecten van prenataal roken terug te dringen kan mogelijk worden vergroot door daaraan gerelateerde risicofactoren aan te pakken en door partners bij het programma te betrekken.

Appendices

Appendix I	Brochure describing NTR survey studies and introducing Survey 8
Appendix II	Letter accompanying the brochure, inviting twins and family members to complete the web-based version of Survey 8
Appendix III	Letter accompanying the paper version of Survey 8
Appendix IV	Items and scales included in Survey 8
Appendix V	Form for incoming phone calls from participants
Appendix VI	Reminder by email
Appendix VII	Reminder card
Appendix VIII	Protocol for reminders by phone
Appendix IX	Example letter responding to participants'comment/question
Appendix X	Thank you card

Persoonsgegevens
Uw gegevens worden zorgvuldig verwerkt en niet aan derden verstrekt. Persoonlijke gegevens, zoals naam en adres, worden apart van uw antwoorden op de vragenlijst bewaard. De onderzoeksresultaten zijn voor niemand toegankelijk behalve voor de onderzoekers. De onderzoeksgegevens worden uitsluitend voor wetenschappelijke doeleinden gebruikt.

Meer informatie?
Als u nog vragen hebt dan kunt u contact opnemen met Lot Geels of Jenny van Beek via 020-5985335 of vragenlijstNTR@psy.vu.nl (de Vrije Universiteit is gesloten van 25-12-08 t/m 4-1-09; wij zijn dan wel per e-mail bereikbaar). Meer informatie over dit onderzoek vindt u ook op onze website www.tweelingenregister.org/ANTR8. Tevens staan op onze website alle publicaties van eerdere onderzoeken van het Nederlands Tweelingen Register.

Oproep
Wij hopen op de medewerking van zoveel mogelijk meerlingen en hun partners, broers, zussen, ouders en kinderen. Iedereen die 18 jaar of ouder is, kan meedoen. Als u een meerling bent en een partner, broer, zus, ouder of kind hebt die wil meedoen aan dit onderzoek, maar die geen uitnodiging van ons heeft ontvangen, neemt u dan alstublieft contact met ons op.

Alvast onze zeer hartelijke dank voor uw medewerking!

vrije Universiteit amsterdam

Familieonderzoek naar Gezondheid, Leefgewoonten en Persoonlijkheid

Portret van een Nederlands gezin rond 1670 (H. ten Oever)

Sinds 1987 wordt in Nederland onderzoek gedaan naar gezondheid, leefgewoonten en persoonlijkheid bij meerlingfamilies die staan ingeschreven bij het Nederlands Tweelingen Register (NTR). Ook u staat ingeschreven bij het NTR. Waarschijnlijk bent u wel eens eerder uitgenodigd voor onderzoek van het NTR en hebt u hieraan meegedaan. Graag willen we u (weer) van harte uitnodigen om een vragenlijst in te vullen. Wat dit nieuwe onderzoek inhoudt, wordt in deze folder beschreven.

Meerlingen en hun familieleden als bijzondere onderzoeksgroep
De vraag die in het NTR-onderzoek centraal staat, is waarom mensen van elkaar verschillen wat betreft gezondheid, leefgewoonten en persoonlijkheid. Hebben deze verschillen te maken met erfelijke aanleg? Of spelen andere factoren een rol? Om hier iets over te kunnen zeggen, wordt het onderzoek gedaan bij meerlingfamilies. Meerlingen zijn een bijzondere groep, omdat eeneiige meerlingen 100% dezelfde erfelijke aanleg hebben. Tweeeiige of drie-eiige meerlingen zijn voor 50% van hun erfelijke aanleg gelijk, net als ouders en kinderen, en andere broers en zussen. Als eeneiige tweelingparen voor een eigenschap meer op elkaar lijken dan twee-eiige paren betekent dit dat erfelijke aanleg voor deze eigenschap een rol speelt. Uit eerder onderzoek van het NTR blijkt dat niet alleen kenmerken zoals gewicht en lengte door genen beïnvloed worden, maar ook gedrag zoals roken en sporten. Naast erfelijke aanleg spelen leefgewoonten en omgeving een belangrijke rol bij gezondheid. Lijken familieleden op elkaar omdat ze dezelfde genen hebben of imiteren zij elkaar? Om dit te onderzoeken, nodigen wij meerlingen en hun partners, volwassen kinderen, ouders, broers en zussen uit om mee te doen.

Alcohol en gezondheid
Het nieuwe onderzoek gaat onder andere over effecten van alcohol op gezondheid. Het gaat hierbij zowel over de positieve rol die alcohol kan spelen als over negatieve gevolgen. Om te weten te komen hoe alcohol bijdraagt aan welbevinden en op welke manier alcohol leidt tot gezondheidsrisico's, stellen we vragen over alcoholgebruik, zoals: Op welke leeftijd beginnen mensen met drinken? Welke factoren bepalen alcoholgebruik? Is drinkgedrag iets dat mensen van huis uit meekrijgen? Om deze vragen te kunnen beantwoorden, is uw deelname van groot belang, niet alleen als u regelmatig alcohol drinkt maar ook als u (bijna) nooit alcohol gebruikt.

Uw deelname aan het langlopend onderzoek
Uw bijdrage aan dit onderzoek is van groot belang, zowel als u al eerder hebt meegedaan als wanneer u voor het eerst meedoet. Velen van u hebben al eerder meegedaan, waarvoor zeer hartelijk dank! Als u eerder hebt meegedaan, zal het u misschien opvallen dat een deel van de vragen al eerder is gesteld. Wij begrijpen dat de lijst hierdoor lang wordt, maar het is van groot belang dat u deze vragen weer beantwoordt. Door regelmatig dezelfde vragen te stellen, onderzoeken we hoe leefgewoonten en gezondheid veranderen als mensen opgroeien en ouder worden. Verder kunnen wij zo onderzoeken of leefgewoonten op jongere leeftijd aspecten van gezondheid op latere leeftijd voorspellen.

Wie kunnen meedoen?
Deze uitnodiging wordt verstuurd aan volwassen meerlingen en hun familieleden (iedereen van 18 jaar en ouder) die staan ingeschreven bij het NTR. Sommigen van u hebben al eerder vragenlijsten ingevuld over zichzelf. Anderen hebben als ouder van een meerling in het verleden vragen over hun jonge kinderen beantwoord. Wij vragen u nu allemaal aan dit nieuwe project mee te doen en een lijst over uzelf in te vullen.

Hoe kunt u meedoen?
U kunt op twee manieren deelnemen aan het onderzoek: u kunt een papieren vragenlijst invullen of u kunt de vragenlijst via een beveiligde internetpagina invullen. In de bijgevoegde brief vindt u uw **persoonlijke** inlognaam en wachtwoord. Hiermee kunt u via www.tweelingenregister.org/ANTR8 inloggen en direct naar de vragenlijst gaan. Als u over enkele weken nog geen gebruik hebt gemaakt van de mogelijkheid de vragenlijst online in te vullen, krijgt u per post een papieren vragenlijst toegestuurd. Hier hoeft u dus niets voor te doen.

APPENDIX II Letter inviting twins and family members to complete the web-based survey

Nederlands Tweelingen Register (NTR)

Datum	Uw contactpersonen	Telefoon	Telefax	Bijlagen
dec.'08/ jan.'09	Lot Geels Jenny van Beek	020-5985335	020-5988832	Informatiefolder

Ons kenmerk	E-mail	Website
Lijst 8 uitnodiging	vragenlijstNTR@psy.vu.nl	www.tweelingenregister.org/ANTR8

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte heer, mevrouw,

Bij het Nederlands Tweelingen Register gaat opnieuw een vragenlijstonderzoek van start. Wij nodigen u van harte uit hieraan mee te doen. Ook als u al eerder meedeed aan vragenlijst- of ander onderzoek hopen we dat we dit keer weer op uw medewerking mogen rekenen!

Informatie over het onderzoek vindt u in de bijgevoegde folder. U kunt meedoen door via internet of op papier een vragenlijst in te vullen. Om de lijst in te vullen via internet gaat u naar de website van het Nederlands Tweelingen Register:

www.tweelingenregister.org/ANTR8

Hier krijgt u toegang tot de vragenlijst. Als u uw inlognaam en wachtwoord invult, kunt u met de lijst beginnen.

Inlognaam:
Wachtwoord:

U kunt op ieder moment het invullen van de vragenlijst stoppen en op een later tijdstip verder gaan. U kunt na het inloggen uw wachtwoord veranderen.

Als u liever een papieren vragenlijst invult, hoeft u op dit moment niets te doen. Wij sturen u over enkele weken een vragenlijst en antwoordenveloppe toe als u geen gebruik hebt gemaakt van de mogelijkheid de vragenlijst via internet in te vullen.

Wij hopen dat u aan het onderzoek wilt meedoen en danken u bij voorbaat zeer hartelijk voor uw medewerking. Als u nog vragen hebt, kunt u contact opnemen met Lot Geels of Jenny van Beek via 020-5985335 of vragenlijstNTR@psy.vu.nl (de Vrije Universiteit is gesloten van 25-12-08 t/m 4-1-09; in deze periode zijn wij wel per e-mail bereikbaar).

Met vriendelijke groet,

NB Als u een papieren vragenlijst met *grotere letters* wilt, neemt u dan a.u.b. contact op via 020-5985335 of vragenlijstNTR@psy.vu.nl.

Mw. prof. dr. D.I. Boomsma
Vrije Universiteit, Amsterdam

Appendix III Letter accompanying the paper version of Survey 8

Nederlands Tweelingenregister (NTR)

Datum	Uw contactpersonen	Telefoon	Telefax	Bijlagen
Februari 2009	Lot Geels Jenny van Beek	020-5985335	020-5988832	Papieren vragenlijst Antwoordenvelop
Ons kenmerk		E-mail		
Lijst 8 papieren vragenlijst		vragenlijstNTR@psy.vu.nl		

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte heer, mevrouw,

Enige tijd geleden bent u uitgenodigd om deel te nemen aan een nieuw vragenlijstonderzoek van het Nederlands Tweelingen Register (NTR). In deze uitnodiging stonden uw persoonlijke inloggegevens om de vragenlijst online te kunnen invullen. Zoals aangekondigd, kunt u de vragenlijst ook op papier invullen. Mogelijk geeft u de voorkeur hieraan. Daarom sturen wij u hierbij de papieren vragenlijst toe. U kunt deze terugsturen in de bijgevoegde antwoordenvelop (een postzegel is niet nodig).

Natuurlijk kunt u de vragenlijst ook nog steeds via internet invullen. Wellicht bent u al aan de internetvragenlijst begonnen en wilt u deze nog afmaken. In dat geval kunt u deze brief met papieren vragenlijst als niet verstuurd beschouwen. Via www.tweelingenregister.org/ANTR8 kunt u de online vragenlijst bereiken. U kunt inloggen met uw persoonlijke inloggegevens:

Inlognaam: Wachtwoord:

Wij hopen van harte dat u wilt deelnemen aan ons onderzoek. Uiteraard is uw deelname geheel vrijwillig. U kunt er ook voor kiezen niet deel te nemen. Wanneer u nog vragen hebt, kunt u contact opnemen met Lot Geels of Jenny van Beek via vragenlijstNTR@psy.vu.nl of 020-5985335. Neemt u alstublieft ook contact op met bovenstaande personen als u een meerling bent en een partner, broer, zus, ouder of kind hebt die wil meedoen aan dit onderzoek, maar die geen uitnodiging heeft ontvangen. Wij sturen hem of haar graag een uitnodiging toe.

Heel hartelijk dank voor uw medewerking!

Met vriendelijke groet,

NB Als u een papieren vragenlijst met *grotere letters* wilt, neemt u dan a.u.b. contact op via 020-5985335 of vragenlijstNTR@psy.vu.nl.

Mw. prof. dr. D.I. Boomsma
Vrije Universiteit, Amsterdam

Afdeling Biologische Psychologie Bezoekadres:
Van der Boechorststraat 1 Transitorium

APPENDIX IV Overview of items and scales included in Survey 8

A	Biographical information	Date of completing the survey; first name; sex; date of birth; role in family; multiples: birth order
B	Family situation	No. of full/half/non-biological brothers/sisters; no. of biological/non-biological children; no. and age range of children living in the home; no. of grandchildren*; biological children: date of birth, sex, breech presentation, primarily breastfed; date of birth mother and father; if mother and/or father no longer alive, age of death; having a steady relationship*; if yes, duration of current relationship; partner's date of birth, sex, and birth country; having had a previous steady relationship ('no', 'yes, ended in divorce/breakup', 'yes, ended due to death partner', 'other'+ specification'); * based on Netherlands Kinship Panel Study (Dykstra et al., 2005)
C	Health	General state of health (1 'poor' – 5 'excellent'); height (cm); weight (kg); highest adult weight (kg) Eating habits: ever been on a diet (1 'never' – 5 'always'); how afraid are you of gaining weight/becoming fat (1 'not afraid' – 5 'extremely afraid'); how fast do you usually eat (1 'very slowly' – 5 'very fast', BMJ); do you usually eat until full ('stop before full', 'stop when full', 'continue even when full', BMJ); twins/multiples: who eats most ('I do', 'just as much', 'co-multiple', 'I don't know') Ever diagnosed/Medication prescribed for ('no', 'yes, not anymore'; 'yes, now'): allergy/hay fever; asthma/bronchitis; migraine; epilepsy; recurring infections groin/armpits; gastroenteritis; serious intestinal disorders; hepatitis; other liver disease; kidney disease; diabetes; persistent back problems/hernia; RSI; rheumatism/rheumatoid arthritis; osteoarthritis; osteoporosis; high blood pressure; cardiovascular disease; thrombosis; stroke; cancer; anxiety disorders; depression; other. Ever had professional counseling for problems unrelated to physical health ('no', 'yes, in the past; not anymore', 'yes, now'); use of sleeping pills/sedatives ('no', 'yes, on doctor's advice', 'yes, at my own initiative'); current medication: name of medication; disease/ailment; frequency; since (date); having a handicap/disease/injury ('no', 'yes, ...'); memory problems ('no', 'sometimes, but it's not a problem', 'yes, it's a problem', 'yes, it's

		a serious problem')
D	Nervous tics	Involuntary, sudden nervous tics: eye movements; nose movements; lip/mouth movements; head shaking; shoulder/neck movements; arm/hand movements; squeaking/whistling noises; growling/throat clearing/coughing/sniffing; 'purposeless' cursing/utterance of rude/obscene language ('never', '0-1 year age', '1-5 years ago', '>5 years ago'); if any of the above: age of onset; duration of tics for more than 1 year at a time ('no', 'yes'); frequency of tics in most severe period ('not daily', 'daily, but tic-free most of the day', 'daily, but with tic-free periods of 3 hrs not uncommon', 'daily, with tic-free periods of at most half an hour') (based on Leckman et al., 1989)
E	Life events	New job/important promotion; retirement; financial problems; dismissal; dropping out of school/college; relational problems with partner/child/other loved one; hospitalization (self); serious illness/injury of self/partner/child/parent/other loved one; death of partner/child/other loved one; traffic accident; theft/burglary/vandalism; violent crime; sexual crime; child leaving parental home; moving house; moving to nursing home self/parents/other loved one; caring for parent(s); other. Answer categories: 'no', 'less than 1 year ago', '1-5 years ago', 'more than 5 years ago' (based on previous surveys); If co-multiple is no longer alive, age at death; parental divorce
F	Duke UNC Functional Support Questionnaire	8 items on social support, answer categories: 1 'much less than I would like' – 5 'as much as I would like'(Broadhead et al., 1988)
G	Religion	Member of church/religious community ('no', 'yes, but not practicing', 'yes, practicing member'); name of religion; comfort and support from religion (0 'none at all' – 8 'much comfort/support'); comfort and support from religious community (0 'none at all' – 8 'much comfort/support')
H	Alcohol use	Alcohol initiation ('no', 'yes, a few times to try', 'yes'); age at initiation; age at onset regular drinking; preferred beverage ('beer', 'wine', 'spirits', 'no preference'); unpleasant physical reactions to alcohol ('never', 'sometimes', 'always'); situation-specific urges to drink alcohol: social/dinner/after work/relaxing/concentrating/stress ('not at all', 'a little', 'strong'); no. of times intoxicated; age first time intoxicated; frequency of alcohol use past

		year (1 ‘never’ – 6 ‘6 times a week/daily’); weekly quantity of alcohol consumed (beer, wine, and spirts each day); reasons for alchol abstinence (‘health’, ’religion’, ‘taste’, ‘other’, ‘n.a.’)
	Alcohol Use Disorders Identification Test (AUDIT)	Risk of hazardous drinking (Babor et al., 2001; Saunders et al., 1993)
	Risk for problem drinking (CAGE)	4 items indexing symptoms of problem drinking, answer categories: ‘no’, ‘yes, not in past year’, ‘yes, in past year’ (Ewing, 1984)
I	Well-being	
	Satisfaction with Life Scale (SWLS)	5 items with answer categories ranging from 1 ‘strongly disagree’ to 7 ‘strongly agree’ (Diener et al., 1985)
	Three-Item Loneliness Scale (TILS)	3 items, answer categories: ‘hardly ever’, ‘sometimes’, ‘often’ (Hughes et al., 2004)
	Stress question	
	Cantril ladder	General quality of life, measured on a scale from 1 ‘the worst life you can imagine’ to 10 ‘the best life you can imagine’ (Cantril, 1965)
J	NEO-Five Factor Inventory (NEO-FFI)	Big Five personality traits (neuroticism, extraversion, agreeableness, openness, conscientiousness), 60 items, answer categories 1 ‘strongly disagree’ – ‘5 ‘strongly agree’ (Costa Jr & McCrae, 1992)
K	Sport and exercise	Regular exercise (‘no’, ‘yes’); if yes, for each sport: name of sports, no. of years practiced, no. of months/year; no. of times/week , average time spent each time (minutes); how are you good at sports, scale from 0 ‘not good at all’ – 8 ‘very good’; highest level ever reached (‘recreational, for my own pleasure only’, ‘competitive for club/sports academy’, ‘selection team of club/sports academy’, ‘regional level’, ‘national level’); time spent cycling in average week; time spent walking in average week
L	Hospital Anxiety and Depression Scale	only items indexing depression included; 7 items, different answering options: (Zigmond & Snaith, 1983)

	(HADS)	
M	Hoarding Rating Scale-Self Report (HRS-SR)	4 items, answered on scale ranging from 0 'not at all' to 8 'very difficult' (Frost et al., 2009)
N	PADUA Inventory Abbreviated	12 items indexing obsessive compulsive symptoms, subscales: washing, rumination, checking, precision, impulses, answer categories on scale from 0 'never' to 4 'very often' (Sanavio, 1988; van Grootheest et al., 2009)
O	Personality Assessment Inventory-Borderline Features Scale (PAI-BOR)	24 items, subscales: Affective Instability, Identity Disturbance, Negative Relationships, Self-Harm, answer categories on scale from 1 'not at all true' to 4 'very true' (Morey, 1991)
P	Munich Chronotype Questionnaire (MCTQ)	9 items about regular work hours, for workdays and days off: time going to bed, time getting ready to sleep, time it takes to fall asleep, time of waking up, time taken to get up, use of alarm clock, waking up before alarm goes off, ability to choose sleeping times on days off (Roenneberg et al., 2007)
Q	Smoking	Smoking initiation ('no', 'yes, a few times to try', 'yes'); age at initiation; current smoking frequency ('never been a regular smoker', 'used to smoke but quit', 'once a week or less', 'several times a week, not every day', 'daily'); age at onset regular smoking; total no. of years smoked; no. of quitting attempts; what is smoked ('cigarettes, at times in combination with cigars/pipe tobacco', 'only cigars/pipe tobacco'); ex-smokers: age at smoking cessation; smokers: quantity of cigarettes smoked on average per day, or if not daily smoker: quantity of cigarettes smoked per week
	Situation-specific urges to smoke	Urge to smoke in social situations; after a meal; during breaks; after getting up in the morning; when relaxing; when concentrating; in stressful situations; with an alcoholic drink. Answer categories were 'not at all', 'a little', and 'strong' (West & Russell, 1985)
	Fagerström Test for Nicotine Dependence (FTND)	6 items, sumscores range between 0-10 (Heatherton et al., 1991)
R	Sensation Seeking Scale	Shortened version based on regression analyses: 21 items, subscales Thrill and Adventure

	(SSS)	Seeking, Experience Seeking, Boredom Susceptibility, Disinhibition, answer categories range from 5 'totally agree' to 1 'strongly disagree', (Zuckerman et al., 1964)
S	Leisure time activities	5 items about frequency of going to the movies/theatre/a play/museum/concert; spending time in nature/going sightseeing/or going to amusement park/zoo; going to a café/restaurant/dancing; participate in neighborhood programs/hobby/social clubs/professional organizations (1 'hardly ever' – 6 'several times a week'); and time spent on leisure activities: physical exercise/sport; reading/intellectual sports; listening to music; computer activities; watching tv (1 'hardly ever' – 4 '>10 hrs a week); based on Netherlands Kinship Panel Study (Dykstra et al., 2005)
T	Conners' Adult ADHD Rating Scales – ADHD Index	12 items, answer categories ranging between 1 'never' and 4 'very often' (Connors et al., 1999)
U	Substance use	Ever experimented with hash/marijuaha; if yes; age at first use; regular use ('yes', 'no'); age at onset regular use; Ever experimented with xtc/amphetamines/cocaine initiation; if yes; age at first use; regular use ('yes', 'no'); age at onset regular use; Ever experimented with other drugs; if yes; age at first use; regular use ('yes', 'no'); age at onset regular use; No. of drugs experimented with or used regularly
V	Questions for women	Pill/other use of hormonal contraception ('no', 'yes, I used to, no. of years', 'yes, now, no. of years'); menopause started ('no', 'yes, naturally', 'yes, induced', 'don't know'); if yes, age at onset menopause; twins/multiples: who started menstruating first ('I did', 'my co-multiple/twin sister; triplet/quadruplet: name', 'don't know/n.a.'); twins/multiples: who reached menopause first ('I did', 'my co-multiple/twin sister; triplet/quadruplet: name', 'don't know/n.a.')
W	Education and Occupation	Highest level of education of self, mother, and father, on scale ranging from 1 'elementary school' to 9 'post-graduate degree or PhD degree'; diploma/degree attained (self, mother, and father, 'yes', 'no'); total no. years education after elementary school; current employment status of self and partner ('paid job' + hrs per week, 'volunteer work' + hrs per week, 'school pupil/student', 'housewife/husband' + year at onset, 'unemployed' + year at onset, 'retired' (early) + year at onset, 'disabled' + year at onset, 'other,

		namely...'); satisfaction with own income, answer categories ranging from 1 'dissatisfied' to 5 'satisfied'; satisfaction with family income, answer categories ranging from 1 'dissatisfied' to 5 'satisfied'; level of financial stress experienced in past year ('none/little', 'moderate', 'high'); detailed description of most recent job; main duties/responsibilities of most recent job; self-employed/freelance ('no, employed', 'self-employed: own business/profession', 'in family business without fixed salary'); management of others ('no', 'yes, no. of people'); freedom to set own working hours/days ('none/hardly', 'some', 'considerable', 'set own hours/days')*; frequency of stress at work over past year (scale from 1 'never' to 4 'constantly', 5 'n.a.') *question based on Netherlands Kinship Panel Study (Dykstra et al., 2005)
X	Adult Self Report	123 items, subscales: Internalizing (Anxious/Depressed, Withdrawn, Somatic Complaints), Externalizing (Aggressive Behavior, Rule Breaking Behavior, Intrusive) Thought Problems, Attention problems, answer categories: 'not at all', 'somewhat/sometimes', 'very much so/often' (Achenbach & Rescorla, 2003)
Y	Remarks and comments	

Appendix V Form for incoming phone calls from participants

☐ Telefoongesprek ☐ Email Datum: _____ - _____ - 200__

☐ Aanmelding ☐ Verhuizing Door: _____

☐ Afmelding ☐ Overlijden

Algemene gegevens (*alleen invullen indien gewijzigd / aangevuld*)

Voornamen: _____

Roepnaam: _____

Achternaam: _____

Geslacht: ☐ man ☐ vrouw

Geboortedatum: ____ - ____ - 19__

Panternummer: _____

Adresgegevens

Straat: _____ Huisnr. _____

Postcode en woonplaats: _____

Telefoonnummer: _____

Emailadres: _____

Opmerkingen

_____

_____

_____

_____

_____

_____

Verwerking

☐ Gewijzigd in Panter: ____ - ____ - 200__ initialen: _____

☐ Aanmelding: datum registratiepakket toegestuurd: ____ - ____ - 200__ initialen: _____

☐ Afgehandeld ____ - ____ - 200__ initialen: _____

Appendix VI Reminder by email

Geachte Dhr./Mw. ___,

Onlangs hebben we u uitgenodigd mee te doen aan het vragenlijstonderzoek van het Nederlands Tweelingen Register (NTR). Het onderzoek richt zich op Gezondheid, Leefgewoonten en Persoonlijkheid.

U kunt nog steeds meedoen!
De verzameling van gegevens zal zeker nog geruime tijd doorlopen en we stellen uw deelname zeer op prijs.

U kunt een internetversie van de vragenlijst bereiken door op de volgende link te klikken of deze te typen in het adresbalk van uw internetbrowser:
www.tweelingenregister.org/ANTR8

Uw persoonlijke inloggegevens zijn:
Inlog:
Wachtwoord:

U kunt ook de papieren vragenlijst invullen. Mocht u deze niet hebben ontvangen, bijvoorbeeld omdat u bent verhuisd in de afgelopen jaren, of heeft u de vragenlijst om andere redenen niet meer in uw bezit, neem dan contact op met de onderzoekers Lot Geels of Jenny van Beek via vragenlijstNTR@psy.vu.nl of 020-5985335.

Het is geweldig als u tijd kunt vrijmaken om de vragenlijst in te vullen. Het Nederlands Tweelingen Register biedt met haar onderzoek een unieke bijdrage aan het onderzoek naar geestelijke en lichamelijke gezondheid en in het specifiek hoe genetische invloeden hierbij een rol spelen.

Heel hartelijk dank voor uw deelname!

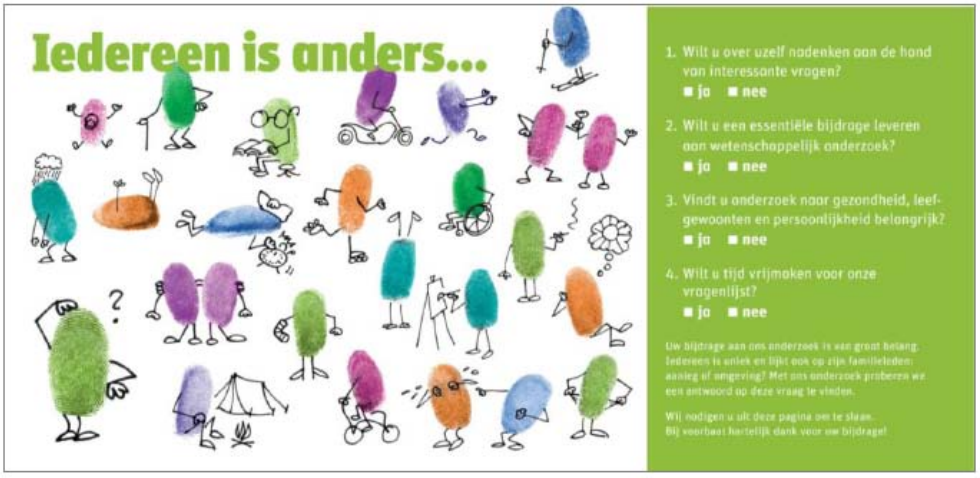
Lot Geels
Jenny van Beek

P.S. Als u de afgelopen tijd bent verhuisd, wilt u dan zo vriendelijk zijn dit aan ons door te geven?

Wij hebben in ons systeem de volgende naam- en adresgegevens van u staan:

Achternaam:
Voorletters:
Voornaam:
Registratienummer:
Adres:

Appendix VII Reminder card



APPENDIX VIII Protocol for reminders by phone

Belactie voor NTR-deelnemers die:
- aan bloedverzamelingsproject hebben deelgenomen
- lijst 8 nog niet hebben ingevuld (en daarvoor een herinneringskaart hebben gekregen)
((- lijst 7 niet hebben ingevuld – dit niet communiceren aan de deelnemers))

Achtergrond Nederlands Tweelingen Register: NTR doet onderzoek bij tweelingfamilies met als doel nagaan in hoeverre genetische invloeden (en in hoeverre omgevingsfactoren) een rol spelen bij aspecten van gezondheid, leefgewoonten en persoonlijkheid. Bij jonge tweelingen richt het onderzoek zich op de ontwikkeling. Hoe meer mensen uit de familie meedoen, des te waardevoller de data. Maar ook de deelname van 1 persoon uit een familie is waardevol (want we doen ook longitudinaal onderzoek + onderzoek naar het krijgen van tweelingen).
Er zijn verschillende routes waarlangs mensen bij het bloedverzamelingsproject terecht kunnen zijn gekomen.

- Meerlingen + hun familieleden die staan ingeschreven bij het volwassen tweelingenregister en eerder hebben meegedaan aan vragenlijstonderzoek.
- Meerlingen + hun familieleden die staan ingeschreven bij het volwassen tweelingenregister en niet eerder hebben meegedaan aan vragenlijstonderzoek.
- Meerlingen + hun familieleden die stonden ingeschreven bij het jong tweelingenregister. Nu de tweeling 18+ is, krijgen zij (meerling + familieleden) voor het eerst een vragenlijst van het volwassen tweelingenregister.
- Moeders + vaders van jonge tweelingen die aan een project naar de erfelijkheid van het krijgen van twee-eiige tweelingen hebben meegedaan

Als beller weet je niet hoe mensen binnenstromen in het onderzoek en aan welk onderzoek mensen hebben meegedaan. Als beller heb je geen toegang tot de database met adresgegevens, maar werk je vanaf een bellijst. Dit in het kader van de privacy.
Als mensen vragen hebben, geef dan aan dat een vaste kracht van het Nederlands Tweelingen Register ze terugbelt. Graag zo precies mogelijk doorgeven waarover het gaat als de persoon dat zelf aangeeft (bijv. over welk project), dan kunnen we de juiste persoon laten terug bellen en gericht

informatie verschaffen.

Uitleg lopend vragenlijstonderzoek 'Familieonderzoek naar Gezondheid, Leefgewoonten en Persoonlijkheid – lijst 8'

- Het betreft vragenlijstonderzoek met vragen over gezondheid, leefgewoonten en persoonlijkheid. De vragenlijst kan via internet worden ingevuld en op papier.
    - Invullen via internet kan via: www.tweelingenregister.org/ANTR8 Op de herinneringskaart die ze begin dec hebben gekregen staan persoonlijke inloggegevens.
    - Als ze liever een papieren vragenlijst ontvangen, kunnen ze dat aan jou doorgeven.

Protocol reminders phone (continued)

- In januari 2009 zijn per brief uitnodigingen verstuurd om de vragenlijst online in te vullen. Als we niet het juiste adres hadden, hebben zij de uitnodiging later ontvangen.
- In februari zijn papieren vragenlijsten verstuurd. Als we niet het juiste adres hadden, hebben zij de uitnodiging later ontvangen.
- Mogelijkerwijs hebben mensen waarvan we een e-mailadres hebben, per email een herinnering gehad om de vragenlijst in te vullen.
- Mogelijkerwijs hebben mensen zelf contact met ons opgenomen nav de herinneringskaart.

Belprotocol

1. Goedemiddag/ goedenavond met ... van het Nederlands Tweelingen Register, komt het gelegen dat ik bel?
2. Vragen naar de persoon uit de bellijst.
3. Als het goed is hebt u begin december kaart ontvangen van het Nederlands Tweelingen Register om via internet vragenlijst in te vullen. Klopt dit?
4. Dataverzameling loopt nog enkele jaren door. We zouden het heel erg op prijs stellen als u de vragenlijst nog zou willen invullen. U hebt eerder aan het bloedverzamelingsproject van het Nederlands Tweelingen Register meegedaan. Daarom is het voor ons extra waardevol als u de vragenlijst zou willen invullen.
5. Als u wilt, kan ik u ook een papieren vragenlijst toe laten sturen.
6. Hartelijk bedanken voor de tijd en moeite.

Mogelijke reacties van mensen.

1. Herinneringskaart niet ontvangen.
    a. Adresgegevens checken + emailadres vragen.
    b. Liever op papier invullen of online?
    c. Als ze vragenlijst online willen invullen, dan sturen wij de inloggegevens per mail op.
    d. Als ze vragenlijst op papier willen invullen, dan sturen wij de vragenlijst op per reguliere post.

    Niet aanbieden om herinneringskaart opnieuw op te sturen.

    Als adresgegevens zijn gewijzigd, navragen of ze wel de eerste uitnodigingen hebben gekregen (in jan + feb).
2. Heb de vragenlijst (lijst 8) al ingevuld.
    a. Bedanken voor het invullen.
    b. Als ze wel perse willen dat we hen laten weten waarom ze de herinneringskaart hebben gekregen, aangeven dat het geruime tijd kan duren voordat ze worden teruggebeld (misschien moeten we bijv wachten op de ingescande vragenlijsten voordat we het kunnen uitzoeken).

3. Geen tijd.
    a. Dataverzameling loopt nog steeds door

Protocol reminders phone (continued)

    b. Hun vragenlijstgegevens zijn extra waardevol voor ons, omdat ze aan bloedverzamelingsproject hebben meegedaan
    c. Vragenlijst hoeft niet in één keer ingevuld te worden

4. Te persoonlijke vragen.
    a. Gegevens worden zorgvuldig beheerd. Komen niet in handen van derden.
    b. Vragenlijstgegevens worden onder een nummer opgeslagen en zijn dus niet gekoppeld aan naam- en adresgegevens.
    c. Vragen die men niet wil invullen, kan men overslaan.

5. Heb de vragen al een keer beantwoord in een vorige vragenlijst.
    a. Longitudinale dataverzameling maakt juist gegevens extra waardevol. Informatie over hoe mensen veranderen over de tijd.
    b. Lijst 8 bevat naast longitudinale vragen ook een groot aantal nieuwe vragen.

6. Wil niet meedoen
    a. Vragen of we hem/haar over een paar jaar weer eens mogen benaderen voor onderzoek. We zullen hen dan niet meer benaderen voor dit onderzoek, maar wel weer over een paar jaar bij een nieuw onderzoek.
    b. Het is mogelijk dat mensen helemaal geen post meer van ons willen ontvangen. Dan zullen we hen ook niet meer benaderen voor een volgend onderzoek (aangezien we natuurlijk geen mensen willen kwijtraken, is dit geen optie waar je zelf over begint). Als mensen helemaal niet meer benaderd willen worden voor onderzoek, vraag dan of ze nog prijsstellen op ons jaarlijkse informatiemagazine. De versturing daarvan staat namelijk los van de versturing van de uitnodigingen voor het onderzoek.

7. Als mensen vragen hebben die je niet kunt beantwoorden, zeggen dat je het na zult vragen en ze erover terug zult bellen.

8. Hebben nog nooit meegedaan aan vragenlijstonderzoek

    a. Deelnemers kunnen op verschillende manieren in het onderzoek ingestroomd zijn. Het is mogelijk dat ze wel hebben meegedaan aan het bloedverzamelingsproject maar niet eerder aan vragenlijstonderzoek.

Bijhouden in belfile:
-Datum gebeld (3x proberen) + resultaat: geen gehoor, familielid/huisgenoot gesproken, persoon zelf gesproken.
-Actie: per email inloggegevens opsturen (1); papieren lijst sturen (2); wil niet meer benaderd worden voor lijst 8 (3)
-Wijzigingen in panter: nieuwe adresgegevens; nieuw emailadres/ tel nr; andere aanhef; overleden familielid etc.
- Vraag: is er een vraag die beantwoord moet worden etc.
-Opmerkingen: overige info.
-Toezegging om lijst 8 nog in te vullen? Ja / nee

Appendix IX Example letter responding to participants'comment/question

Nederlands Tweelingenregister (NTR)

Datum	Uw contactpersonen	Telefoon	Telefax	Bijlagen
Juli 2011	Lot Geels Jenny van Beek	020-5985335	020-5988832	

Ons kenmerk	E- mail	Website
Lijst 8	vragenlijstNTR@psy.vu.nl	www.tweelingenregister.org/ANTR8

Postadres: Van der Boechorststraat 1, 1081 BT Amsterdam

vrije* Universiteit *amsterdam

Geachte mevrouw ,

Allereerst wil ik u graag heel hartelijk bedanken voor het invullen van de vragenlijst van het Nederlands Tweelingen Register (NTR). Aan het eind van deze vragenlijst konden deelnemers vragen en opmerkingen kwijt. U hebt hier aangegeven dat uw vader kort geleden is overleden. Hierbij wil ik graag onze oprechte deelneming betuigen voor dit verlies en u laten weten hoe geweldig ik het vind, dat u toch de moeite hebt genomen mee te doen aan het NTR onderzoek.

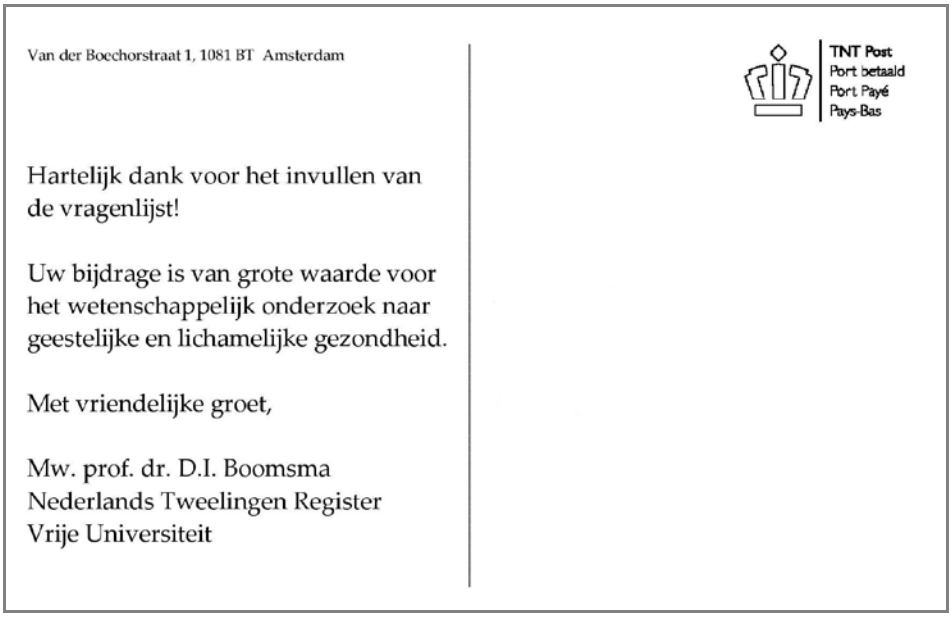
Met vriendelijke groet, namens alle NTR medewerkers.

Hoogachtend,

Dorret Boomsma

Mw. prof. dr. D.I. Boomsma
Vrije Universiteit, Amsterdam
Afdeling Biologische Psychologie

Appendix X Survey 8 thank you card



List of publications

List of publications

Articles

Geels, LM, Groen-Blokhuis, MM, van Beijsterveldt, CEM, Vink, JM, Middeldorp, CM, Bartels, M, Nelson, KA, Huizenga, PE, Davies, GE, Boomsma, DI. Maternal prenatal smoking and offspring emotional problems: No moderating effect of maternal or child 5-HTTLPR genotype. American Journal of Medical Genetics part B: Neuropsychiatric Genetics, 2012 October 22 [epub].

Abdellaoui, A., de Moor, M. H., **Geels, L. M.**, van Beek, J. H., Willemsen, G., & Boomsma, D. I. (2012). Thought problems from adolescence to adulthood: measurement invariance and longitudinal heritability. *Behavior Genetics, 42*(1), 19-29.

Beek van, J. H., Kendler, K. S., de Moor, M. H., **Geels, L. M.**, Bartels, M., Vink, J. M., et al. (2012). Stable genetic effects on symptoms of alcohol abuse and dependence from adolescence into early adulthood. *Behavior Genetics, 42*(1), 40-56.

Distel, M. A., Trull, T. J., de Moor, M. M., Vink, J. M., **Geels, L. M.**, van Beek, J. H., et al. (2012). Borderline Personality Traits and Substance Use: Genetic Factors Underlie the Association with Smoking and Ever Use of Cannabis, but Not with High Alcohol Consumption. *J Pers Disord*. Epub.

Geels, L. M., Bartels, M., van Beijsterveldt, T. C. E. M., Willemsen, G., van der Aa, N., Boomsma, D. I., et al. (2012). Trends in adolescent alcohol use: effects of age, sex and cohort on prevalence and heritability. *Addiction, 107*(3), 518-527.

Vink, J. M., Bartels, M., van Beijsterveldt, T. C. E. M., van Dongen, J., van Beek, J. H. D. A., Distel, M. A., de Moor, M. H. M., Smit, D. J. A., Minica, C. C., Ligthart, R. S. L., **Geels, L. M.**, Abdellaoui, A., Middeldorp, C. M., Hottenga, J. J., Willemsen, G., de Geus, E. J. C., & Boomsma, D. I. Sex differences in genetic architecture of complex phenotypes? *PLOS One, in press*.

Willemsen, G., Vink, J. M., Abdellaoui, A., Den Braber, A., van Beek, J. H. D. A., Draisma, H. H. M., van Dongen, J., van 't End, D., **Geels, L. M.**, van Lien, R., Ligthart, R. S. L., Kattenberg, M. V., Mbarek, H., de Moor, M. H. M., Neijts, M., Pool, R., Stroo, N., Kluft, K., Suchiman, H. E. D., Slagboom, E. P., de Geus, E. J. C., & Boomsma, D. I. The Adult Netherlands Twin Register: 25 years of survey and biological data collection. *Twin Research and Human Genetics, in press*.

de Moor, M. H., Vink, J. M., van Beek, J. H., **Geels, L. M.**, Bartels, M., de Geus, E. J., et al. (2011). Heritability of problem drinking and the genetic overlap with personality in a general population sample. *Front Genet, 2*, 76.

Boomsma, D. I., Saviouk, V., Hottenga, J. J., Distel, M. A., de Moor, M. H., Vink, J. M., **Geels, L. M.**, van Beek, J. H. D. A., Bartels, M., de Geus, E. J. C., & Willemsen, G. (2010). Genetic epidemiology of attention deficit hyperactivity disorder (ADHD index) in adults. *PLoS One, 5*(5), e10621.

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References

Achenbach, T. M. (1992). *Manual for the Child Behavior Checklist/2-3 and 1992 Profile*. Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M., & Rescorla, L. A. (2000). *Manual for the ASEBA Preschool Forms & Profiles*. Burlington, VT: University of Vermont; Research Center for Children Youth & Families.

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA School-Age Forms & Profiles*. Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M., & Rescorla, L. A. (2003). *Manual for the ASEBA Adult Forms & Profiles*. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.

Agrawal, A., Balasubramanian, S., Smith, E. K., Madden, P. A. F., Bucholz, K. K., Heath, A. C., et al. (2010a). Peer substance involvement modifies genetic influences on regular substance involvement in young women. *Addiction, 105*(10), 1844-1853.

Agrawal, A., Sartor, C. E., Lynskey, M. T., Grant, J. D., Pergadia, M. L., Grucza, R., et al. (2009). Evidence for an Interaction Between Age at First Drink and Genetic Influences on DSM-IV Alcohol Dependence Symptoms. *Alcoholism-Clinical and Experimental Research, 33*(12), 2047-2056.

Agrawal, A., Scherrer, J. F., Grant, J. D., Sartor, C. E., Pergadia, M. L., Duncan, A. E., et al. (2010b). The effects of maternal smoking during pregnancy on offspring outcomes. *Preventive Medicine, 50*(1-2), 13-18.

Agrawal, A., Verweij, K. J., Gillespie, N. A., Heath, A. C., Lessov-Schlaggar, C. N., Martin, N. G., et al. (2012). The genetics of addiction-a translational perspective. *Translational Psychiatry, 2*, e140.

Ait-Daoud, N., Roache, J. D., Dawes, M. A., Liu, L., Wang, X.-Q., Javors, M. A., et al. (2009). Can Serotonin Transporter Genotype Predict Craving in Alcoholism? *Alcoholism: Clinical and Experimental Research, 33*(8), 1329-1335.

Ait-Daoud, N., Seneviratne, C., Smith, J. B., Roache, J. D., Dawes, M. A., Liu, L., et al. (2012). Preliminary Evidence for cue-induced Alcohol Craving Modulated by Serotonin Transporter Gene Polymorphism rs1042173. *Frontiers in Psychiatry, 3*, 6.

Akaike, H. (1987). Factor-Analysis and Aic. *Psychometrika, 52*(3), 317-332.

Alati, R., Al Mamun, A., Williams, G. M., O'Callaghan, M., Najman, J. M., & Bor, W. (2006). In utero alcohol exposure and prediction of alcohol disorders in early adulthood - A birth cohort study. *Archives of General Psychiatry, 63*(9), 1009-1016.

Alati, R., Macleod, J., Hickman, M., Sayal, K., May, M., Smith, G. D., et al. (2008). Intrauterine exposure to alcohol and tobacco use and childhood IQ: findings from a parental-offspring comparison within the Avon Longitudinal Study of Parents and Children. *Pediatric Research, 64*(6), 659-666.

Aldridge-Gerry, A. A., Roesch, S. C., Villodas, F., McCabe, C., Leung, Q. K., & Da Costa, M. (2011). Daily stress and alcohol consumption: modeling between-person and within-person ethnic variation in coping behavior. *Journal of Studies on Alcohol and Drugs, 72*(1), 125-134.

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)* (4th ed.). Washington, D.C.: APA.

Anderson, K. G., & Brown, S. A. (2011). Middle School Drinking: Who, Where, and When. *Journal of Child & Adolescent Substance Abuse, 20*(1), 48-62.

Anderson, K. G., Tomlinson, K., Robinson, J. M., & Brown, S. A. (2011). Friends or Foes: Social Anxiety, Peer Affiliation, and Drinking in Middle School. *Journal of Studies on Alcohol and Drugs, 72*(1), 61-69.

Anderson, P., de Bruijn, A., Angus, K., Gordon, R., & Hastings, G. (2009). Impact of alcohol advertising and media exposure on adolescent alcohol use: a systematic review of longitudinal studies. *Alcohol and Alcoholism, 44*(3), 229-243.

Anderson, P., Møller, L., & Galea, G. (2012). Alcohol in the European Union: Consumption, harm and policy approaches: WHO Regional office for Europe.

Arts, K. (2009). Ontwikkelingen in kerkelijkheid en kerkbezoek (1999-2008) [Developments in religiousness and churchgoing (1999-2008)] *Religie aan het begin van de 21ste eeuw [Religion at the beginning of the 21st century]*. Den Haag/Heerlen, the Netherlands: Statistics Netherlands

Ashford, J., van Lier, P. A. C., Timmermans, M., Cuijpers, P., & Koot, H. M. (2008). Prenatal smoking and internalizing and externalizing problems

in children studied from childhood to late adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry, 47*(7), 779-787.

Baba, S., Wikström, A.-K., Stephansson, O., & Cnattingius, S. (2012). Influence of smoking and snuff cessation on risk of preterm birth. *European Journal of Epidemiology, 27*(4), 297-304.

Babor, T. F., Higgins-Biddle, J. C., Saunders, J. B., & Monteiro, M. G. (2001). *AUDIT: The Alcohol Use Disorders Identification Test - Guidelines for Use in Primary Care* (2 ed.). Geneva, Switzerland: World Health Organization, Department of Mental Health and Substance Dependence.

Bartels, M., Boomsma, D. I., Hudziak, J. J., van Beijsterveldt, T. C. E. M., & van den Oord, E. J. C. G. (2007a). Twins and the study of rater (dis) agreement. *Psychological Methods, 12*(4), 451-466.

Bartels, M., van Beijsterveldt, C. E. M., Derks, E. M., Stroet, T. M., Polderman, T. J. C., Hudziak, J. J., et al. (2007b). Young Netherlands Twin Register (Y-NTR): A longitudinal multiple informant study of problem behavior. *Twin Research and Human Genetics, 10*(1), 3-11.

Bartels, M., van der Aa, N., van Beijsterveldt, C. E. M., Middeldorp, C. M., & Boomsma, D. I. (2011). Adolescent Self-Report of Emotional and Behavioral Problems; Interactions of Genetic Factors with Sex and Age. *Journal of the Canadian Academy of Child and Adolescent Psychiatry, 20*(1), 35-52.

Bava, S., & Tapert, S. F. (2010). Adolescent Brain Development and the Risk for Alcohol and Other Drug Problems. *Neuropsychology Review, 20*(4), 398-413.

Bergen, S. E., Gardner, C. O., & Kendler, K. S. (2007). Age-related changes in heritability of behavioral phenotypes over adolescence and young adulthood: A meta-analysis. *Twin Research and Human Genetics, 10*(3), 423-433.

Boden, J. M., & Fergusson, D. M. (2011). Alcohol and depression. *Addiction, 106*(5), 906-914.

Boelens, A., & Sinkeldam, I. (2000, 2000/09//). Werk om te leren - bijbaantjes vooral op lager beroepsniveau Retrieved 2011/07/14/, from http://www.cbs.nl/NR/rdonlyres/D93DBE53-71E1-45BA-B2AA-2E9D66D93A96/0/index1085.pdf
http://www.webcitation.org/60Abf2C0G

Boomsma, D. I., de Geus, E. J. C., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: From twins to twin families. *Twin Research and Human Genetics, 9*(6), 849-857.

Boomsma, D. I., Koopmans, J. R., Van Doornen, L. J. P., & Orlebeke, J. F. (1994). Genetic and social influences on starting to smoke: a study of Dutch adolescent twins and their parents. *Addiction, 89*(2), 219-226.

Boomsma, D. I., Martin, N. G., D'Haenen, H., den Boer, J. A., & Willner, P. (2002a). Gene-Environment Interactions *Biological Psychiatry* (pp. 181-187): John Wiley & Sons.

Boomsma, D. I., Vink, J. M., van Beijsterveldt, T. C., de Geus, E. J., Beem, A. L., Mulder, E. J., et al. (2002b). Netherlands Twin Register: a focus on longitudinal research. *Twin Research and Human Genetics, 5*(5), 401-406.

Boschloo, L., Vogelzangs, N., Smit, J. H., van den Brink, W., Veltman, D. J., Beekman, A. T., et al. (2010). The performance of the Alcohol Use Disorder Identification Test (AUDIT) in detecting alcohol abuse and dependence in a population of depressed or anxious persons. *Journal of Affective Disorders, 126*(3), 441-446.

Boutwell, B. B., Beaver, K. M., Gibson, C. L., & Ward, J. T. (2011). Prenatal exposure to cigarette smoke and childhood externalizing behavioral problems: a propensity score matching approach. *International Journal of Environmental Health Research, 21*(4), 248-259.

Brennan, P. A., Hammen, C., Sylvers, P., Bor, W., Najman, J., Lind, P., et al. (2011). Interactions between the COMT Val108/158Met polymorphism and maternal prenatal smoking predict aggressive behavior outcomes. *Biological Psychology, 87*(1), 99-105.

Breslow, R. A., & Smothers, B. A. (2005). Drinking Patterns and Body Mass Index in Never Smokers. *American Journal of Epidemiology, 161*(4), 368-376.

Brion, M. J., Victora, C., Matijasevich, A., Horta, B., Anselmi, L., Steer, C., et al. (2010). Maternal smoking and child psychological problems: disentangling causal and noncausal effects. *Pediatrics*, e57-e65.

Broadhead, W. E., Gehlbach, S. H., de Gruy, F. V., & Kaplan, B. H. (1988). The Duke-UNC Functional Social Support Questionnaire. Measurement of social support in family medicine patients. *Medical Care, 26*(7), 709-723.

Brook, D. W., Zhang, C., Rosenberg, G., & Brook, J. S. (2006). Maternal cigarette smoking during pregnancy and child aggressive behavior. *American Journal on Addictions, 15*(6), 450-456.

Buchmann, A. F., Schmid, B., Blomeyer, D., Becker, K., Treutlein, J., Zimmermann, U. S., et al. (2009). Impact of age at first drink on vulnerability to alcohol-related problems: Testing the marker hypothesis in a prospective study of young adults. *Journal of Psychiatric Research, 43*(15), 1205-1212.

Button, T. M. M., Hewitt, J. K., Rhee, S. H., Corley, R. P., & Stallings, M. C. (2010). The Moderating Effect of Religiosity on the Genetic Variance of Problem Alcohol Use. *Alcoholism-Clinical and Experimental Research, 34*(9), 1619-1624.

Cantril, H. (1965). *The pattern of human concerns*. New Brunswick, NJ: Rutgers University Press.

Caspi, A., Hariri, A. R., Holmes, A., Uher, R., & Moffitt, T. E. (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *American Journal of Psychiatry, 167*(5), 509-527.

Cents, R. A. M., Tiemeier, H., Velders, F. P., Jaddoe, V. W. V., Hofman, A., Verhulst, F. C., et al. (2012). Maternal smoking during pregnancy and child emotional problems: The relevance of maternal and child 5-HTTLPR genotype. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 159B*(3), 289-297.

Chen, H., Cohen, P., & Chen, S. (2010). How Big is a Big Odds Ratio? Interpreting the Magnitudes of Odds Ratios in Epidemiological Studies. [doi: 10.1080/03610911003650383]. *Communications in Statistics - Simulation and Computation, 39*(4), 860-864.

Chun, T. H., & Linakis, J. G. (2012). Interventions for adolescent alcohol use. *Current Opinion in Pediatrics, 24*(2), 238-242.

Cnattingius, S. (2004). The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine & Tobacco Research, 6 Suppl 2*, S125-140.

Connors, C. K., Erhardt, D., & Sparrow, E. (1999). *Conners' Adult ADHD Rating Scales (CAARS)*. North Tonawanda, NY: Multi-Health systems Inc.

Cornelius, M. D., & Day, N. L. (2009). Developmental consequences of prenatal tobacco exposure. *Current Opinion in Neurology, 22*(2), 121-125.

Cornelius, M. D., De Genna, N. M., Leech, S. L., Willford, J. A., Goldschmidt, L., & Day, N. L. (2011). Effects of prenatal cigarette smoke exposure on neurobehavioral outcomes in 10-year-old children of adolescent mothers. *Neurotoxicology and Teratology, 33*(1), 137-144.

Costa Jr, P. T., & McCrae, R. R. (1992). *Revised NEO Personality Inventory (NEO PI-R) and NEO Five-Factor Inventory (NEO-FFI) Professional Manual*. Odessa, FL: Psychological Assessment Resources, Inc.

D'Onofrio, B. M., Hulle, C. A., Waldman, I. D., Rodgers, J. L., Harden, K. P., Rathouz, P. J., et al. (2008). Smoking during pregnancy and offspring externalizing problems: An exploration of genetic and environmental confounds. *Development and Psychopathology, 20*(01), 139-164.

D'Onofrio, B. M., Van Hulle, C. A., Waldman, I. D., Rodgers, J. L., Rathouz, P. J., & Lahey, B. B. (2007). Causal inferences regarding prenatal alcohol exposure and childhood externalizing problems. *Archives of General Psychiatry, 64*(11), 1296-1304.

Dawson, D. A., Goldstein, R. B., Chou, S. P., Ruan, W. J., & Grant, B. F. (2008). Age at first drink and the first incidence of adult-onset DSM-IV alcohol use disorders. *Alcoholism: Clinical and Experimental Research, 32*(12), 2149-2160.

De Coole, R. L., & Jansma, J. B. M. (1983). *G.K.S. Gezinsklimaatschaal Handleiding*. Lisse: Swets & Zeitlinger.

de Moor, M. H. M. (2009). *Exercise Behavior and Mental Health - A Genetic perspective*. Amsterdam, the Netherlands: VU University Amsterdam.

Dear, K., Henderson, S., & Korten, A. (2002). Well-being in Australia--findings from the National Survey of Mental Health and Well-being. *Social Psychiatry and Psychiatric Epidemiology, 37*(11), 503-509.

Dick, D. M., Aliev, F., Krueger, R. F., Edwards, A., Agrawal, A., Lynskey, M., et al. (2010). Genome-wide association study of conduct disorder symptomatology. *Molecular Psychiatry*.

Dick, D. M., Bierut, L., Hinrichs, A., Fox, L., Bucholz, K. K., Kramer, J., et al. (2006). The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behavior Genetics, 36*(4), 577-590.

Dick, D. M., Meyers, J. L., Latendresse, S. J., Creemers, H. E., Lansford, J. E., Pettit, G. S., et al. (2011). CHRM2, parental monitoring, and adolescent externalizing behavior: evidence for gene-environment interaction. *Psychological Science, 22*(4), 481-489.

Dick, D. M., Pagan, J. L., Viken, R., Purcell, S., Kaprio, J., Pulkkinen, L., et al. (2007a). Changing environmental influences on substance use across development. *Twin Research and Human Genetics, 10*(2), 315-326.

Dick, D. M., Prescott, C. A., McGue, M., & Kim, Y. K. (2009). The Genetics of Substance Use and Substance Use Disorders *Handbook of Behavior Genetics* (Vol. 1st, pp. 433-453). New York, NY: Springer Science+Business Media, LLC.

Dick, D. M., Viken, R., Purcell, S., Kaprio, J., Pulkkinen, L., & Rose, R. J. (2007b). Parental monitoring moderates the importance of genetic and environmental influences on adolescent smoking. *Journal of Abnormal Psychology, 116*(1), 213-218.

Diener, E., Emmons, R. A., Larsen, R. J., & Griffin, S. (1985). The Satisfaction with Life Scale. *Journal of Personality Assessment, 49*(1), 71-75.

Disney, E. R., Iacono, W., McGue, M., Tully, E., & Legrand, L. (2008). Strengthening the Case: Prenatal Alcohol Exposure Is Associated With Increased Risk for Conduct Disorder. *Pediatrics, 122*(6), E1225-E1230.

Donath, C., Grassel, E., Baier, D., Pfeiffer, C., Karagulle, D., Bleich, S., et al. (2011). Alcohol consumption and binge drinking in adolescents: comparison of different migration backgrounds and rural vs. urban residence-a representative study. *Bmc Public Health, 11*, 84.

Donovan, J. E. (2004). Adolescent alcohol initiation: a review of psychosocial risk factors. *Journal of Adolescent Health, 35*(6), 529 e527-518.

Donovan, J. E., & Molina, B. S. (2011). Childhood risk factors for early-onset drinking. *Journal of Studies on Alcohol and Drugs, 72*(5), 741-751.

Drerup, M. L., Johnson, T. J., & Bindl, S. (2011). Mediators of the relationship between religiousness/spirituality and alcohol problems in an adult community sample. [doi: 10.1016/j.addbeh.2011.07.013]. *Addictive Behaviors, 36*(12), 1317-1320.

Duffy, D. L. (2012). Sib-pair: A program for simple genetic analysis, version 1.0.0: User Manual. Herston, Australia: Queensland Institute of Medical Research.

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal of Psychiatry, 168*(10), 1041-1049.

Dutch Centre for Crime Prevention and Safety. (2010). www.hetccv.nl.

Dutch Institute for Alcohol Policy (STAP). (2008, 2008/05/09/). Selection supermarket stimulates alcohol use Retrieved 2011/07/14/, from Archived at http://www.webcitation.org/60AYuM6Fc
http://www.stap.nl/nl/nieuws/persberichten.html/3490/744/aanbod-supermarkt-stimuleert-alcoholmisbruik#p3490

Dutch Institute for Alcohol Policy (STAP). (2009). Feiten en cijfers [Facts and figures], from http://www.stap.nl/nl/home/feiten-en-cijfers.html

Dwyer, J. B., McQuown, S. C., & Leslie, F. M. (2009). The dynamic effects of nicotine on the developing brain. *Pharmacology & Therapeutics, 122*(2), 125-139.

Dykstra, P. A., Kalmijn, M., Knijn, T. C. M., Komter, A. E., Liefbroer, A. C., & Mulder, C. H. (2005). *Codebook of the Netherlands Kinship Panel Study, Wave 1* (Vol. No. 4). The Hague: Netherlands Interdisciplinary Demographic Institute.

Eaves, L. J. (1984). The resolution of genotype x environment interaction in segregation analysis of nuclear families. *Genetic Epidemiology, 1*(3), 215-228.

Eaves, L. J. (1987). Including the environment in models for genetic segregation. *J Psychiatr Res, 21*(4), 639-647.

Eaves, L. J. (2006). Genotype Environment Interaction in Psychopathology: Fact or Artifact? [doi:10.1375/183242706776403073]. *Twin Research and Human Genetics, 9*(1), 1-8.

Edwards, A. C., Maes, H. H., Pedersen, N. L., & Kendler, K. S. (2011). A population-based twin study of the genetic and environmental relationship of major depression, regular tobacco use and nicotine dependence. *Psychological Medicine, 41*(2), 395-405.

Edwards, A. C., Sihvola, E., Korhonen, T., Pulkkinen, L., Moilanen, I., Kaprio, J., et al. (2010). Depressive Symptoms and Alcohol Use are Genetically and Environmentally Correlated Across Adolescence. *Behavior Genetics, 41*(4), 476-487.

Ehli, E. A., Abdellaoui, A., Hu, Y., Hottenga, J. J., Kattenberg, M., van Beijsterveldt, T., et al. (2012). De novo and inherited CNVs in MZ twin pairs selected for discordance and concordance on Attention Problems. *Eur J Hum Genet*.

Ekblad, M., Gissler, M., Lehtonen, L., & Korkeila, J. (2010). Prenatal smoking exposure and the risk of psychiatric morbidity into young adulthood. *Archives of General Psychiatry, 67*(8), 841-849.

Enoch, M. A. (2012). The influence of gene-environment interactions on the development of alcoholism and drug dependence. *Curr Psychiatry Rep, 14*(2), 150-158.

Epstein, N. B., Baldwin, L. M., & Bishop, D. S. (1983). The Mcmaster Family Assessment Device. *Journal of Marital and Family Therapy, 9*(2), 171-180.

Erikson, R., Goldthorpe, J. H., & Portocarero, L. (1979). Intergenerational Class Mobility in Three Western European Societies: England, France and Sweden. *The British Journal of Sociology, 30*(4), 415-441.

Ernst, M., Moolchan, E. T., & Robinson, M. L. (2001). Behavioral and neural consequences of prenatal exposure to nicotine. *Journal of the American Academy of Child and Adolescent Psychiatry, 40*(6), 630-641.

European Commission. (2010). EU citizen's attitudes towards alcohol *Special Eurobarometer* (Vol. 72.3). Brussels.

Everett, K. D., Bullock, L., Longo, D. R., Gage, J., & Madsen, R. (2007). Men's tobacco and alcohol use during and after pregnancy. *American Journal of Men's Health, 1*(4), 317-325.

Ewing, J. A. (1984). Detecting alcoholism. *JAMA: The Journal of the American Medical Association, 252*(14), 1905-1907.

Falconer, D. S., & Mackay, T. F. C. (1996). *Quantitative Genetics*. Essex: Longman Group Ltd.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods, 41*(4), 1149-1160.

Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods, 39*(2), 175-191.

Field, A. (2009). *Discovering Statistics Using SPSS* (3 ed.). London: Sage Publications.

Fisher, L. B., Miles, I. W., Austin, S. B., Camargo, C. A., Jr., & Colditz, G. A. (2007). Predictors of initiation of alcohol use among US adolescents: findings from a prospective cohort study. *Archives of Pediatrics & Adolescent Medicine, 161*(10), 959-966.

Fleming, C. B., White, H. R., Oesterle, S., Haggerty, K. P., & Catalano, R. F. (2010). Romantic relationship status changes and substance use among 18- to 20-year-olds. *Journal of Studies on Alcohol and Drugs, 71*(6), 847-856.

Fowler, T., Lifford, K., Shelton, K., Rice, F., Thapar, A., Neale, M. C., et al. (2007). Exploring the relationship between genetic and environmental influences on initiation and progression of substance use. *Addiction, 102*(3), 413-422.

Freathy, R. M., Ring, S. M., Shields, B., Galobardes, B., Knight, B., Weedon, M. N., et al. (2009). A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5–CHRNA3–CHRNB4) is associated with a reduced ability of women to quit smoking in pregnancy. *Human Molecular Genetics, 18*(15), 2922-2927.

Frost, R. O., Tolin, D. F., Steketee, G., Fitch, K. E., & Selbo-Bruns, A. (2009). Excessive acquisition in hoarding. [doi: 10.1016/j.janxdis.2009.01.013]. *Journal of Anxiety Disorders, 23*(5), 632-639.

Garmiene, A., Zemaitiene, N., & Zaborskis, A. (2006). Family time, parental behaviour model and the initiation of smoking and alcohol use by ten-year-old children: an epidemiological study in Kaunas, Lithuania. *Bmc Public Health, 6*, 287.

Garwood, F. (1941). The Application of Maximum Likelihood to Dosage-Mortality Curves. *Biometrika, 32*(1), 46-58.

Geels, L. M., Bartels, M., van Beijsterveldt, T. C. E. M., Willemsen, G., van der Aa, N., Boomsma, D. I., et al. (2012). Trends in adolescent alcohol use: effects of age, sex and cohort on prevalence and heritability. *Addiction, 107*(3), 518-527.

Gezondheidsraad. (2006). Richtlijnen goede voeding 2006 (pp. 90). Den Haag.

Goldschmidt, L., Cornelius, M. D., & Day, N. L. (2011). Prenatal Cigarette Smoke Exposure and Early Initiation of Multiple Substance Use.

Nicotine & Tobacco Research. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22180590 doi:10.1093/ntr/ntr280

Grant, J. D., Scherrer, J. F., Lynskey, M. T., Lyons, M. J., Eisen, S. A., Tsuang, M. T., et al. (2006). Adolescent alcohol use is a risk factor for adult alcohol and drug dependence: evidence from a twin design. *Psychological Medicine, 36*(1), 109-118.

Grucza, R. A., Norberg, K. E., & Bierut, L. J. (2009). Binge drinking among youths and young adults in the United States: 1979-2006. *J Am Acad Child Adolesc Psychiatry, 48*(7), 692-702.

Guerri, C., & Pascual, M. (2010). Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol, 44*(1), 15-26.

Guo, G., Elder, G. H., Cai, T. J., & Hamilton, N. (2009). Gene-environment interactions: Peers' alcohol use moderates genetic contribution to adolescent drinking behavior. *Social Science Research, 38*(1), 213-224.

Guttmannova, K., Bailey, J. A., Hill, K. G., Lee, J. O., Hawkins, J. D., Woods, M. L., et al. (2011). Sensitive periods for adolescent alcohol use initiation: predicting the lifetime occurrence and chronicity of alcohol problems in adulthood. *Journal of Studies on Alcohol and Drugs, 72*(2), 221-231.

Han, C., McGue, M. K., & Iacono, W. G. (1999). Lifetime tobacco, alcohol and other substance use in adolescent Minnesota twins: univariate and multivariate behavioral genetic analyses. *Addiction, 94*(7), 981-993.

Hanewinkel, R., Sargent, J. D., Poelen, E. A., Scholte, R., Florek, E., Sweeting, H., et al. (2012). Alcohol consumption in movies and adolescent binge drinking in 6 European countries. *Pediatrics, 129*(4), 709-720.

Hayes, B., & Sharif, F. (2009). Behavioural and emotional outcome of very low birth weight infants – literature review. *Journal of Maternal-Fetal and Neonatal Medicine, 22*(10), 849-856.

Health Council of the Netherlands. (2006). Guidelines for a healthy diet 2006 (pp. 90). The Hague, the Netherlands.

Heath, A. C., & Martin, N. G. (1988). Teenage Alcohol-Use in the Australian Twin Register - Genetic and Social Determinants of Starting to Drink. *Alcoholism-Clinical and Experimental Research, 12*(6), 735-741.

Heath, A. C., Meyer, J., Eaves, L. J., & Martin, N. G. (1991). The Inheritance of Alcohol-Consumption Patterns in A General-Population Twin Sample .1. Multidimensional-Scaling of Quantity Frequency Data. *Journal of Studies on Alcohol, 52*(4), 345-352.

Heatherton, T. F., Kozlowski, L. T., & Frecker, R. C. (1991). The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom tolerance questionnaire. *Br J Addict, 86*(9), 1119-1127.

Hellström-Lindahl, E. (2000). Nicotinic acetylcholine receptors during prenatal development and brain pathology in human aging. *Behavioural Brain Research, 113*(1-2), 159-168.

Hemsing, N., Greaves, L., O'Leary, R., Chan, K., & Okoli, C. (2012). Partner support for smoking cessation during pregnancy: a systematic review. *Nicotine Tob Res, 14*(7), 767-776.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., et al. (2009). The 2007 ESPAD Report - Substance Use Among Students in 35 European Countries Stockholm: The European School Survey Project on Alcohol and Other Drugs.

Hicks, B. M., Schalet, B. D., Malone, S. M., Iacono, W. G., & McGue, M. (2011). Psychometric and Genetic Architecture of Substance Use Disorder and Behavioral Disinhibition Measures for Gene Association Studies. *Behavior Genetics, 41*(4), 459-475.

Hill, J., Emery, R., Harden, K., Mendle, J., & Turkheimer, E. (2008). Alcohol Use in Adolescent Twins and Affiliation with Substance Using Peers. *Journal of Abnormal Child Psychology, 36*(1), 81-94.

Homish, G. G., Eiden, R. D., Leonard, K. E., & Kozlowski, L. T. (2012). Social-environmental factors related to prenatal smoking. *Addictive Behaviors, 37*(1), 73-77.

Hopfer, C. J., Crowley, T. J., & Hewitt, J. K. (2003). Review of twin and adoption studies of adolescent substance use. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(6), 710-719.

Hopper, J. L., White, V. M., Macaskill, G. T., Hill, D. J., & Clifford, C. A. (1992). Alcohol-Use, Smoking-Habits and the Junior Eysenck Personality Questionnaire in Adolescent Australian Twins. *Acta Geneticae Medicae et Gemellologiae, 41*(4), 311-324.

Hsieh, C. J., Jeng, S. F., Su, Y. N., Liao, H. F., Hsieh, W. S., Wu, K. Y., et al. (2010). CYP1A1 modifies the effect of maternal exposure to environmental tobacco smoke on child behavior. *Nicotine & Tobacco Research, 12*(11), 1108-1117.

Hughes, M. E., Waite, L. J., Hawkley, L. C., & Cacioppo, J. T. (2004). A Short Scale for Measuring Loneliness in Large Surveys: Results From Two Population-Based Studies. *Res Aging, 26*(6), 655-672.

Huizink, A. C. (2009). Moderate use of alcohol, tobacco and cannabis during pregnancy: New approaches and update on research findings. *Reproductive Toxicology, 28*(2), 143-151.

Huizink, A. C., & Mulder, E. J. H. (2006). Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neuroscience & Biobehavioral Reviews, 30*(1), 24-41.

Hung, C. C., Yen, L. L., & Wu, W. C. (2009). Association of parents' alcohol use and family interaction with the initiation of alcohol use by sixth graders: a preliminary study in Taiwan. *Bmc Public Health, 9*, 172.

Hunter, D. J. (2005). Gene-environment interactions in human diseases. *Nat Rev Genet, 6*(4), 287-298.

Hussong, A. M., Jones, D. J., Stein, G. L., Baucom, D. H., & Boeding, S. (2011). An internalizing pathway to alcohol use and disorder. *Psychology of Addictive Behaviors, 25*, 390-404.

Iacono, W. G., Malone, S. M., & McGue, M. (2008). Behavioral disinhibition and the development of early-onset addiction: common and specific influences. *Annu.Rev.Clin.Psychol., 4*, 325-348.

IBM. (2011). *IBM SPSS Statistics 20 Command Syntax Reference*. Chicago, Il: IBM

Inc, S. (2006). SPSS for Windows (15.0 ed.). Chicago: SPSS Inc.

Indredavik, M. S., Brubakk, A. M., Romundstad, P., & Vik, T. (2007). Prenatal smoking exposure and psychiatric symptoms in adolescence. *Acta Paediatrica, 96*(3), 377-382.

Institute of Alcohol Studies. (2002). IAS Fact Sheet - What is problem drinking? St Ives, UK.

Irons, D. E., Iacono, W. G., Oetting, W. S., & McGue, M. (2012). Developmental Trajectory and Environmental Moderation of the Effect of ALDH2 Polymorphism on Alcohol Use. *Alcoholism: Clinical and Experimental Research*.

Johnson, E. O., & Schultz, L. (2005). Forward telescoping bias in reported age of onset: an example from cigarette smoking. *Int J Methods Psychiatr Res, 14*(3), 119-129.

Jonkman, H. B., Haggerty, K. P., Steketee, M., Fagan, A., Hanson, K., & Hawkins, J. D. (2009). Communities That Care, core elements and context: Research of implementation in two countries (Vol. 30, pp. 42): NIH Public Access.

Jöreskog, K. G., & Sörbom, D. (1993). *New Features in PRELIS 2*. Chicago, IL: Scientific Software International, Inc.

Kabir, Z., Connolly, G. N., & Alpert, H. R. (2011). Secondhand smoke exposure and neurobehavioral disorders among children in the United States. *Pediatrics, 128*(2), 263-270.

Kahn, R. S., Khoury, J., Nichols, W. C., & Lanphear, B. P. (2003). Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. *Journal of Pediatrics, 143*(1), 104-110.

Kaprio, J., Rose, R. J., Romanov, K., & Koskenvuo, M. (1991). Genetic and environmental determinants of use and abuse of alcohol: the Finnish Twin Cohort studies. *Alcohol Alcohol Suppl, 1*, 131-136.

Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-httlpr), stress, and depression meta-analysis revisited: Evidence of genetic moderation. [doi: 10.1001/archgenpsychiatry.2010.189]. *Archives of General Psychiatry, 68*(5), 444-454.

Karlsson, T., & Osterberg, E. (2006). Alcohol Policies in EU Member States and Norway - A Collection of Country Reports. Helsinki, Finland: National Research and Development Centre for Welfare and Health, .

Kendler, K. S., Chen, X., Dick, D., Maes, H., Gillespie, N., Neale, M. C., et al. (2012). Recent advances in the genetic epidemiology and molecular genetics of substance use disorders. *Nat Neurosci, 15*(2), 181-189.

Kendler, K. S., Gardner, C., & Dick, D. M. (2011a). Predicting alcohol consumption in adolescence from alcohol-specific and general externalizing genetic risk factors, key environmental exposures and their interaction. *Psychological Medicine, 41*(7), 1507-1516.

Kendler, K. S., & Gardner, C. O. (2010). Interpretation of interactions: guide for the perplexed. *British Journal of Psychiatry, 197*(3), 170-171.

Kendler, K. S., Gardner, C. O., & Prescott, C. A. (2011b). Toward a Comprehensive Developmental Model for Alcohol Use Disorders in Men. *Twin Research and Human Genetics, 14*(1), 1-15.

Kendler, K. S., Neale, M. C., MacLean, C. J., Heath, A. C., Eaves, L. J., & Kessler, R. C. (1993). Smoking and major depression. A causal analysis. *Archives of General Psychiatry, 50*(1), 36-43.

Kendler, K. S., & Prescott, C. A. (2006). Externalizing and Substance Use Disorders *Genes, Environment, and Psychopathology: Understanding the causes of Psychiatric and Substance Use Disorders*. New York: Guilford Press.

Kendler, K. S., Prescott, C. A., Jacobson, K., Myers, J., & Neale, M. C. (2002). The joint analysis of personal interview and family history diagnoses: evidence for validity of diagnosis and increased heritability estimates. *Psychological Medicine, 32*(5), 829-842.

Kendler, K. S., Prescott, C. A., Myers, J., & Neale, M. C. (2003). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry, 60*(9), 929-937.

Kendler, K. S., Schmitt, E., Aggen, S. H., & Prescott, C. A. (2008). Genetic and Environmental Influences on Alcohol, Caffeine, Cannabis, and Nicotine Use From Early Adolescence to Middle Adulthood. *Archives of General Psychiatry, 65*(6), 674-682.

King, K. M., & Chassin, L. (2007). A prospective study of the effects of age of initiation of alcohol and drug use on young adult substance dependence. *Journal of Studies on Alcohol and Drugs, 68*(2), 256-265.

Knopik, V. S. (2009). Maternal Smoking During Pregnancy and Child Outcomes: Real or Spurious Effect? *Developmental Neuropsychology, 34*(1), 1-36.

Koivumaa-Honkanen, H., Kaprio, J., Korhonen, T., Honkanen, R. J., Heikkilä, K., & Koskenvuo, M. (2012). Self-reported Life Satisfaction and Alcohol use: A 15-year follow-up of Healthy Adult Twins. *Alcohol and Alcoholism, 47*(2), 160-168.

Koopmans, J. R. (1997). *The genetics of health-related behaviors. PhD Thesis.* VU University, Amsterdam.

Koopmans, J. R., & Boomsma, D. I. (1996). Familial resemblances in alcohol use: Genetic or cultural transmission? *Journal of Studies on Alcohol, 57*(1), 19-28.

Koopmans, J. R., Doornen van, L. J. P., Boomsma, D. I., Goldbourt, U., DeFaire, U., & Berg, K. (1994). Smoking and Sports Participation *Genetic Factors in Coronary Heart Disease* (pp. 217-235). Dordrecht, The Netherlands: Kluwer.

Koopmans, J. R., Slutske, W. S., Heath, A. C., Neale, M. C., & Boomsma, D. I. (1999a). The genetics of smoking initiation and quantity smoked in Dutch adolescent and young adult twins. *Behavior Genetics, 29*(6), 383-393.

Koopmans, J. R., Slutske, W. S., van Baal, G. C. M., & Boomsma, D. I. (1999b). The influence of religion on alcohol use initiation: Evidence for genotype x environment interaction. *Behavior Genetics, 29*(6), 445-453.

Kruse, M. I., Radnovich, A. J., Kalapatapu, R. K., Mehdiyoun, N., Chambers, R. A., & Davidson, D. (2012). Effects of alcohol availability, access to alcohol, and naltrexone on self-reported craving and patterns of drinking in response to an alcohol-cue availability procedure. *Journal of Studies on Alcohol and Drugs, 73*(2), 205-215.

Laar van, M., Cruts, A. A. N., Ooyen-Houben van, M. M. J., Meijer, R. F., & Brunt, T. (2010). National Drug Monitor - Year Report 2009 Utrecht, the Netherlands.

Laar van, M., Cruts, A. A. N., Van Ooyen-Houben, M. M. J., Meijer, R. F., Croes, E. A., Brunt, T., et al. (2011a). National Drug Monitor - Year Report 2010 *National Drug Monitor*. Utrecht, the Netherlands: Trimbos Institute/WODC (Research and Documentation Centre).

Laar van, M., Cruts, A. A. N., Van Ooyen-Houben, M. M. J., Meijer, R. F., Croes, E. A., Brunt, T., et al. (2011b). Nationale Drug Monitor - Jaarverslag 2010 *Nationale Drug Monitor*. Utrecht: Trimbos Instituut/WODC.

Laar van, M. W. (2010). Afhankelijkheid van alcohol: Zijn er verschillen naar sociaaleconomische status? [Alcohol dependency: Are there differences by socioeconomic status?] *Nationaal Kompas Volksgezondheid*. Bilthoven, the Netherlands.

Lancaster, C. A., Gold, K. J., Flynn, H. A., Yoo, H., Marcus, S. M., & Davis, M. M. (2010). Risk factors for depressive symptoms during pregnancy: a systematic review. *American Journal of Obstetrics & Gynecology, 202*(1), 5-14.

Langley, K., Turic, D., Rice, F., Holmans, P., van den Bree, M. B. M., Craddock, N., et al. (2008). Testing for Gene x Environment Interaction Effects in Attention Deficit Hyperactivity Disorder and Associated Antisocial Behavior. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics, 147B*(1), 49-53.

Lanting, C. I., Segaar, D., Crone, M. R., & Van Wouwe, J. P. (2007). Slight decrease in the prevalence of smoking around pregnancy. *Nederlands Tijdschrift voor Geneeskunde, 151*(46), 2566-2569.

Lauria, L., Lamberti, A., & Grandolfo, M. (2012). Smoking behaviour before, during, and after pregnancy: the effect of breastfeeding. *ScientificWorldJournal, 2012*, 154910.

Lavigne, J. V., Hopkins, J., Gouze, K. R., Bryant, F. B., LeBailly, S. A., Binns, H. J., et al. (2011). Is smoking during pregnancy a risk factor for psychopathology in young children? A methodological caveat and report on preschoolers. *Journal of Pediatric Psychology, 36*(1), 10-24.

Lawrence, R. J., & Fudge, C. (2009). Healthy Cities in a global and regional context. *Health Promotion International, 24*(suppl 1), i11-i18.

Leckman, J. F., Riddle, M. A., Hardin, M. T., Ort, S. I., Swartz, K. L., Stevenson, J., et al. (1989). The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *J Am Acad Child Adolesc Psychiatry, 28*(4), 566-573.

Lee, L. O., Wolff, K. C., Kendler, K. S., & Prescott, C. A. (2012). The effects of age at drinking onset and stressful life events on alcohol use in adulthood: a replication and extension using a population-based twin sample. *Alcoholism: Clinical and Experimental Research, 36*(4), 693-704.

Legrand, L. N., Keyes, M., McGue, M., Iacono, W. G., & Krueger, R. F. (2008). Rural environments reduce the genetic influence on adolescent

substance use and rule-breaking behavior. *Psychological Medicine, 38*(9), 1341-1350.

Lehn, H., Derks, E. M., Hudziak, J. J., Heutink, P., van Beijsterveldt, T. C. E. M., & Boomsma, D. I. (2007). Attention problems and attention-deficit/hyperactivity disorder in discordant and concordant monozygotic twins: Evidence of environmental mediators. *Journal of the American Academy of Child and Adolescent Psychiatry, 46*(1), 83-91.

Ligthart, L., & Boomsma, D. I. (2012). Causes of Comorbidity: Pleiotropy or Causality? Shared Genetic and Environmental Influences on Migraine and Neuroticism. *Twin Research and Human Genetics, 15*(2), 158-165.

Lin, M., Hrabovsky, A., Pedrosa, E., Wang, T., Zheng, D., & Lachman, H. M. (2012). Allele-biased expression in differentiating human neurons: implications for neuropsychiatric disorders. *PLoS One, 7*(8), e44017.

Little, H. J. (2000). Behavioral mechanisms underlying the link between smoking and drinking. *Alcohol Research & Health, 24*(4), 215-224.

Liu, T., Gatsonis, C. A., Baylin, A., Kubzansky, L. D., Loucks, E. B., & Buka, S. L. (2011). Maternal smoking during pregnancy and anger temperament among adult offspring. *Journal of Psychiatric Research, 45*(12), 1648-1654.

Loehlin, J. (2010). Is There an Active Gene-Environment Correlation in Adolescent Drinking Behavior? *Behavior Genetics, 40*(4), 447-451.

Luby, J. L., Heffelfinger, A. K., Mrakotsky, C., Brown, K. M., Hessler, M. J., Wallis, J. M., et al. (2003). The clinical picture of depression in preschool children. *Journal of the American Academy of Child and Adolescent Psychiatry, 42*(3), 340-348.

Lumley, J., Chamberlain, C., Dowswell, T., Oliver, S., Oakley, L., & Watson, L. (2009). Interventions for promoting smoking cessation during pregnancy. *Cochrane Database Syst Rev*(3), CD001055.

Lynskey, M. T., Heath, A. C., Bucholz, K. K., Slutske, W. S., Madden, P. A., Nelson, E. C., et al. (2003). Escalation of drug use in early-onset cannabis users vs co-twin controls. *JAMA: The Journal of the American Medical Association, 289*(4), 427-433.

Lyubomirsky, S., & Lepper, H. S. (1999). A measure of subjective happiness: Preliminary reliability and construct validation. *Social Indicators Research, 46*(2), 137-155.

Maas van der, E. (2006). Factsheet Alcohol en Werk [Factsheet Alcohol and Profession]. Woerden, the Netherlands.

MacArthur, G. J., Smith, M. C., Melotti, R., Heron, J., Macleod, J., Hickman, M., et al. (2012). Patterns of alcohol use and multiple risk behaviour by gender during early and late adolescence: the ALSPAC cohort. *Journal of Public Health, 34*(suppl 1), i20-i30.

Maes, H. H., Woodard, C. E., Murrelle, L., Meyer, J. M., Silberg, J. L., Hewitt, J. K., et al. (1999). Tobacco, alcohol and drug use in eight- to sixteen-year-old twins: The Virginia Twin Study of Adolescent Behavioral Development. *Journal of Studies on Alcohol, 60*(3), 293-305.

Malone, P. S., Northrup, T. F., Masyn, K. E., Lamis, D. A., & Lamont, A. E. (2012). Initiation and persistence of alcohol use in United States Black, Hispanic, and White male and female youth. *Addictive Behaviors, 37*(3), 299-305.

Mayzer, R., Fitzgerald, H. E., & Zucker, R. A. (2009). Anticipating Problem Drinking Risk From Preschoolers' Antisocial Behavior: Evidence for a Common Delinquency-Related Diathesis Model. *Journal of the American Academy of Child and Adolescent Psychiatry, 48*(8), 820-827.

McCarty, C. A., Rhew, I. C., Murowchick, E., McCauley, E., & Stoep, A. V. (2011). Emotional health predictors of substance use initiation during middle school. *Psychology of Addictive Behaviors*. Retrieved from doi:10.1037/a0025630

McEvoy, B. P., & Visscher, P. M. (2009). Genetics of human height. *Economics & Human Biology, 7*(3), 294-306.

McGue, M., Iacono, W. G., Legrand, L. N., & Elkins, I. (2001a). Origins and consequences of age at first drink. II. Familial risk and heritability. *Alcoholism-Clinical and Experimental Research, 25*(8), 1166-1173.

McGue, M., Iacono, W. G., Legrand, L. N., Malone, S., & Elkins, I. (2001b). Origins and consequences of age at first drink. 1. Associations with substance-use disorders, disinhibitory behavior and psychopathology, and P3 amplitude. *Alcoholism: Clinical and Experimental Research, 25*(8), 1156-1165.

Meyers, J. L., & Dick, D. M. (2010). Genetic and Environmental Risk Factors for Adolescent-Onset Substance Use Disorders. *Child and Adolescent Psychiatric Clinics of North America, 19*(3), 465-+.

Mick, E., Biederman, J., Faraone, S. V., Sayer, J., & Kleinman, S. (2002). Case-control study of attention-deficit hyperactivity disorder and maternal smoking, alcohol use, and drug use during pregnancy. *J Am Acad Child Adolesc Psychiatry, 41*(4), 378-385.

Ministerie van Volksgezondheid Welzijn en Sport. (2012a). Alcohol Retrieved 23-07-2012, from http://www.rijksoverheid.nl/onderwerpen/alcohol

Ministerie van Volksgezondheid Welzijn en Sport. (2012b). Jongeren en Alcohol Retrieved 19-07-2012, from http://www.rijksoverheid.nl/onderwerpen/alcohol/jongeren-en-alcohol

Ministry of Education Culture and Science. (2012). Aantal studenten in hoger onderwijs als percentage van de bevolking van 18-25 jaar [Number of students in higher education as percentage of the 18-25 year old population]. *Trendontwikkelingen onderwijsdeelname [Trend developments education participation]* Retrieved 17-06-2012, from http://www.trendsinbeeld.minocw.nl/grafieken/3_1_1_3.php

Ministry of Health Welfare & Sport. (2009, 2009/01/12/). Dutch Alcohol Policy Retrieved 2010/08/16/, from http://english.minvws.nl/en/themes/alcohol/default.asp

Ministry of Security and Justice. (2012). Soft Drugs - Dutch Drugs Policy, from http://www.government.nl/issues/alcohol-and-drugs/drugs

Monshouwer, K., Huizink, A. C., Harakeh, Z., Raaijmakers, Q. A., Reijneveld, S. A., Oldehinkel, A. J., et al. (2011). Prenatal Smoking Exposure and the Risk of Behavioral Problems and Substance Use in Adolescence: the TRAILS Study. *European Addiction Research, 17*(6), 342-350.

Moos, R. H., Brennan, P. L., Schutte, K. K., & Moos, B. S. (2006). Older adults' coping with negative life events: common processes of managing health, interpersonal, and financial/work stressors. *Int J Aging Hum Dev, 62*(1), 39-59.

Morey, L. C. (1991). *The Personality Assessment Inventory: Professional manual*. Odessa, FL: Psychological Assessment Resources.

Munafò, M. R., Durrant, C., Lewis, G., & Flint, J. (2009). Gene X environment interactions at the serotonin transporter locus. *Biological Psychiatry, 65*(3), 211-219.

Muthén, L. K., & Muthén, B. O. (2010). *Mplus User's Guide*. Los Angeles, CA: Muthén & Muthén.

National Institute on Alcohol Abuse and Alcoholism. (2011). Women and Alcohol. Bethesda, MD.

National Reference Point. (2009). Current educational system Retrieved 2011/08/17, from http://www.nlnrp.nl/current-educational-system.html

National Statistics. (2004). Focus on Health *Focus on* (pp. 7). London, UK.

Nazareth, I., Walker, C., Ridolfi, A., Aluoja, A., Bellon, J., Geerlings, M., et al. (2011). Heavy episodic drinking in Europe: a cross section study in primary care in six European countries. *Alcohol and Alcoholism, 46*(5), 600-606.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2006). *Mx: Statistical Modeling*: VCU Box 900126, Richmond, VA 23298: Department of Psychiatry.

Neale, M. C., & Cardon, L. (1992). *Methodology for Genetic Studies of Twins and Families*. Dordrecht, The Netherlands: Kluwer Academic Publishers B.V.

Nederlands Instituut voor Alcoholbeleid (STAP). (2009). Nationaal Beleid, from http://www.stap.nl/nl/home/nationaal-beleid.html

Nederlands Instituut voor Alcoholbeleid (STAP). (2012). Initiatiefwetsvoorstel om alcoholleeftijd naar 18 te brengen, from http://www.stap.nl/nl/nieuws/laatste-nieuws.html/3454/1839/initiatiefwetsvoorstel-om-alcoholleeftijd-naar-18-te-brengen-#p3454

Netherlands Institute of Mental Health and Addiction. (2011). Alcohol preventie [Alcohol prevention]. *Alcohol en Drugs [Alcohol and Drugs]* Retrieved 19-06-2012, from http://www.trimbos.nl/onderwerpen/alcohol-en-drugs/alcohol/preventie

Netherlands Institute of Mental Health and Addiction. (2012). Preventie - Ouderen [Prevention - Elderly population], from http://www.trimbos.nl/onderwerpen/preventie/ouderen

Netherlands Institute on Mental Health and Addiction. (2009). Studenten - Alcoholgebruik [Students - Alcohol use], from http://www.alcoholinfo.nl/index.cfm?act=esite.tonen&a=3&b=52&c=238

Neuman, R. J., Lobos, E., Reich, W., Henderson, C. A., Sun, L. W., & Todd, R. D. (2007). Prenatal smoking exposure and dopaminergic genotypes interact to cause a severe ADHD subtype. *Biological Psychiatry, 61*(12), 1320-1328.

Neville, M. J., Johnstone, E. C., & Walton, R. T. (2004). Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat, 23*(6), 540-545.

. Nibud Scholierenonderzoek 2008-2009. (2009, 2009) Retrieved 2011/07/14/, from http://www.nibud.nl/fileadmin/user_upload/Documenten/PDF/onderzoeken/NSO_2008-2009.pdf
http://www.webcitation.org/60AeHQ6c4

Nomura, Y., Gilman, S. E., & Buka, S. L. (2011). Maternal Smoking During Pregnancy and Risk of Alcohol Use Disorders Among Adult Offspring. *Journal of Studies on Alcohol and Drugs, 72*(2), 199-209.

Nunez-Smith, M., Wolf, E., Huang, H. M., Chen, P. G., Lee, L., Emanuel, E. J., et al. (2010). Media Exposure and Tobacco, Illicit Drugs, and Alcohol Use Among Children and Adolescents: A Systematic Review. [doi: 10.1080/08897077.2010.495648]. *Substance Abuse, 31*(3), 174-192.

Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American Journal of Human Genetics, 74*(4), 765-769.

Oliva, E., Koenig, L. B., & Laudet, A. (2012). Commentary on Geels et al. (2012): The search for G spurs questions about E. *Addiction, 107*(3), 528-529.

Orlebeke, J. F., Knol, D. L., & Verhulst, F. C. (1999). Child behavior problems increased by maternal smoking during pregnancy. *Archives of Environmental Health, 54*(1), 15-19.

Otten, F., Bos, W., Vrooman, C., & Hoff, S. (2006). Armoedebericht 2006 [Poverty report 2006]. Voorburg, the Netherlands: Statistics Netherlands / The Netherlands Institute for Social Research.

Ouwehand, A. W., Wisselink, D. J., Kuijpers, W. G. T., van Delden, E. B., & Mol, A. (2011). Kerncijfers Verslavingszorg 2010 [Figures Addiction health care 2010]. Houten, the Netherlands: Landelijk Alcohol en Drugs Informatie Systeem (LADIS).

Paradis, A. D., Fitzmaurice, G. M., Koenen, K. C., & Buka, S. L. (2011). Maternal smoking during pregnancy and criminal offending among adult offspring. *Journal of Epidemiology and Community Health, 65*(12), 1145-1150.

Pei, Y. F., Zhang, L., Yang, T. L., Han, Y., Hai, R., Ran, S., et al. (2012). Genome-wide association study of copy number variants suggests LTBP1 and FGD4 are important for alcohol drinking. *PLoS One, 7*(1), e30860.

Pickett, K. E., Rathouz, P. J., Dukic, V., Kasza, K., Niessner, M., Wright, R. J., et al. (2009). The complex enterprise of modelling prenatal exposure to cigarettes: what is enough? *Paediatric and Perinatal Epidemiology, 23*(2), 160-170.

Pickett, K. E., Wood, C., Adamson, J., DeSouza, L., & Wakschlag, L. S. (2008). Meaningful differences in maternal smoking behaviour during pregnancy: implications for infant behavioural vulnerability. *Journal of Epidemiology and Community Health, 62*(4), 318-324.

Piper, B. J., Gray, H. M., & Birkett, M. A. (2012). Maternal smoking cessation and reduced academic and behavioral problems in offspring. *Drug and Alcohol Dependence, 121*(1-2), 62-67.

Plomin, R., DeFries, J C, McClearn, G E, McGuffin, P. (2008). *Behavioral Genetics* (5 ed.). New York: Worth.

Poelen, E. A. P., Scholte, R. H. J., Engels, R. C. M. E., Boomsma, D. I., & Willemsen, G. (2005). Prevalence and trends of alcohol use and misuse among adolescents and young adults in the Netherlands from 1993 to 2000. *Drug and Alcohol Dependence, 79*(3), 413-421.

Poelen, E. A. P., Scholte, R. H. J., Willemsen, G., Boomsma, D. I., & Engels, R. C. M. E. (2007). Drinking by parents, siblings, and friends as predictors of regular alcohol use in adolescents and young adults: A longitudinal twin-family study. *Alcohol and Alcoholism, 42*, 362-369.

Prescott, C. A., & Kendler, K. S. (1999). Age at first drink and risk for alcoholism: a noncausal association. *Alcoholism: Clinical and Experimental Research, 23*(1), 101-107.

Purcell, S. (2002). Variance components models for gene-environment interaction in twin analysis. *Twin Res, 5*(6), 554-571.

Rebollo, I., de Moor, M. H., Dolan, C. V., & Boomsma, D. I. (2006). Phenotypic factor analysis of family data: correction of the bias due to dependency. *Twin Research and Human Genetics, 9*(3), 367-376.

Rhee, S. H., Hewitt, J. K., Young, S. E., Corley, R. P., Crowley, T. J., & Stallings, M. C. (2003). Genetic and environmental influences on substance initiation, use, and problem use in adolescents. *Archives of General Psychiatry, 60*(12), 1256-1264.

Rietveld, M. J., van Der Valk, J. C., Bongers, I. L., Stroet, T. M., Slagboom, P. E., & Boomsma, D. I. (2000). Zygosity diagnosis in young twins by parental report. *Twin Res, 3*(3), 134-141.

Risch, N., Herrell, R., Lehner, T., Liang, K. Y., Eaves, L., Hoh, J., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA: The Journal of the American Medical Association, 301*(23), 2462-2471.

Robinson, M., McLean, N. J., Oddy, W. H., Mattes, E., Bulsara, M., Li, J., et al. (2010). Smoking cessation in pregnancy and the risk of child behavioural problems: a longitudinal prospective cohort study. *Journal of Epidemiology & Community Health, 64*(7), 622-629.

Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., et al. (2007). Epidemiology of the human circadian clock. [doi: 10.1016/j.smrv.2007.07.005]. *Sleep Medicine Reviews, 11*(6), 429-438.

Rogers, J. M. (2009). Tobacco and pregnancy. *Reproductive Toxicology, 28*(2), 152-160.

Room, R. (1992). The impossible dream?--Routes to reducing alcohol problems in a temperance culture. *J Subst Abuse, 4*(1), 91-106.

Rose, R. J., Dick, D. M., Viken, R. J., & Kaprio, J. (2001a). Gene-environment interaction in patterns of adolescent drinking: Regional residency moderates longitudinal influences on alcohol use. *Alcoholism: Clinical and Experimental Research, 25*(5), 637-643.

Rose, R. J., Dick, D. M., Viken, R. J., Pulkkinen, L., & Kaprio, J. (2001b). Drinking or abstaining at age 14? A genetic epidemiological study. *Alcoholism-Clinical and Experimental Research, 25*(11), 1594-1604.

Roza, S. J., Verhulst, F. C., Jaddoe, V. W. V., Steegers, E. A. P., Mackenbach, J. P., Hofman, A., et al. (2008). Maternal smoking during pregnancy

and child behaviour problems: the Generation R Study. *International Journal of Epidemiology, 38*(3), 680-689.

Rückinger, S., Rzehak, P., Chen, C. M., Sausenthaler, S., Koletzko, S., Bauer, C. P., et al. (2009). Prenatal and Postnatal Tobacco Exposure and Behavioral Problems in 10-Year-Old Children: Results from the GINI-plus Prospective Birth Cohort Study. *Environmental Health Perspectives, 118*(1), 150-154.

Ryan, S. M., Jorm, A. F., & Lubman, D. I. (2010). Parenting factors associated with reduced adolescent alcohol use: a systematic review of longitudinal studies. *Aust N Z J Psychiatry, 44*(9), 774-783.

Salmasi, G., Grady, R., Jones, J., McDonald, S. D., & , O. b. o. t. K. S. G. (2010). Environmental tobacco smoke exposure and perinatal outcomes: a systematic review and meta-analyses. *Acta Obstetricia et Gynecologica Scandinavica, 89*(4), 423-441.

Sanavio, E. (1988). Obsessions and compulsions: the Padua Inventory. *Behaviour research and therapy, 26*(2), 169-177.

Sartor, C. E., Bucholz, K. K., Nelson, E. C., Madden, P. A., Lynskey, M. T., & Heath, A. C. (2011). Reporting bias in the association between age at first alcohol use and heavy episodic drinking. *Alcoholism: Clinical and Experimental Research, 35*(8), 1418-1425.

Sartor, C. E., Lynskey, M. T., Bucholz, K. K., Madden, P. A. F., Martin, N. G., & Heath, A. C. (2009). Timing of first alcohol use and alcohol dependence: evidence of common genetic influences. *Addiction, 104*(9), 1512-1518.

Sartor, C. E., Lynskey, M. T., Heath, A. C., Jacob, T., & True, W. (2007). The role of childhood risk factors in initiation of alcohol use and progression to alcohol dependence. *Addiction, 102*(2), 216-225.

Saunders, J. B., Aasland, O. G., Babor, T. F., Fuente, J. R., & Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption II. *Addiction, 88*(6), 791-804.

Savelkoul, M., Verweij, A., & Zantinge, E. M. (2011). Alcoholgebruik: Zijn er verschillen naar sociaaleconomische status? [Alcohol use: Are there differences by socioeconomic status?] *Volksgezondheid Toekomst Verkenning, Nationaal Kompas Volksgezondheid*. Bilthoven, the Netherlands: National Institute for Public Health and the environment.

Sayon-Orea, C., Martinez-Gonzalez, M. A., & Bes-Rastrollo, M. (2011). Alcohol consumption and body weight: a systematic review. *Nutrition Reviews, 69*(8), 419-431.

Schermelleh-Engel, K., Moosbrugger, H., & Müller, H. (2003). Evaluating the Fit of Structural Equation Models: Tests of Significance and Descriptive Goodness-of-Fit Measures. *Methods of Psychological Research Online, 8*(2), 23-74.

Schlotz, W., & Phillips, D. I. W. (2009). Fetal origins of mental health: evidence and mechanisms. *Brain, Behavior, and Immunity, 23*(7), 905-916.

Schumann, G., Coin, L. J., Lourdusamy, A., Charoen, P., Berger, K. H., Stacey, D., et al. (2011). Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. *Proc Natl Acad Sci U S A, 108*(17), 7119-7124.

Schutten, M., Eijnden van den, R. J. J. M., & Knibbe, R. A. (2003). Onderzoeksrapportage Alcohol en Werk [Research report Alcohol and Profession]. The Hague, the Netherlands.

Schwarz, N. (2007). Retrospective and Concurrent Self-Reports: The Rationale for Real-Time Data Capture. In A. Stone, S. Shiffman, A. Atienza & L. Nebeling (Eds.), *The Science of Real-Time Data Capture - Self-Reports in Health Research* (pp. 11-26). New York, NY: Oxford University Press.

Silventoinen, K. (2003). Determinants of variation in adult body height. *Journal of Biosocial Science, 35*(2), 263-285.

Smit, E., Verdurmen, J., Monshouwer, K., & Smit, F. (2008). Family interventions and their effect on adolescent alcohol use in general populations; a meta-analysis of randomized controlled trials. [doi: 10.1016/j.drugalcdep.2008.03.032]. *Drug and Alcohol Dependence, 97*(3), 195-206.

Smith, D. (2008). Assessing intrauterine influences on offspring health outcomes: can epidemiological findings yield robust results? *Basic & Clinical Pharmacology & Toxicology, 102*(5), 245-256.

Smith, L., & Foxcroft, D. (2009). Drinking in the UK - An exploration of trends. UK: Joseph Rowntree Foundation.

Smits, J. P. (1999). Economische ontwikkeling, 1800-1995 [Economical development, 1800-1995]. In R. Bie van der & P. Dehing (Eds.), *Nationaal goed - Feiten en cijfers over onze samenleving, (ca.)1800-1999 [National good - Facts and figures on our society, (ca.) 1800-1999]*. Voorburg/Heerlen, the Netherlands.

SPSS Inc. (2009). PASW Statistics 18 (18.0.0 ed.). Chicago, Il.

Statistics Netherlands. (1999). De geest uit de fles: Gaat kerkelijke betrokkenheid samen met drankgebruik? [The spirit out of the bottle: Does religious involvement co-occur with alcohol use?] Voorburg/Heerlen, the Netherlands.

Statistics Netherlands. (2001). Standard Classification of Occupations (SBC). Voorburg/Heerlen, the Netherlands Statistics Netherlands.

Statistics Netherlands. (2003, 2003). Jeugd 2003, feiten en cijfers [Youth 2003, facts and figures] Retrieved 2011/04/05/, from http://www.cbs.nl/NR/rdonlyres/9CF19548-A0B9-42BF-9D67-15C6C903AFE6/0/2003g87p051art.pdf
http://www.webcitation.org/5xiZU2wJw

Statistics Netherlands. (2007). Meeste rokers gestopt rond hun veertigste [Most smokers quit around age 40 years]. *Health and well-being*, from http://www.cbs.nl/nl-NL/menu/themas/gezondheid-welzijn/publicaties/artikelen/archief/2007/2007-2204-wm.htm

Statistics Netherlands. (2008). Opleidingsniveau en stedelijkheid naar leeftijd 2008 en 1998 [Educational attainment and urbanization by age 2008 and 1998]. Heerlen/Voorburg, the Netherlands.

Statistics Netherlands. (2010a). Drugsgebruik; leeftijd en geslacht gebruiker [Drug use; age and sex user]. Voorburg/Heerlen, the Netherlands: Statistics Netherlands,.

Statistics Netherlands. (2010b). Gezondheid, leefstijl, zorggebruik; t/m 2009 [Health, lifestyle, health care use; until 2009]. Voorburg/Heerlen, the Netherlands: Statistics Netherlands.

Statistics Netherlands. (2012a). Beroepsbevolking; behaalde onderwijs naar herkomst geslacht en leeftijd [Working population; educational attainment by etnicity sex and age]. Voorburg/Heerlen, the Netherlands: Statistics Netherlands.

Statistics Netherlands. (2012b). Beroepsbevolking; geslacht en leeftijd [Working population; sex and age]. Voorburg/Heerlen, the Netherlands: Statistics Netherlands.

Statistics Netherlands. (2012c). Leeftijd ouders bij geboorte kind, 1996 en 2000-2006 [Parental age at birth of child, 1996 and 2000-2006] Retrieved 04-01-2012, from http://www.cbs.nl/nl-NL/menu/themas/bevolking/cijfers/incidenteel/maatwerk/2388-wm.htm

Stephens, S. H., Hoft, N. R., Schlaepfer, I. R., Young, S. E., Corley, R. C., McQueen, M. B., et al. (2012). Externalizing behaviors are associated with SNPs in the CHRNA5/CHRNA3/CHRNB4 gene cluster. *Behavior Genetics, 42*(3), 402-414.

Substance Abuse and Mental Health Services Administration. (2011). State Estimates of Substance Use and Mental Disorders from the 2008-2009 National Surveys on Drug Use and Health. Rockville, MD.

Swaim, R. C., & Stanley, L. R. (2011). Rurality, Region, Ethnic Community Make-Up and Alcohol Use Among Rural Youth. *Journal of Rural Health, 27*(1), 91-102.

Terry-McElrath, Y. M., O'Malley, P. M., & Johnston, L. D. (2011). Exercise and Substance Use Among American Youth, 1991-2009. *American Journal of Preventive Medicine, 40*(5), 530-540.

Thapar, A., Rice, F., Hay, D., Boivin, J., Langley, K., van den Bree, M., et al. (2009). Prenatal Smoking Might Not Cause Attention-Deficit/Hyperactivity Disorder: Evidence from a Novel Design. *Biological Psychiatry, 66*(8), 722-727.

Thapar, A., & Rutter, M. (2009). Do prenatal risk factors cause psychiatric disorder? Be wary of causal claims. *British Journal of Psychiatry, 195*(2), 100-101.

Tiesler, C. M., Chen, C. M., Sausenthaler, S., Herbarth, O., Lehmann, I., Schaaf, B., et al. (2011). Passive smoking and behavioural problems in children: results from the LISAplus prospective birth cohort study. *Environmental Research, 111*(8), 1173-1179.

Tong, V. T. (2009). *Trends in Smoking Before, During, and After Pregnancy: Pregnancy Risk Assessment Monitoring System (PRAMS), United States, 31 Sites, 2000-2005*: Dept. of Health and Human Services, Centers for Disease Control and Prevention.

Trimbos, I. (2010). National Drug Monitor 2009. Available at: http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2010/05/27/nationale-drugmonitor-2009.html. Accessed: 2011-01-20. (Archived by WebCite© at http://www.webcitation.org/5vsO0yYfu) (pp. 151-160). Utrecht.

Tripodi, S. J., Bender, K., Litschge, C., & Vaughn, M. G. (2010). Interventions for reducing adolescent alcohol abuse: A meta-analytic review. [doi: 10.1001/archpediatrics.2009.235]. *Archives of Pediatrics & Adolescent Medicine, 164*(1), 85-91.

Trucco, E. M., Colder, C. R., & Wieczorek, W. F. (2011). Vulnerability to peer influence: a moderated mediation study of early adolescent alcohol use initiation. *Addictive Behaviors, 36*(7), 729-736.

Tucker, L. R., & Lewis, C. (1973). A reliability coefficient for maximum likelihood factor analysis. *Psychometrika, 38*(1), 1-10.

Valenzuela, C. F., Morton, R. A., Diaz, M. R., & Topper, L. (2012). Does moderate drinking harm the fetal brain? Insights from animal models. [doi: 10.1016/j.tins.2012.01.006]. *Trends in Neurosciences, 35*(5), 284-292.

van Beek, J. H., Willemsen, G., de Moor, M. H., Hottenga, J. J., & Boomsma, D. I. (2010). Associations between ADH gene variants and alcohol phenotypes in Dutch adults. *Twin Research and Human Genetics, 13*(1), 30-42.

van Beek, J. H. D. A., Kendler, K. S., de Moor, M. H., Geels, L. M., Bartels, M., Vink, J. M., et al. (2012). Stable genetic effects on symptoms of alcohol abuse and dependence from adolescence into early adulthood. *Behavior Genetics, 42*(1), 40-56.

van Beijsterveldt, C. E. M., Bartels, M., Groen-Blokhuis, M. M., Lamb, D., Huppertz, C., de Zeeuw, E., et al. (2012). The Young Netherlands Twin Registry (Y-NTR): Longitudinal twin and family studies in over 70,784 children.

van den Berg Jeths, A., Timmermans, J. M., Hoeymans, N., & Woittiez, I. B. (2004). Ouderen nu en in de toekomst: Gezondheid, verpleging en verzorging 2000 - 2020 [Elderly now and in the future: Health, nursing and care taking 2000 - 2020]. Bilthoven, the Netherlands: National Institute for Public Health and the Environment, .

Van den Brink, W., & Koele, P. (2002). *Statistiek deel 3: Toepassingen* (7e ed.). Amsterdam: Boom.

van der Aa, N., Bartels, M., Te Velde, S. J., Boomsma, D. I., de Geus, E. J., & Brug, J. (2012). Genetic and Environmental Influences on Individual Differences in Sedentary Behavior During Adolescence: A Twin-Family Study. *Archives of Pediatrics & Adolescent Medicine*.

van der Zwaluw, C. S., Engels, R. C., Vermulst, A. A., Franke, B., Buitelaar, J., Verkes, R. J., et al. (2010a). Interaction between dopamine D2 receptor genotype and parental rule-setting in adolescent alcohol use: evidence for a gene-parenting interaction. *Mol Psychiatry, 15*(7), 727-735.

van der Zwaluw, C. S., Engels, R. C. M. E., Vermulst, A. A., Rose, R. J., Verkes, R. J., Buitelaar, J., et al. (2010b). A serotonin transporter polymorphism (5-HTTLPR) predicts the development of adolescent alcohol use. *Drug and Alcohol Dependence, 112*(1-2), 134-139.

van der Zwaluw, C. S., Scholte, R. H., Vermulst, A. A., Buitelaar, J., Verkes, R. J., & Engels, R. C. (2009). The crown of love: intimate relations and alcohol use in adolescence. *Eur Child Adolesc Psychiatry, 18*(7), 407-417.

van Grootheest, D. S., Cath, D., Hottenga, J. J., Beekman, A. T., & Boomsma, D. I. (2009). Genetic factors underlie stability of obsessive-compulsive symptoms. *Twin Research and Human Genetics, 12*(5), 411-419.

van Hoof, J. J., Gosselt, J. F., & Baas, N. (2011). Beschikbaarheid alcohol voor jongeren onder de 16 - minutenwerk [Alcohol availability to adolescents under age 16 years - a matter of minutes]. Retrieved from http://www.stap.nl/nl/nieuws/laatste-nieuws.html/3454/1118/alcohol-kopen-door-minderjarigen-even-eenvoudig-als-kopen-frisdrank#p3454

van Laar, M., Cruts, A. A. N., Ooyen-Houben van, M. M. J., Meijer, R. F., & Brunt, T. (2010). National Drug Monitor - Year Report 2009 Utrecht, the Netherlands.

Van Laar, M., Cruts, A. A. N., Van Ooyen-Houben, M. M. J., Meijer, R. F., Croes, E. A., Brunt, T., et al. (2011). National Drug Monitor - Year Report 2010 *National Drug Monitor*. Utrecht, the Netherlands: Trimbos Institute/WODC (Research and Documentation Centre).

van Rooij, A. J., Schoenmakers, T. M., & van de Mheen, D. (2011). Nationaal Prevalentie Onderzoek Middelengebruik 2009: De kerncijfers [National

Prevalence Study on Substance Use 2009: Core Statistics]. Rotterdam, the Netherlands: IVO.

Verdurmen, J., Monshouwer, K., Dorsselaer van, S., Lokman, S., Vermeulen-Smit, E., & Vollebergh, W. (2012). Jeugd en Riskant gedrag - Kerngegevens uit het peilstationsonderzoek scholieren. Utrecht: Trimbos Instituut.

Verhulst, F. C., Van der Ende, J., & Koot, H. M. (1997). *Handleiding voor de Youth Self Report (YSR) [Manual for the Youth Self Report (YSR)].* Rotterdam: Afdeling Kinder- en Jeugdpsychiatrie, Sophia Kinderziekenhuis/ Academisch Ziekenhuis Rotterdam/ Erasmus Universiteit Rotterdam.

Viken, R. J., Kaprio, J., Koskenvuo, M., & Rose, R. J. (1999). Longitudinal analyses of the determinants of drinking and of drinking to intoxication in adolescent twins. *Behavior Genetics, 29*(6), 455-461.

Vink, J., Willemsen, G., & Boomsma, D. (2005). Heritability of smoking initiation and nicotine dependence. *Behavior Genetics, 35*(4), 397-406.

Vink, J. M., Nawijn, L., Boomsma, D. I., & Willemsen, G. (2007). Personality differences in monozygotic twins discordant for cannabis use. *Addiction, 102*(12), 1942-1946.

Wakschlag, L. S., Kistner, E. O., Pine, D. S., Biesecker, G., Pickett, K. E., Skol, A. D., et al. (2009). Interaction of prenatal exposure to cigarettes and MAOA genotype in pathways to youth antisocial behavior. *Molecular Psychiatry, 15*(9), 928-937.

Wang, J. C., Kapoor, M., & Goate, A. M. (2012). The Genetics of Substance Dependence. *Annu Rev Genomics Hum Genet*.

Weingart, S. (2009). Alcohol en ouderen in de verslavingszorg in Nederland (1999-2007) [Alcohol and the elderly in addiction health care in the Netherlands (1999-2007)]. Utrecht, the Netherlands.

West, R. J., & Russell, M. A. H. (1985). Pre-abstinence smoke intake and smoking motivation as predictors of severity of cigarette withdrawal symptoms. *Psychopharmacology, 87*(3), 334-336.

WHO-HFA. (2012, 15-05-2012). European health for all database, from http://data.euro.who.int/hfadb/

WHO. (2004, 2004). WHO Global Status Report: Alcohol Policy Retrieved 2011/04/08/, from http://www.who.int/substance_abuse/publications/en/Alcohol%20Policy%20Report.pdf

WHO. (2009). Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks. Geneva, Switzerland: WHO.

WHO. (2011). Global Status Report on Alcohol and Health 2011. Geneva, Switzerland.

Wiles, N. J., Lingford-Hughes, A., Daniel, J., Hickman, M., Farrell, M., Macleod, J., et al. (2007). Socio-economic status in childhood and later alcohol use: a systematic review. *Addiction, 102*(10), 1546-1563.

Willemsen, G., Posthuma, D., & Boomsma, D. I. (2005). Environmental factors determine where the Dutch live: Results from the Netherlands Twin Register. *Twin Research and Human Genetics, 8*(4), 312-317.

Willemsen, G., Vink, J. M., Abdellaoui, A., Den Braber, A., van Beek, J. H. D. A., Draisma, H. H. M., et al. (in press). The Adult Netherlands Twin Register: 25 years of survey and biological data collection. *Twin Research and Human Genetics*.

Willemsen, M. C., Feenstra, D., Kranen van, H. J., & Zantinge, E. M. (2011). Wat zijn de mogelijke oorzaken van roken? [Which are the possible causes of smoking?] *Nationaal Kompas Volksgezondheid*. Bilthoven, the Netherlands.

Witt, E. D. (2010). Research on alcohol and adolescent brain development: opportunities and future directions. *Alcohol, 44*(1), 119-124.

World Health Organization. (2011). Global Status Report on Alcohol and Health 2011. Geneva, Switzerland.

Young-Wolff, K. C., Kendler, K. S., & Prescott, C. A. (2012). Shared genetic contributions to early-onset drinking and drinking to cope motives. *Addict Behav, 37*(10), 1176-1180.

Zantinge, E. M., & van Laar, M. W. (2011). Hoeveel mensen gebruiken alcohol? [How many people use alcohol?]. Bilthoven, the Netherlands National Institute for Public Health and the Environment.

Zigmond, A. S., & Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta psychiatrica scandinavica, 67*(6), 361-370.

Zucker, R. A., Donovan, J. E., Masten, A. S., Mattson, M. E., & Moss, H. B. (2008). Early developmental processes and the continuity of risk for underage drinking and problem drinking. *Pediatrics, 121 Suppl 4*, S252-272.

Zuckerman, M., Kolin, E. A., Price, L., & Zoob, I. (1964). Development of a sensation-seeking scale. *Journal of Consulting Psychology, 28*(6), 477.