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

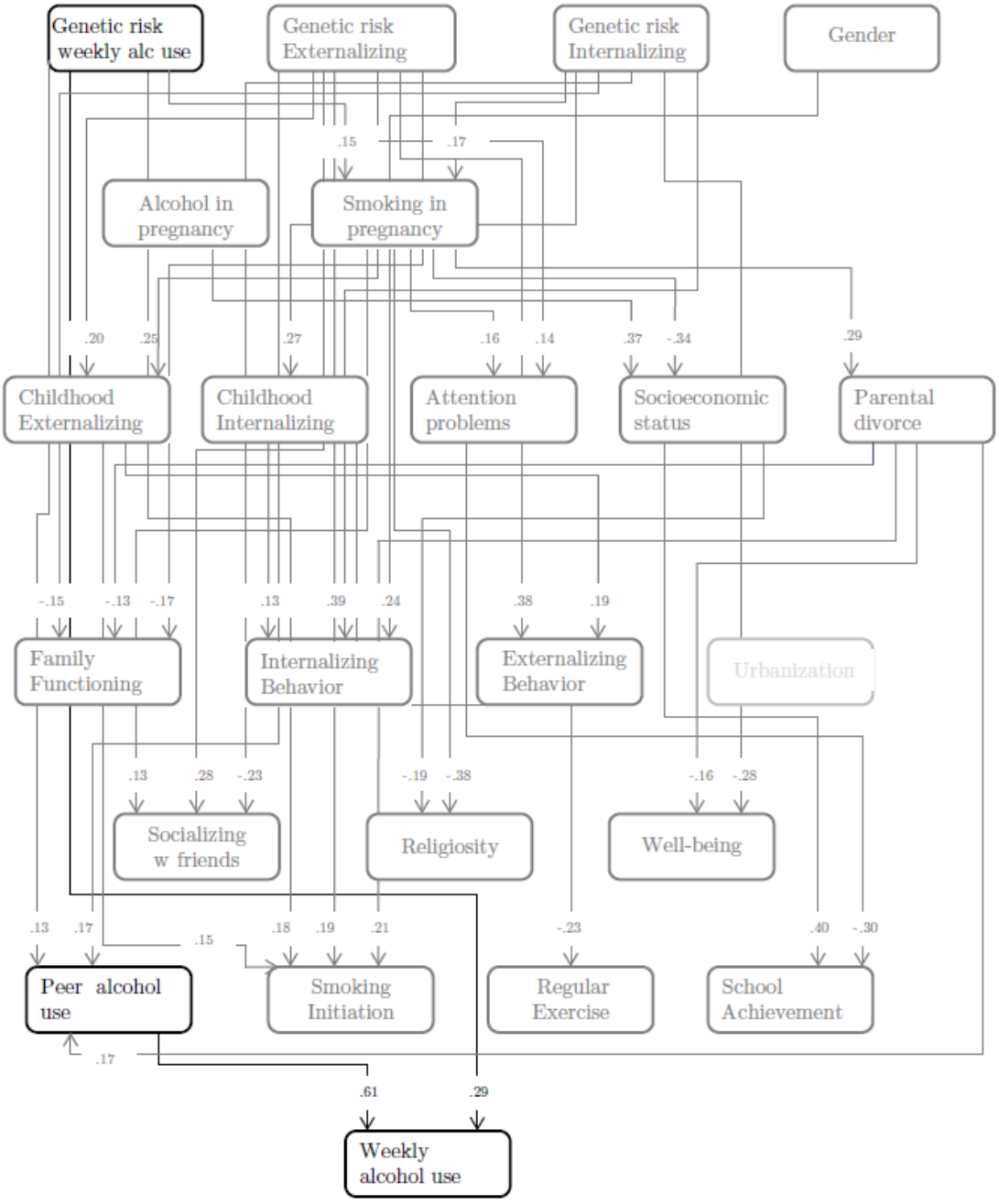

FIGURE 1 Standardized partial regression coefficients estimated under the best fitting model predicting weekly alcohol use. Variables are grouped by developmental timing (genetic risk, prenatal, childhood, and adolescence). Pathways directly related to alcohol initiation are depicted in black, the indirect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

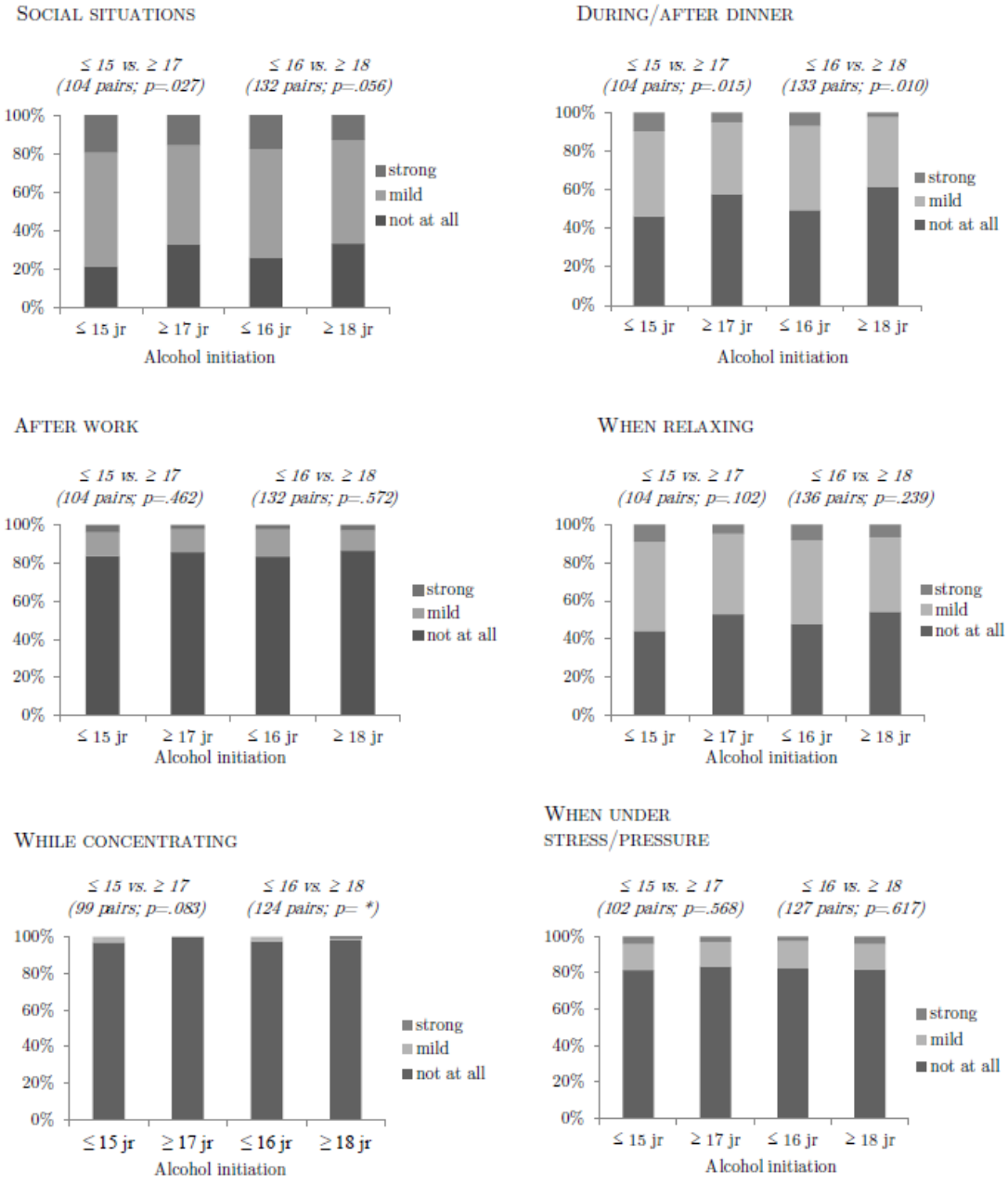

FIGURE 2 Situation-specific urges to drink in twins discordant for early alcohol initiation. Lifelong abstainers were excluded from the analyses. * p-value could not be computed due to empty cells.

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	