



A serotonin transporter polymorphism (5-HTTLPR) predicts the development of adolescent alcohol use

Carmen S. van der Zwaluw^{a,*}, Rutger C.M.E. Engels^a, Ad A. Vermulst^a, Richard J. Rose^b,
Robbert J. Verkes^c, Jan Buitelaar^c, Barbara Franke^{c,d}, Ron H.J. Scholte^a

^a Behavioural Science Institute, P.O. Box 9014, 6500 HE Nijmegen, Radboud University, Nijmegen The Netherlands

^b Department of Psychological & Brain Sciences, Indiana University, Bloomington, IN 47405, USA

^c Radboud University Nijmegen Medical Centre, Department of Psychiatry, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

^d Radboud University Nijmegen Medical Centre, Department of Human Genetics, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

ARTICLE INFO

Article history:

Received 11 January 2010

Received in revised form 28 May 2010

Accepted 3 June 2010

Available online 3 July 2010

Keywords:

Alcohol
Adolescence
5-HTTLPR
Development
Serotonin transporter

ABSTRACT

Background: Because the effects of susceptibility genes on alcohol use may differ as a function of age throughout adolescence and young adulthood, prospective study designs, in addition to cross-sectional ones are needed in genetic association studies. The short, low activity allele of a polymorphism (5-HTTLPR) in the serotonin transporter gene (*SLC6A4*) has been related to alcohol dependence. In the current study we tested whether 5-HTTLPR genotype was associated with adolescent alcohol use both cross-sectionally and longitudinally.

Methods: Non-regular drinkers ($n=202$) were selected from Dutch, nationwide sample of adolescents (mean age 13.4 at baseline) who were assessed across five annual waves. Latent growth curve modeling was applied to examine individual development of alcohol use over time, by estimating the initial level of alcohol use at Wave 2 (intercept), and the rate of change in alcohol use across time (slope).

Results: The 5-HTTLPR short allele predicted adolescent's growth (slope) in alcohol use over time. Adolescents with the 5-HTTLPR short allele showed larger increase in alcohol consumption than those without the 5-HTTLPR short allele. 5-HTTLPR genotype was not related to the initial level (intercept) of alcohol consumption. In all analyses we controlled for sex and personality.

Conclusions: To gain more insight into the etiological role of genetic determinants of adolescent alcohol use, developmental approaches that distinguish between onset and continuation of drinking should be applied.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

In most Western societies, alcohol use is widespread among adolescents and young adults. The average adolescent drinks his first alcoholic beverage at the age of 13 (Hibell et al., 2004). Subsequently, alcohol use increases during adolescence and young adulthood (Duncan et al., 1997), until it stabilizes or decreases at the age of approximately 25 (Poelen et al., 2005). Most youngsters experiment with alcohol at one point during adolescence. Some, however, progress easily into elevated levels of drinking (Duncan et al., 1997), putting themselves at risk for deleterious problems, such as work-related problems, physical and mental health problems, and drug addiction later in life (e.g., Duncan et al., 1997; Newcomb, 1992). In the search for etiological factors, twin studies have con-

vincingly demonstrated genetic influences on regular alcohol use, alcohol dependence, and on the transition from onset of alcohol use to alcohol dependence (e.g., Goldman et al., 2005; Liu et al., 2004; Pagan et al., 2006).

The serotonergic system has been one of the key targets in examining the genetic bases for alcohol use and dependence. Although the exact role of serotonin (5-HT) in alcohol use and abuse remains unclear, evidence exists that serotonin deficits in the brain result in alcohol-seeking behavior in humans and animals (for overviews, see LeMarquand et al., 1994a,b). Serotonin availability in the brain is partly influenced by the serotonin transporter protein (5-HTT), which terminates synaptic serotonergic activity by the reuptake of serotonin into presynaptic neurons. A 44-bp insertion/deletion polymorphism (5-HTTLPR) located in the promoter region of the serotonin transporter gene (*SLC6A4/SERT/5-HTT*) has been a prime candidate for genetic association studies on alcohol abuse risk. Relative to the 5-HTTLPR long (l) allele, the short (s) allele decreases the transcriptional activity of 5-HTTLPR, resulting in reduced

* Corresponding author. Tel.: +31 24 3612076; fax: +31 24 3612776.
E-mail address: C.vanderZwaluw@bsi.ru.nl (C.S. van der Zwaluw).

5-HTT binding, expression and 5-HT uptake in lymphoblasts (Hariri and Holmes, 2006; Lesch et al., 1996). In a meta-analysis on 17 studies involving 3489 adult alcohol-dependent subjects and 2325 adult control participants, Feinn et al. (2005) showed that the 5-HTTLPR s allele was significantly associated with alcohol dependence (OR = 1.18, 95% CI = 1.03–1.33).

With respect to the association between 5-HTTLPR genotype and alcohol use in adolescence and young adulthood, findings are mixed. Some studies have shown relationships between the 5-HTTLPR s allele and early alcohol use (Kaufman et al., 2007), binge drinking (Herman et al., 2003, 2005), and drinking to get drunk (Herman et al., 2003). Nilsson et al. (2005) found that adolescents with the heterozygous 5-HTTLPR s/l genotype consumed more alcohol than those with the s/s or l/l genotypes. Others, however, did not find an effect of 5-HTTLPR genotype on drinking behavior (Gacek et al., 2008; Hopfer et al., 2005). Guo et al. (2007) examined both adolescents and young adults, and found that the 5-HTTLPR s allele was significantly related to higher levels of alcohol use in young adulthood, but not in adolescence. Lastly, Olsson et al. (2005) even found a protective effect of the 5-HTTLPR s allele on binge drinking in young adulthood.

As the magnitude of genetic influences on substance use differs with age and stage of substance use (Kendler et al., 2008; Pagan et al., 2006), a developmental perspective needs to be adopted (Van der Zwaluw and Engels, 2009). Because cross-sectional designs do not distinguish between onset and continuation of drinking, genetic effects may be overlooked, especially if they differ by stage of use. With sophisticated statistical approaches like growth curve modeling at hand (Bollen and Curran, 2006), differential effects of genetic markers on different stages of use can be assessed. So far, however, no longitudinal research has tested the role of 5-HTTLPR genotype in actual development of alcohol consumption in adolescence.

In the current study we examined the association between the 5-HTTLPR genotype and development of alcohol use throughout adolescence and young adulthood, fitting a longitudinal growth curve model to five waves of annual assessments. Because both alcohol misuse and the 5-HTTLPR genotype have been frequently associated with affective disorders and neuroticism (e.g., Greenberg et al., 2000; Lesch, 2005; Schinka et al., 2004; Sullivan et al., 2005), we controlled for depressive feelings and Big Five personality traits (i.e., extraversion, agreeableness, conscientiousness, neuroticism, and openness) in our analyses.

2. Methods

2.1. Participants and procedure

Participants were 428 Dutch adolescents (52% female) of mainly Caucasian descent, with an average age of 13.4 years (range: 13–15 years, SD = .50) at Time 1 (T1). The adolescents were recruited for the longitudinal Family and Health study (see for more details on the sample selection, Harakeh et al., 2005; Van der Vorst et al., 2005). Across the five assessments, 416 (97%), 401 (94%), 338 (79%), and 305 (71%) adolescents participated at Time 2 (T2), Time 3 (T3), Time 4 (T4), and Time 5 (T5), respectively. At T4, saliva samples were collected using Oragene containers (Oragene, DNA Genotek Inc., Ottawa, Ontario, Canada). Of the 338 adolescents who participated at T4, 311 consented to genotyping. Attrition analyses were conducted to examine whether adolescents who remained participants in the study at T5 ($n = 305$; 71%) differed from those who did not (drop-outs; $n = 123$; 29%). No significant differences ($p > .05$) in alcohol use at T1, gender, ethnicity or age were found between participating and drop-out adolescents. Participating adolescents did report a higher level of education at T1 than drop-outs ($\chi^2(5) = 20.18, p = .001$).¹

¹ The Dutch school system differs from that of other countries in Europe, Asia or the US. In the Netherlands, after primary school (at the age of 12), children can continue their school career in four different levels of secondary education. They are selected for one of these levels, or a combination of two levels, mainly based on their achievements in primary school. The different levels are comparable with the different tracks of a public high school in the US, although they may not be completely interchangeable. In the current study the level of education was measured with one

Table 1
Descriptives.

	Non-regular drinkers T1 (N = 202)
Gender, male	97 (48%)
Education, median (range)	3 (1–4)
5-HTTLPR l/l	65 (32%)
5-HTTLPR s/l	95 (47%)
5-HTTLPR s/s	42 (21%)
Alcohol use (# glasses) T1	0
Extraversion, mean (SD)	4.73 (1.03)
Agreeableness, mean (SD)	5.35 (.76)
Conscientiousness, mean (SD)	4.07 (1.09)
Emotional stability, mean (SD)	4.17 (.99)
Openness, mean (SD)	4.79 (.85)
Depressive feelings, mean (SD)	2.45 (.69)

To examine whether model variables predicted the transition from non-regular drinking to initiation and subsequent development of regular alcohol consumption, we selected those adolescents at T1 who were non-regular drinkers (i.e., those who had stayed abstinent in the week preceding the questionnaire) (cf. Van der Zwaluw et al., 2009). From this sample of non-regular drinkers ($n = 290$), genetic data were available for two-third of the sample ($n = 202$). Approval for the data collection was obtained from the Central Committee on Research Involving Human Subjects in The Netherlands.

2.2. Genotyping

Genotyping of the 5-HTTLPR polymorphism in the *SLC6A4* (5-HTT, *SERT*) gene was performed by simple sequence length analysis. PCR was carried out on 50 ng genomic DNA using 10 pmol of forward primer (5'-GGCGTTGCCCTCTGAATGC-3') and 10 pmol reverse primer (5'-GAGGGACTGAGCTGGACAACCAC-3'), 0.25 mM dNTPs, 0.5 U Taq DNA polymerase (Invitrogen, Breda, The Netherlands) in a PCR buffer containing 0.3 M Tris-HCl (pH 8.5), 75 mM ammonium sulfate and 7.5 mM MgCl₂. The cycling conditions for the PCR started with 5 min at 92 °C, followed by 35 cycles of 1 min at 92 °C, 1 min at the optimized annealing temperature (57.5 °C), and 1 min 72 °C, then followed by an extra 5 min at 72 °C. PCR products were analyzed on a 2% agarose gel. The amplification yielded distinct bands at 484 bp (short "s" allele) and 528 bp (long "l" allele). 5% duplicates and blanks were taken along as quality controls during genotyping. No deviations from Hardy-Weinberg equilibrium (HWE) were detected ($p = .96$), as was estimated with the GENEPOP Markov-Chain Monte-Carlo approximation of the exact test (Raymond and Rousset, 1995). To maximize the power of the analyses, 5-HTTLPR genotype was dummy-coded into 1 (long/long) and 2 (short/long and short/short) (cf. Greenberg et al., 2000; Schinka et al., 2004; Skowronek et al., 2006).

2.3. Measures

2.3.1. Adolescent alcohol use. Alcohol use of adolescents was self-reported and measured with one measure, which consisted of four sub-questions (Engels et al., 1999): 'How many alcoholic drinks did you consume in the past week at home on weekdays?', 'How many alcoholic drinks did you consume in the past week at home during the weekend?', 'How many alcoholic drinks did you consume in the past week when you were out on weekdays?', and 'How many alcoholic drinks did you consume in the past week when you were out during the weekend?'. Asking about these four specific situations forces respondents to actively 'search' their memory, which is supposed to increase the reliability of their response (Van der Vorst et al., 2006). The four answers were summed to represent the amount of alcohol consumed in the past week. This measure has been used frequently and reliably in other studies (e.g., Spijkerman et al., 2007; Van der Vorst et al., 2006; Van Zundert et al., 2006), and it has shown high validity (Bot et al., 2005). Because of the skewed distribution of this variable, total scores were categorized into 7 groups (0 = 0 glasses, 1 = 1–2 glasses, 2 = 3–5 glasses, 3 = 6–10 glasses, 4 = 11–20 glasses, 5 = 21–30 glasses, 6 = 31 glasses and above) (cf. Van der Zwaluw et al., 2008).

2.3.2. Personality. Adolescent personality was measured at T1 with the Quick Big Five (Vermulst and Gerris, 2005). Adolescents had to indicate to what extent 30 personality items (e.g., nervous, quiet, friendly) fitted their own personality on a scale from 1 'does not fit at all' to 7 'fits totally'. Internal consistencies for the five personality dimensions (extraversion, agreeableness, conscientiousness, neuroticism, and openness) were sufficient ($.65 \leq \alpha \leq .84$).

question: What kind of education do you currently follow? Because the population study of Monshouwer et al. (2008) showed that substance use is significantly higher among Dutch pupils who follow lower educational levels, we controlled for this possibly confounding factor in the analyses.

Table 2
Pearson's correlations.

	1	2	3	4	5	6	7	8	9	10	11
1. Alcohol time 2											
2. Alcohol time 3	.37***										
3. Alcohol time 4	.22**	.42***									
4. Alcohol time 5	.02	.36***	.45***								
5. 5-HTTLPR	-.09	.08	.02	.09							
6. Extraversion	.04	.05	.01	.07	.03						
7. Conscientiousness	-.08	-.05	-.02	.00	.04	.01					
8. Agreeableness	-.08	-.06	-.14*	-.17*	.17*	.20***	.29***				
9. Emotional stability	.01	-.05	-.01	.05	-.08	.45***	.02	-.02			
10. Openness	.04	.04	-.03	-.03	-.00	.09	.30***	.52***	-.07		
11. Depressive feelings	-.04	.07	-.03	-.03	-.03	-.31	-.17**	-.15*	-.47***	-.12*	

Note: 5-HTTLPR: 1 = long/long genotype, 2 = long/short and short/short genotypes.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

2.3.3. *Depressive feelings.* The Depressive Mood List (Kandel and Davies, 1982) was used to measure depressive feelings at T1. Adolescents reported how often they had experienced negative feelings (e.g., feeling unhappy, sad or depressed) in the past 12 months, on a scale from 1 'never' to 5 'always'. Cronbach's alpha was .77.

2.4. Statistical analyses

Descriptives and Pearson's correlation coefficients of the model variables were calculated with the 17th version of the Statistical Package of Social Sciences (SPSS). Structural equation modeling (SEM) in Mplus package 5.1 (Muthén and Muthén, 1998–2007) was used to carry out subsequent analyses. With a latent growth curve model, we examined individual development of alcohol use over time, by estimating the initial level of alcohol use at T2 (intercept), and the rate of change in alcohol use from T2 to T5 across time (slope). In the linear latent growth curve model, the relation between the observed repeated measures and the latent factors intercept and slope is expressed in the factor loading matrix. The loadings are fixed to 1 for the intercept factor and fixed to 0, 1, 2, and 3 for the slope factor. In this way individual variation in both the onset of alcohol consumption and the progression of drinking is captured. Adolescent alcohol use, however, did not augment in a linear way, causing an insufficient model fit. Adding cubic or quadratic trends to the model did not improve model fit. We applied an alternative method of non-linear curve fitting by estimating factor loadings of the slope factor: the freed-loading latent growth curve model. The first loading was set to zero, and the second to 1 to set the metric of the latent factor. The remaining two factor loadings were freely estimated (Bollen and Curran, 2006, p. 98). In a non-linear model such as this, the intercept can still be interpreted as a starting value. The slope, however, is time-dependent, meaning that a positive mean slope indicates that the trajectory is increasing over time as long as the corresponding factor loading is positive (Bollen and Curran, 2006, p. 102). Inspection of factor loadings shows the exact development from time point to time point. To deal with missing values, the model parameters were estimated with the Full Information Maximum Likelihood (ML) estimator.

In the first growth model without predictors, the intercept and slope of adolescent alcohol use were determined. In the second model we examined whether gender, education (at T1), and 5-HTTLPR genotype predicted onset and slope of alcohol use. The interaction between gender and 5-HTTLPR genotype was added in a second step to examine whether the model differed between males and females. In an additional model, we controlled for possible confounding effects of the adolescent's personality and depressive feelings. Again, the interaction between gender and 5-HTTLPR genotype was added in the second step. Model fit was assessed by the chi-square test, the Comparative Fit Index (CFI; Bentler, 1990) and the Root Mean Square of Approximation (RMSEA; Byrne, 1998).

3. Results

3.1. Descriptives and correlations

Descriptives of model variables are shown in Table 1. Genotype frequencies did not differ significantly between males and females ($\chi^2(2) = 3.07$, n.s.) and were consistent with frequency distributions in other Caucasian samples (Covault et al., 2007; Nilsson et al., 2005; Olsson et al., 2005). Table 2 shows that 5-HTTLPR genotype was not significantly associated with alcohol use at any time point ($-.09 \leq r \leq .09$, n.s.). Alcohol use was moderately correlated with alcohol consumption at later waves ($.22 \leq r \leq .45$, $p < .05$). Agreeableness was negatively related with alcohol use at T4 and T5

($r = -.14$ and $r = -.17$ respectively, $p < .05$) and positively with 5-HTTLPR genotype ($r = .17$, $p < .05$). The other personality traits and depressive feelings showed no significant correlations with alcohol use or 5-HTTLPR genotype (see Table 2).

3.2. Growth models

The basic growth model without predictors showed a reasonable fit to the data (see Table 3 for all model fits). The mean intercept and slope were significant ($I = .76$ and $S = .62$, $p < .001$), indicating that the average starting value was significantly different from zero, and that levels of adolescent alcohol use increased significantly over time. Slope factor loadings were .00, 1.00, 2.46, and 2.51 at T2, T3, T4, and T5, respectively, which would have been 0, 1, 2, and 3 in a linear model. These slope factor loadings indicate that the increase in alcohol use from T3 to T4 was substantial (1.46), while growth from T4 to T5 was close to zero (.05). Variances of intercept and slope were .90 ($p < .001$) and .32 ($p < .05$), respectively, indicating individual differences in intercept and nonlinear rate of change.

The second model showed that neither adolescents' gender, level of education nor 5-HTTLPR genotype was significantly related to alcohol use intercept (Table 3). Also the interaction between 5-HTTLPR genotype and gender was not significant, indicating that the model was similar for males and females. Adolescent gender and 5-HTTLPR significantly predicted development (slope) of adolescent alcohol use ($\beta = -.32$, $p < .001$ and $\beta = .20$, $p < .05$, respectively); males and adolescents with the 5-HTTLPR s allele showed larger increases in alcohol consumption than girls and adolescents without the 5-HTTLPR s allele. Level of education and the interaction between gender and 5-HTTLPR genotype were not significantly related to drinking development.

Model three showed that adding adolescents' personality and depressive feelings to the model did not change the effect of 5-HTTLPR genotype on the development of alcohol use, which remained positive and significant ($\beta = .22$, $p < .05$). Depressive feelings and personality did not significantly predict intercept or slope of adolescent alcohol use (Table 3).²

² The analyses were carried out on a subsample of adolescents who reported to be non-regular drinkers at the age of thirteen, to allow for the starting point and the development of regular drinking over time to be predicted. Additionally, the same analyses were carried out on the entire sample of genotyped adolescents ($n = 311$). However, 5-HTTLPR genotype did not predict the slope of adolescents' alcohol use over time in this sample. An explanation for this might be that adolescents who are already weekly drinkers at the age of 13, may be considered a somewhat more deviant subgroup of early onset drinkers. For example, a study by McGue et al. (2001) showed that age at first drink is associated with a wide range of psychopathology and behavioral disinhibition, such as illicit drug use and dependence, conduct disorder, and hyperactivity/impulsivity. So it is likely that other (genetic) factors play a role in

Table 3
Growth model, standardized estimates.

	Basic model		5-HTTLPR		Personality	
	I	S	I	S	I	S
Growth estimates	.763	.623				
Step 1						
Gender			-.02	-.32***	.01	-.32***
Education			-.01	.13	-.01	.15
5-HTTLPR			-.09	.20*	-.07	.22*
Extraversion					.08	.08
Agreeableness					-.15	-.18
Conscientiousness					-.10	.11
Emotional stability					-.05	-.05
Openness					.14	.02
Depressive feelings					-.04	.04
Step 2						
Gender × 5-HTTLPR			-.05	-.15	-.05	-.18
R ²			.01	.16*	.04	.19*
χ ² (df)	9.79 ^a (3)		15.90 (9)		30.23 (21)	
CFI	.94		.95		.93	
RMSEA	.09		.05		.04	

Note: I = intercept, S = slope, CFI = Comparative Fit Index, RMSEA = Root Mean Square of Approximation. Gender: 1 = male, 2 = female. 5-HTTLPR: 1 = long/long genotype, 2 = long/short and short/short genotypes.

^a $p < .05$.

*** $p < .001$.

4. Discussion

Because the effects of susceptibility genes on alcohol use may differ as a function of age throughout adolescence and young adulthood (Guo et al., 2007; Kendler et al., 2008), genetic association studies including a developmental design are highly informative (Van der Zwaluw et al., 2009). Therefore, we tested whether a polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene influenced the development of adolescent alcohol use over time. Results showed that adolescents with the 5-HTTLPR short (s) allele developed higher levels of alcohol consumption over time than long (l) allele carriers. 5-HTTLPR genotype did neither affect the initial level of adolescent alcohol use, nor was it associated with alcohol consumption at any separate time point, when examined cross-sectionally.

Although several cross-sectional studies have reported an increased risk for alcohol (ab)use in youths with the 5-HTTLPR s allele (Herman et al., 2003, 2005; Nilsson et al., 2005), negative results from other studies (e.g., Gacek et al., 2008; Hopfer et al., 2005) do not enable us to draw firm conclusions about the role of 5-HTTLPR genotype in adolescents' and young adults' alcohol use. The current study extends the existing literature by showing that the 5-HTTLPR polymorphism is associated with the development of alcohol use from early to late adolescence. We are not aware of other studies that have examined relationships between genetic polymorphisms in the serotonin transporter gene and adolescents' alcohol use from a developmental perspective, leaving ample room for future prospective studies.

It is important to emphasize that we did not find an effect of 5-HTTLPR genotype on the initial level (intercept) of alcohol use, while the 5-HTTLPR s allele did influence the course of alcohol use throughout adolescence. This implies that cross-sectional levels of behavior and its longitudinal development should not be considered similar, as they possibly are preceded by different biological mechanisms. Several scholars have proposed that (early) changes in

serotonin homeostasis are involved in the physiopathology of later psychiatric diseases, such as mood disorders and alcohol addiction (Ansoorge et al., 2004; Gaspar et al., 2003). These psychiatric disorders may reflect changes in brain structures or mis-wiring of brain connections caused by genetically driven variation in serotonin functioning (Hariri and Holmes, 2006). As the 5-HTTLPR low activity alleles (s and l_g) result in a relative loss of 5-HTT functioning and in decreased 5-HT reuptake, this may influence brain structures and brain functioning. Neuro-imaging studies have demonstrated that 5-HTTLPR s allele carriers show reduced grey matter volume in limbic brain areas, in the prefrontal cortex and in structures connecting the amygdala to prefrontal areas (Frodl et al., 2008; Pezawas et al., 2005). This might result in abnormalities in functional connectivity between the prefrontal cortex and the amygdala during emotional processing (Hariri and Holmes, 2006). Pacheco et al. (2009) found that the 5-HTTLPR low activity alleles (s and l_g) were associated with a decrease in white matter in the pathway that connects the anterior temporal lobe and the amygdala to the inferior parts of the frontal lobes (for a summary, see Jasinska and Perkins, 2009). In short, these findings indicate that the 5-HTTLPR s allele affects several brain structures that are engaged in (a.o.) anxiety and emotion regulation. Disorders in these areas have, in turn, been associated with alcohol abuse and dependence in numerous population studies (e.g., Davidson and Ritson, 1993; Kessler et al., 1997; Kushner et al., 1990; Schuckit, 1986).

Changes in brain structures and functioning may also be caused by serotonergic gene–environment interactions (Young et al., 2007). For example, Andersen and Teicher (2009) proposed that (early) life stress predisposes individuals to an increased risk for substance abuse via a highly reactive hypothalamic–pituitary–adrenal axis (HPA) and via structural changes in brain structures such as the hippocampus, nucleus accumbens and prefrontal cortex. Andersen and Teicher (2009) also state that a certain level of maturation of the brain is needed for the effects of the stress exposure to manifest, making adolescence, in which particularly the prefrontal lobes and white matter cohesion are still maturing (e.g., Casey et al., 2000), a very vulnerable period for the initiation of substance addiction. In addition, evidence is emerging that indicates that 5-HTTLPR s allele carriers are less able to cope with stress than individuals homozygous for the l

the etiology of alcohol consumption of this subgroup of adolescents. Future studies should focus on more risk drinking behaviors, such as early onset and alcohol abuse and dependence.

allele. In the study of Caspi et al. (2003) 5-HTTLPR s allele carriers exhibited a higher risk for depression if they experienced several stressful life events. Also among rhesus macaques, s allele carriers showed an increased risk for depression if they were reared in a stressful environment (Barr et al., 2004). Also on a neurological level it has been found that persons with the 5-HTTLPR low activity allele respond differently to environmental stimuli. For example, s allele carriers generally show a heightened amygdala reactivity to fearful and angry faces, compared to individuals with the l/l genotype (Hariri et al., 2002, 2005). This exaggerated amygdala response has been shown in healthy individuals, as well as in persons with panic disorder or social phobia (see Hariri and Holmes (2006) for an overview).

Although we controlled for depressive feelings and neuroticism in our analyses it remains plausible that the mechanism behind the 5-HTTLPR-alcohol association is at least partly represented by developmental serotonin-related changes in the emotional system in the teenage years. Future longitudinal studies examining the relationships between 5-HTTLPR genotypes and the development of adolescents' alcohol use are likely to shed more light on these puzzles, for example by including (physiological) measures of stress response as an endophenotype (Lesch, 2005).

The results of our study should be interpreted in the context of its limitations. First, as the adolescents self-reported on their alcohol consumption, under- or over-reporting may have occurred. Engels et al. (2007), however, showed that self-reports on alcohol use are a reliable source of information. Additionally, the assessment of alcohol consumption was limited to a single measure (i.e. intensity of alcohol use in the past week). We do not know whether the results translate to other measures of (risky) alcohol use, such as binge drinking. Future studies should attempt to elaborate on this issue. Also, in the growth curve analysis, gender, level of education, and 5-HTTLPR genotype together explained 16% of the variance of the development of adolescent alcohol use over time. Although this is similar to the amount of variance explained in other studies (e.g., 11% in Nilsson et al., 2005), it should be recognized that the effect of the examined polymorphism is small and should not be over-interpreted. Further, we cannot rule out that the 5-HTTLPR variable number of tandem repeats (VNTR) polymorphism is in linkage disequilibrium with another functional variant in *SLC6A4*, which might be causing the significant effect on adolescent alcohol use. Gelernter et al. (1999) found the 5-HTTLPR VNTR to be in linkage disequilibrium with the *STin2* VNTR polymorphism of *SLC6A4* in several populations from different ancestries. In addition, it is very plausible that other genetic loci and environmental factors add to the risk for alcohol misuse during adolescence by epistasis (e.g., Herman et al., 2005) and gene-environment effects (e.g., Nilsson et al., 2005). The next step should be to examine these interaction effects in a longitudinal design. For example, Dick et al. (2009) found that the *GABRA2* gene interacted with parental monitoring in influencing different developmental trajectories of adolescents' externalizing behavior.

In conclusion, in a multi-wave longitudinal study, we found that the short allele of the 5-HTTLPR polymorphism is associated with a steeper increase in adolescent alcohol use over time than the 5-HTTLPR long allele. As this is, to our best knowledge, the first study to examine these effects prospectively with a latent growth curve method, replication studies are essential. Future longitudinal studies will be needed to comprehend how the risk for alcohol misuse unfolds across developmental stages.

Role of funding source

Funding for this study was provided by the Dutch Organization for Scientific Research (NWO) Grant 400-05-051. NWO had

no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Contributors

Rutger Engels contributed to writing the introduction and discussion sections. Ad Vermulst contributed to the statistical analysis and to writing the methods section. Barbara Franke contributed to the genotyping analyses and interpretation of the genetic data. Richard Rose contributed to the methods and results sections. All authors contributed to the editing and final review of the manuscript. All authors approved the final paper.

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgements

We wish to thank Remco Makkinje, Marlies Naber and Johanne Groothuisink for planning, setting up and carrying out molecular analysis of 5-HTTLPR.

References

- Andersen, S.L., Teicher, M.H., 2009. Desperately driven and no brakes: developmental stress exposure and subsequent risk for substance abuse. *Neurosci. Biobehav. Rev.* 33, 516–524.
- Ansgorge, M.S., Zhou, M., Lira, A., Hen, R., Gingrich, J.A., 2004. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* 306, 879–881.
- Barr, C.S., Newman, T.K., Lindell, S., Shannon, C., Champoux, M., Lesch, K.P., Suomi, S.J., Goldman, D., Higley, D., 2004. Interaction between serotonin transporter gene variation and rearing condition in alcohol preference and consumption in female primates. *Arch. Gen. Psychiatry* 61, 1146–1152.
- Bentler, P.M., 1990. Comparative fit indexes in structural equation models. *Psych. Bull.* 107, 238–246.
- Bollen, K.A., Curran, P.J., 2006. *Latent Curve Models. A Structural Equation Perspective*. John Wiley and Sons, Hoboken, New Jersey.
- Bot, S.M., Engels, R.C., Knibbe, R.A., 2005. The effects of alcohol expectancies on drinking behaviour in peer groups: observations in a naturalistic setting. *Addiction* 100, 1270–1279.
- Byrne, B.M., 1998. *Structural Equation Modeling with LISREL, PRELIS and SIMPLIS: Basic Concepts, Applications, and Programming*. Lawrence Erlbaum Associates, NJ, Mahwah.
- Casey, B.J., Giedd, J.N., Thomas, K.M., 2000. Structural and functional brain development and its relation to cognitive development. *Biol. Psychol.* 54, 241–257.
- Caspi, A., Sugden, K., Moffit, T.E., Taylor, A., Craig, I.W., Harrington, H.L., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301, 386–389.
- Covault, J., Tennen, H., Armeli, S., Conner, T.S., Herman, A.I., Cillesen, A.H.N., Kranzler, H.R., 2007. Interactive effects of the serotonin transporter 5-HTTLPR polymorphism and stressful life events on college student drinking and drug use. *Biol. Psychiatry* 61, 609–616.
- Davidson, K.M., Ritson, E.B., 1993. The relationship between alcohol dependence and depression. *Alcohol. Alcohol.* 28, 147–155.
- Dick, D.M., Latendresse, S.J., Lansford, J.E., Budde, J.P., Goate, A., Dodge, K.A., Pettit, G.S., Bates, J.E., 2009. Role of *GABRA2* in trajectories of externalizing behavior across development and evidence of moderation by parental monitoring. *Arch. Gen. Psychiatry* 66, 649–657.
- Duncan, S.C., Alpert, A., Duncan, T.E., Hops, H., 1997. Adolescent alcohol use development and young adult outcomes. *Drug Alcohol Depend.* 49, 39–48.
- Engels, R.C.M.E., Knibbe, R.A., Drop, M.J., 1999. Why do late adolescents drink at home? A study on psychological well-being, social integration and drinking context. *Addict. Res.* 7, 31–46.
- Engels, R.C.M.E., Van der Vorst, H., Dekovic, M., Meeus, W., 2007. Correspondence in collateral and self reports on alcohol consumption: a within family analysis. *Addict. Behav.* 32, 1016–1030.
- Feinn, R., Nellissery, M., Kranzler, H.R., 2005. Meta-analysis of the association of a functional serotonin transporter promoter polymorphism with alcohol dependence. *Am. J. Med. Genet. B* 133, 79–84.
- Frodl, T., Koutsouleris, N., Bottlender, R., Born, C., Jäger, M., Mergenthaler, M., Scheuerecker, J., Zill, P., Baghai, T., Schule, C., Rupprecht, R., Bondy, B., Reiser, M., Moller, H.-J., Meisenzahl, E.M., 2008. Reduced gray matter brain volumes are associated with variants of the serotonin transporter gene in major depression. *Mol. Psychiatry* 13, 1093–1101.

- Gacek, P., Conner, T.S., Tennen, H., Kranzler, H.R., Covault, J., 2008. Tryptophan hydroxylase 2 gene and alcohol use among college students. *Addict. Biol.* 13, 440–448.
- Gaspar, P., Cases, O., Maroteaux, L., 2003. The developmental role of serotonin: news from mouse molecular genetics. *Nat. Neurosci.* 4, 1002–1012.
- Gelernter, J., Cubells, J.F., Kidds, J.R., Pakstic, A.J., Kidd, K.K., 1999. Population studies of polymorphisms of the serotonin transporter protein gene. *Am. J. Med. Genet.* 88, 61–66.
- Goldman, D., Oroszi, G., Ducci, F., 2005. The genetics of addictions: uncovering the genes. *Nat. Rev. Gen.* 6, 521–532.
- Greenberg, B.D., Li, Q., Lucas, F.R., Hu, S., Sirota, L.A., Benjamin, J., Lesch, K.P., Hamer, D., Murphy, D.L., 2000. Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *Am. J. Med. Genet.* 96B, 202–216.
- Guo, G., Wilhelmsen, K., Hamilton, N., 2007. Gene–lifecourse interaction for alcohol consumption in adolescence and young adulthood: five monoamine genes. *Am. J. Med. Genet.* 144B, 417–423.
- Harakeh, Z., Scholte, R.H.J., De Vries, H., Engels, R.C.M.E., 2005. Parental rules and communication: their association with adolescent smoking. *Addiction* 100, 862–870.
- Hariri, A.R., Drabant, E.M., Munoz, K.E., Kolachana, B.S., Mattay, V.S., Egan, M.F., Weinberger, D.R., 2005. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch. Gen. Psychiatry* 62, 146–152.
- Hariri, A.R., Holmes, A., 2006. Genetics of emotional regulation: the role of the serotonin transporter in neural function. *Trends Cogn. Sci.* 10, 182–191.
- Hariri, A.R., Mattay, V.S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin transporter genetic variation and the response of the human amygdala. *Science* 297, 400–403.
- Herman, A.I., Kaiss, K.M., Ma, R., Philbeck, J.W., Hasan, A., Dasti, H., DePetrillo, P.B., 2005. Serotonin transporter promoter polymorphism and monoamine oxidase type A VNTR allelic variants together influence alcohol binge drinking risk in young women. *Am. J. Med. Genet.* 133B, 74–78.
- Herman, A.I., Philbeck, J.W., Vasilopoulos, N.L., Depetrillo, P.B., 2003. Serotonin transporter promoter polymorphism and differences in alcohol consumption behaviour in a college student population. *Alcohol. Alcohol.* 38, 446–449.
- Hibell, B., Andersson, B., Bjarnasson, T., Ahlström, S., Balakireva, O., Kokkevi, A., Morgen, M., 2004. The ESPAD report 2003. Alcohol and other drug use among students in 35 European countries. The Swedish council for information on alcohol and other drugs (CAN), council of Europe, co-operation group to combat drug abuse and illicit trafficking in drugs (Pompidou group). Modintryckoffset AB, Stockholm.
- Hopfer, C.J., Timberlake, D., Haberstick, B., Lessem, J.M., Ehringer, M.A., Smolen, A., Hewitt, J.K., 2005. Genetic influence on quantity of alcohol consumed by adolescents and young adults. *Drug Alcohol Depend.* 78, 187–193.
- Jasinska, A.J., Perkins, S.C., 2009. Impact of the tri-allelic serotonin transporter polymorphism on the white-matter tract connecting the amygdala and the prefrontal cortex. *J. Neurosci.* 29, 10461–10462.
- Kandel, D.B., Davies, M., 1982. Epidemiology of depressive mood in adolescents: an empirical study. *Arch. Gen. Psychiatry* 39, 1205–1212.
- Kaufman, J., Yang, B., Douglas-Palumber, H., Crouse-Artus, M., Lipschitz, D., Krystal, J., Gelernter, J., 2007. Genetic and environmental predictors of early alcohol use. *Biol. Psychiatry* 61, 1228–1234.
- 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. *Arch. Gen. Psychiatry* 65, 674–682.
- Kessler, R.C., Crum, R.M., Warner, L.A., Nelson, C.B., Schulenburg, J., Anthony, J.C., 1997. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. *Arch. Gen. Psychiatry* 54, 313–321.
- Kushner, M.G., Sher, K.J., Beitman, B.D., 1990. The relation between alcohol problems and the anxiety disorder. *Am. J. Psychiatry* 147, 685–695.
- LeMarquand, D., Phil, R.O., Benkelfat, C., 1994a. Serotonin and alcohol intake, abuse and dependence: clinical evidence. *Biol. Psychiatry* 36, 326–337.
- LeMarquand, D., Phil, R.O., Benkelfat, C., 1994b. Serotonin and alcohol intake, abuse and dependence: findings of animal studies. *Biol. Psychiatry* 36, 395–421.
- Lesch, K.P., 2005. Alcohol dependence and gene x environment interaction in emotion regulation: is serotonin the link? *Eur. J. Pharmacol.* 526, 113–124.
- Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531.
- Liu, I.C., Blacker, D.L., Xu, R., Fitzmaurice, G., Lyons, M.J., Tsuang, M.T., 2004. Genetic and environmental contributions to the development of alcohol dependence in male twins. *Arch. Gen. Psychiatry* 61, 897–903.
- McGue, M., Iacono, W.G., Legrand, L.N., Malone, S., Elkins, I., 2001. Origins and consequences of age at first drink. I. Associations with substance-use disorders, disinhibitory behavior and psychopathology, and P300 amplitude. *Alcohol. Clin. Exp. Res.* 25, 1156–1165.
- Monshouwer, K., Verdurmen, J.E.E., Van Dorsselaer, S.A.F.M., Smit, E., Gorter, A.F., Vollebergh, W.A.M., 2008. Jeugd en risikant gedrag 2007. Kerngegevens uit het peilstationsonderzoek scholieren. [Youth and Risky Behavior 2007. Key Data from the Sentinel Research Among Pupils]. Trimbos-instituut, Utrecht.
- Muthén, L.A., Muthén, B.O., 1998–2007. Mplus user's guide, fifth ed. Muthén and Muthén, Los Angeles.
- Newcomb, M.D., 1992. Understanding the multidimensional nature of drug use and abuse: the role of consumption, risk factors and protective factors. In: Glantz, M., Pickens, R. (Eds.), *Vulnerability to Drug Abuse*. American Psychiatric Association, Washington, DC, pp. 255–297.
- Nilsson, K.W., Sjöberg, R.L., Damberg, M., Alm, P.O., Öhrvik, J., Leppert, J., Lindström, L., Öreland, L., 2005. Role of serotonin transporter gene and family function in adolescent alcohol consumption. *Alcohol. Clin. Exp. Res.* 29, 564–570.
- Olsson, C.A., Byrnes, G.B., Lotfi-Miri, M., Collins, V., Williamson, R., Patton, C., Anney, R.J.L., 2005. Association between 5-HTTLPR genotypes and persisting patterns of anxiety and alcohol use: results for a 10-year longitudinal study of adolescent mental health. *Mol. Psychiatry* 10, 868–876.
- Pacheco, J., Beevers, C.G., Benavides, C., McGeary, J., Stice, E., Schnyder, D.M., 2009. Frontal-limbic white matter pathway associations with the serotonin transporter gene promoter region (5-HTTLPR) polymorphism. *J. Neurosci.* 29, 6229–6233.
- Pagan, J.L., Rose, R.J., Viken, R.J., Pulkkinen, L., Kaprio, J., Dick, D.M., 2006. Genetic and environmental influences on stages of alcohol use across adolescence and into young adulthood. *Behav. Genet.* 36, 483–497.
- Pezawas, L., Meyer-Lindberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, H.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nat. Neurosci.* 6, 828–834.
- Poelen, E.A.P., Scholte, R.H.J., Engels, R.C.M.E., Boomsma, D.I., Willemssen, G., 2005. Prevalence and trends of alcohol use and misuse among adolescents and young adults in the Netherlands from 1993 to 2000. *Drug Alcohol Depend.* 79, 413–421.
- Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.
- Schinka, J.A., Busch, R.M., Robichaux-Keene, N., 2004. A meta-analysis of the association between the serotonin transporter gene polymorphism (5-HTTLPR) and trait anxiety. *Mol. Psychiatry* 9, 197–202.
- Schuckit, M.A., 1986. Genetic and clinical implications of alcoholism and affective disorder. *Am. J. Psychiatry* 143, 140–147.
- Skowronek, M.H., Laucht, M., Hohm, E., Becker, K., Schmidt, M.H., 2006. Interaction between the dopamine D4 receptor and the serotonin transporter promoter polymorphisms in alcohol and tobacco use among 15-year-olds. *Neurogenetics* 7, 239–246.
- Spijkerman, R., Van den Eijnden, R.J.J.M., Overbeek, G., Engels, R.C.M.E., 2007. The impact of peer and parental norms and behavior on adolescent drinking: The role of drinker prototypes. *Psychol. Health* 22, 29–35.
- Sullivan, L.E., Fiellin, D.A., O'Connor, P.G., 2005. The prevalence and impact of alcohol problems in major depression: a systematic review. *Am. J. Med.* 118, 330–341.
- Van der Vorst, H., Engels, R.C.M.E., Meeus, W., Dekovic, M., Van Leeuwe, J., 2005. The role of alcohol-specific socialization in adolescents' drinking behavior. *Addiction* 100, 1464–1476.
- Van der Vorst, H., Engels, R.C.M.E., Meeus, W., Dekovic, M., Vermulst, A., 2006. Parental attachment, parental control and early development of alcohol use: a longitudinal study. *Psychol. Addict. Behav.* 20, 107–116.
- Van der Zwaluw, C.S., Engels, R.C.M.E., 2009. Gene–environment interactions and alcohol use and dependence: current status and future challenges. *Addiction* 104, 907–914.
- Van der Zwaluw, C.S., Engels, R.C.M.E., Vermulst, A.A., Franke, B.F., Buitelaar, J., Verkes, R.J., Scholte, R.H.J., 2009. Interaction between the dopamine D2 genotype and parental rule-setting in adolescent alcohol use: evidence for a gene–parenting interaction. *Mol. Psychiatry* (E-pub ahead of print).
- Van der Zwaluw, C.S., Scholte, R.H.J., Vermulst, A.A., Buitelaar, J.K., Verkes, R., Engels, R.C.M.E., 2008. Parental problem drinking, parenting, and adolescent alcohol use. *J. Behav. Med.* 31, 189–200.
- Van Zundert, R.M.P., Van der Vorst, H., Vermulst, A.A., Engels, R.C.M.E., 2006. Pathways to alcohol use among Dutch students in regular education and education for adolescents with behavioral problems: the role of parental alcohol use, general parenting practices, and alcohol-specific parenting practices. *J. Fam. Psychol.* 20, 456–467.
- Vermulst, A.A., Gerris, J.R.M., 2005. QBF: Quick big five persoonlijkheidstest handleiding [Quick Big Five Personality Test Manual]. LDC Publications, Leeuwarden, The Netherlands.
- Young, K.A., Holcomb, L.A., Bonkale, W.L., Hicks, P.B., Yazdani, U., German, D.C., 2007. 5-HTTLPR polymorphism and enlargement of the pulvinar: unlocking the backdoor to the limbic system. *Biol. Psychiatry* 61, 813–818.