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THESIS

No gene-environment interaction found between *5-HTTLPR* and parental behavioral control on alcohol problem drinking among youth.

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Abstract

Present study aimed to investigate a gene-environment interaction between the *5-HTTLPR* and parental control on alcohol problem drinking among youth. We hypothesized that adolescents who experience high levels of behavioral control will engage in less alcohol use and that this effect is stronger for children who are carriers of the short allele of *5-HTTLPR* compared to carriers of the long allele. A longitudinal design consisting of five waves was used on a Dutch sample. The sample consisted of 194 boys (55.2%) and girls that were aged between 14 and 17 years during the first wave (M = 15.26, SD = .62). Children's saliva was taken to determine their *5-HTTLPR* genotype and self-report questionnaires were completed by both parents and children. A hierarchical multiple regression showed that parental behavioral control predicted alcohol consumption among adolescents in the first wave and in the last two waves, however these effects were small. In contrast to the hypothesis, carriers and non-carriers of the short allele of the *5-HTTLPR* did not differ in their susceptibility to parental behavioral control as regards to alcohol consumption. The present results show that parents can contribute in the prevention of excessive alcohol use among their children by exercising behavioral control. Every adolescent in this study seems to benefit by this parenting style because this effect does not vary for the different alleles of the *5-HTTLPR*.

Keywords: 5-HTTLPR, behavioral control, family, alcohol, adolescents, gene-environment interaction

Abstract

Dit onderzoek heeft een gen-omgevingsinteractie tussen het *5-HTTLPR* genotype en ouderlijke gedragscontrole op de mate van ervaren negatieve consequenties door alcohol consumptie onder jongeren onderzocht. Er was voorspeld dat adolescenten die een hoge mate van ouderlijke gedragscontrole ervaren, minder alcohol zullen nuttigen en dat dit effect sterker is voor dragers van het korte allel van het *5-HTTLPR* genotype dan dragers van het lange allel. Een vijfjarig longitudinaal onderzoek was uitgevoerd met een Nederlandse steekproef. De steekproef bestond uit 194 jongens (55.2%) en meisjes wier leeftijd varieerde tussen 14 en 17 jaar in het eerste meetmoment (M = 15.26, SD = .62). Wangslijmvlies van de adolescenten was afgenomen om het genotype te bepalen en vragenlijsten werden individueel ingevuld door ouders en kinderen. Een hiërarchische multipele regressieanalyse toonde aan dat ouderlijke gedragscontrole de mate van ervaren negatieve consequenties door alcohol consumptie onder jongeren voorspelde in het eerste, vierde en vijfde meetmoment. In tegenstelling tot de hypothese, verschilde het effect van ouderlijke gedragscontrole niet voor dragers van het korte allel van het *5-HTTLPR* genotype en dragers van het lange allel. Resultaten van het huidige onderzoek tonen aan dat ouders mogelijk kunnen bijdragen aan de vermindering van alcoholgebruik onder jongeren door controle op hun gedrag uit te oefenen. Daarnaast blijkt elke adolescent uit dit onderzoek te profiteren van deze ouderschapsstijl, ongeacht het *5–HTTLPR* genotype.

Trefwoorden: 5-HTTLPR, gedragscontrole, familie, alcohol, adolescenten, gen-omgevingsinteractie

No gene-environment interaction found between *5-HTTLPR* and parental behavioral control on alcohol problem drinking among youth.

Alcohol drinking is a well-known problem among adolescents. A Dutch reporting system revealed that in 2011, 762 teenagers ended up in emergency rooms because of binge drinking (‘‘NVK: Alcoholgrens op 18 jaar’’, 2012). These numbers showed that the average age of a binge-drinker was 15.3 years and teenagers were unconscious for at least three hours. Alcohol drinking among adolescents not only constitutes a problem in the Netherlands. Across Europe, 60.4% of adolescents aged 12 to 16 years drank alcohol at least once (Steketee, Jonkman, Berten & Vettenburg, 2013) and 28.1% of these teenagers reported heavy episodic drinking of alcoholic beverages. Early alcohol use has negative consequences, including academic failure, traffic accidents, delinquency, and unwanted teenage pregnancy (Kandel & Yamaguchi, 1993; Donovan, 2004). Furthermore, damage to the developing brain, cognitive impairment, and symptoms of depression and anxiety have also been associated with adolescent alcohol use (Townshend & Duka, 2005). Especially adolescence is a vulnerable period for developing future Alcohol Use Disorders (AUD) later in life (Enoch, 2006). Considering these risks, it is important to discover trajectories of alcohol use among adolescents. By getting more knowledge about possible risk factors, interventions can be focused on the proper aspects on time.

Alcohol consumption is a complex behavior and cannot be predicted by one factor (Rutter, 2002). Therefore, it is recommended to take a multidisciplinary approach and focus not only on genetic or environmental influences, but rather on the interplay between these two. Studying such interplay has two main advantages (Heath & Nelson, 2002). First, studies on environmental risk factors that ignore genetic interplay may draw wrong conclusions about the role of shown environmental risk factors. Secondly, research that focuses exclusively on genetic effects on alcohol consumption might miss the real mechanism behind the effect of these specific genes, because these effects could be only at present in a certain adverse situation. The diathesis-stress view can illustrate this. According to this theory, some children are more vulnerable than others to negative influences from their environment, often for genetic reasons (Zuckerman, 1999; Belsky & Pluess, 2009). The

interplay of this genetic vulnerability with a certain adverse environmental situation could cause an individual to exercise problem behavior. Especially in the case of complex behavior like alcohol use it seems promising to take a perspective that incorporates multiple domains.

One genetic factor that seems to play an important role in the brain reward circuitry and is released in response to alcohol is serotonin (*5-HT*) (Yoshimoto, McBride, Lumeng & Li, 1992). Serotonin influences our capability to inhibit impulses. A dysfunctional serotonergic system has been associated with alcohol dependence and genes controlling this system are therefore of interest (Koob, 2003). An important regulator of the level of serotonin (*5-HT*) in the brain is the serotonin transporter protein (*5-HTT*) (Heils et al., 1996). This protein terminates active serotonin in the synaptic cleft by reuptake of serotonin into presynaptic neurons. The gene that codes for the serotonin transporter is known as *SLC6A4* and there is a polymorphism located in the promoter region of this gene, called the *5-HTTLPR*. Two functional alleles of *5-HTTLPR* have been examined, a short allele and a long allele. In comparison with the long allele, the short allele has been associated with a reduced transcriptional activity of the transporter, resulting in reduced efficiency of *5-HT* reuptake and more active serotonin (Lesch, et al., 1995; Whale, et al., 2000). This serotonin deficiency has an effect on all kinds of behaviors, including interference with impulse inhibition (Hallikainen et al., 1999). The effect of the short allele has been examined and appears to be significantly associated with alcohol disorder (AD) (Feinn, Nellissery & Kranzler, 2005), although findings are contradictory. In some studies for example, substance use has been higher in adolescents with an s/s genotype (e.g., Feinn et al., 2005; Gerra et al., 2005; Herman, Philbeck, Vasilopoulos & Depetrillo, 2003; Van der Zwaluw et al., 2010) whereas in other studies higher alcohol use has been reported in the l/l genotype (Buchmann et al., 2009; Laucht et al., 2009). The inability to withhold a response has been identified as a strong predictor for binge drinking (Henges & Marczinski, 2012). Since the short version of *5-HTTLPR* seems to impair the ability to control impulses, it might be that carriers of the short allele could also experience problems with controlling their alcohol use. In this way, the short allele of *5-HTTLPR* could serve as a genetic vulnerability for developing alcohol related problems, in comparison with the long allele.

Since the diathesis-stress view states that a genetic vulnerability might cause an individual to

be more vulnerable for a certain environmental situation, it seems promising to examine the effect of *5-HTTLPR* in combination with a possible environmental factor involved in the ability to control impulses. Evidence from the literature indicates that the *5-HTTLPR* genotype can serve as a genetic vulnerability. For example, one study investigated the relationship between *5-HTTLPR* genotype variants and an experience with maltreatment among children on alcohol use (Kaufman et al., 2007). Results from this study showed that the risk to start using alcohol early was greatest among children with short allele genotype and an experience with maltreatment, implying a gene-environment interaction. Another study investigated possible interactions between *5-HTTLPR* genotype and family relation as concerned to adolescent alcohol consumption (Nilsson et al., 2006). An interaction between these two factors was found, in which possessing the l/s genotype caused children to be more vulnerable for poor family relations and led them consume more alcohol. Another important study on the variance of vulnerability for certain environmental situations was performed with a non-human primate sample (Barr, et al., 2004). Results indicated that *5-HTTLPR* caused a difference in serotonin levels among the monkeys, that in turn interacted with their rearing condition. Subjects that were identified with the short allele and that were reared by peers instead of their mothers, showed more preference towards alcohol. In general, the results of the aforementioned studies serve as a confirmation of the diathesis stress view, by showing that *5-HTTLPR* functioned as vulnerability for a certain environmental influence as regards to alcohol intake. Thereby, *5-HTTLPR* seems a suited genetic candidate to investigate in gene-environment interactions.

As an environmental social influence on alcohol problem drinking, parenting style has often been associated with this behavior. Regularly, adolescents still live in their parents' house which makes the family environment part of their ecological system (Bronfenbrenner, 1994). The ecological system where an adolescent is developing in should be taken into account when someone tries to understand adolescents' behaviors, including alcohol use. This view is supported by some studies which looked among other things at the effect of parental monitoring. Parental monitoring can be understood as 'the extent to which parents engage in activities designed to obtain information about their children's activities, whereabouts and associates' (Fletcher, Steinberg & Williams-Wheeler, 2004) and is negatively linked with substance use. Parental monitoring can be subdivided in specific

behaviors, including 'behavioral control' (Fletcher et al., 2004). Behavioral control implies the extent to which parents exercise control over the behavior of their children by means of setting rules and limitations on behavior. Besides that parental monitoring and behavioral control act together on substance use, behavioral control appears to have an influence of its own on drinking behavior among adolescents (Gray & Steinberg, 1999). Parents, who exercise control on the behavior of their children, seem to be able to decrease their children's alcohol intake.

It is clear that with examining the development of alcohol problems, gene-environment interactions should be taken into account. With the relationships of the above-mentioned factors on alcohol use, it seems promising to investigate a possible interaction between a polymorphism of one of the main important serotonin regulators and the control parents exercise on the behavior of their children. If the short version of *5-HTTLPR* interferes with the ability to control impulses, perhaps adolescents who are carriers of this allele are in more need of behavioral control performed by their parents than adolescents who do not carry this allele. Returning to the diathesis-stress view, *5-HTTLPR* would then function as a vulnerable factor for parental behavioral control. Empirical evidence from the literature can confirm the finding that parents' involvement matter in developing the ability to control impulses (Barr, et al., 2004). This might mean that by exercising control on the behavior of their children and being informed about their whereabouts, parents are enabled to discourage their children's alcohol related problems (Koning et al., 2009).

This study aims to examine a possible moderator effect of *5-HTTLPR* on the effect of parental behavior control on alcohol problem drinking among adolescents. In this way, there can be verified whether the effect of parental behavioral control differs for the different alleles of the *5-HTTLPR* genotype. First of all, we expect that parental behavioral control has an effect on alcohol problem drinking among adolescents, whereby high levels of behavioral control serve as a protection and low levels lead to more alcohol problem drinking. Secondly, we expect that the short allele of *5-HTTLPR* is related to more alcohol problem drinking among adolescents. Furthermore, we hypothesize that adolescents who are carriers of the *5-HTTLPR* short allele and who experience low levels of parental behavioral control experience more alcohol problem drinking, compared to carriers who experience high levels of parental behavioral control.

Method

Procedure

Invitations for a longitudinal study with five years and a reply form were sent to families in 22 different municipalities that consisted of a mother, a father and two 13- to 17-year old children. A number of 885 families returned this reply form with an informed consent. Of all these families, 765 families fulfilled the following inclusion criteria: the children were biologically related to each other, the children were not twins, the father and mother were the biological parents of the children, the parents lived together or were married and the children did not have a mental or physical disability. Finally, 428 families were selected so that the educational level of the children was equally distributed and an equal number of all four possible sibling dyads were provided. The families were visited and interviewed at their homes and filled in questionnaires individually and separately for approximately 1.5 hours. As regards to the genetic information, saliva from each family member was collected at the end of the house visit. When all the necessary data was gathered from the four family members, the family received 30 euros.

Participants

The group of participants consisted of 428 Dutch families with a father, a mother and two adolescent children. All these families fulfilled the above-mentioned inclusion criteria. In this study we only use information of the older siblings, whose ages ranged from 14 to 17 years old (M = 15.26; SD = .62) in the first wave.

Materials

Genotype. Genotyping of the 5-HTTLPR polymorphism in the SLC6A4 (5-HTT, SERT) gene was performed by simple sequence length analysis. PCR was on 50 ng genomic DNA using 10 pmol of forward primer (5'-GGCGTTGCCGCTCTGAATGC-3') and 10 pmol reverse primer (5'-GAGGGACTGAGCTGGACAACCAC-3'), 0.25 mmol/l dNTPs, and 0.5 U Taq DNA polymerase (Invitrogen, Breda, the Netherlands) in a PCR buffer containing 0.3 mol/l Tris-HCl (pH 8.5), 75 mmol/l ammonium sulfate, and 7.5 mmol/l 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, 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). No deviations from HWE were detected (P=0.89) (van Roekel, Scholte, Verhagen, Goossens & Engels, 2010). Distinction was made between two groups of *5-HTTLPR*: 133 (68.6%) carriers of the s-allele (s/s, s/l and l/s) and 61 non-carriers of the s-allele (l/l).

Parental control. A Dutch translated version of the original questionnaire of Darling & Steinberg (1993) was used to assess parental behavioral control (Beyers & Goossens, 1999). Fathers and mothers indicated on a 5-points Likert scale how much of their behavioral control corresponded to the items (1= *never* and 5= *always*). An example of an item is ''Do you need your fathers permission to go out for a night through the week?'' Sum scores were calculated for both parents. The mean score of these two values served as the measurement of parental control. In the first wave cronbach's alpha was found to be .58, in the second wave .71, in the third wave .79, in the fourth wave .85 en in the fifth wave .84. This means that in the present study the internal reliability of the questionnaire can be seen as reasonably high.

Alcohol problem drinking. To assess the extent of problematic alcohol drinking among the oldest children, the Rutgers Alcohol Problem Index (RAPI) was used (White & Labouvie, 1989). RAPI is a self-reported questionnaire whereby adolescents have to report the frequency of 18 negative consequences followed by alcohol drinking they experienced during the past year (e.g. ''Not able to do your homework or study for a test''). Participants responded the items on a 3-points Likert scale ranging from (0= *none* and 3= *more than 5 times*). In ascending order of the five waves, cronbach's alpha appeared to be .72, .80, .76, .80 and .88, which can be conceived as a high internal reliability of the questionnaire in the present study.

Results

Preliminary analyses

Participants whose values for genotype or education were missing, or who lacked a complete questionnaire, were removed from the dataset. To avoid population stratification, different ethnicities were removed too, which resulted in a complete Caucasian sample. As regards the remaining missing

values, mean scores of the concerning items were calculated and entered as the new value for each participant. Extreme values on alcohol problem drinking (n = 20) and parental control (n = 19) were modified to three times plus or minus the standard deviation. Although, it was chosen to perform the analysis one time without extreme values as well, so that the influence of these values could be checked. The gender of the participants was distributed into 107 (55.2%) boys and 87 girls. As regards the education of the adolescents, participants were distributed in two groups: 'low educated' or 'high educated'. The distribution between these groups were for the first wave through the fifth wave respectively 41 (21.1%), 39 (20.1%), 47 (24.2%), 56 (28.9%) and 50 (25.8%) in the low educated group. Further, the correlations between the different variables were inspected and the values of the correlations in the first wave are shown in Table 1. The descriptives of the continuous variables age, alcohol problem drinking and parental control are presented in Table 2, where it is displayed that for each genotype alcohol problem drinking increases over the years and parental control decreases.

Table 1

Pearson correlations for the control variables, predictor variables and response variable in the first wave.

	1	2	3	4	5	6	7
1.Age	--	-.06	.11	-.09	-.16*	.01	-.13
2. Gender		--	.15*	-.05	.10	-.08	.06
3. Education			--	-.01	-.06	-.15*	.05
4. 5-HTTLPR				--	.07	.01	.09
5. Parental control					--	-.18*	.49**
6. Alcohol problem drinking						--	-.06
7. Parental control X 5-HTTLPR							--

Note. *$p < .05$ ** $p < .001$

Table 2

Descriptives of the continuous variables for each condition of genotype

		Wave				
		1	2	3	4	5
Genotype		*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
s/s, s/l	Age	15.29 (0.61)	16.29 (0.61)	17.29 (0.61)	18.29 (0.61)	19.29 (0.61)
	Problem drinking	21.54 (3.93)	22.34 (5.05)	22.68 (4.91)	23.03 (5.12)	23.57 (5.80)
	Parental control	22.08 (2.27)	20.66 (2.65)	18.16 (3.31)	13.95 (3.53)	11.79 (3.68)
l/l	Age	15.18 (0.63)	16.18 (0.63)	17.18 (0.63)	18.18 (0.63)	19.18 (0.63)
	Alcohol	21.64 (3.49)	22.31 (4.57)	22.51 (4.41)	23.37 (5.29)	23.56 (6.25)
	Parental control	22.41 (1.85)	20.63 (2.86)	18.46 (3.89)	14.43 (4.01)	12.10 (3.89)
Total	Age	15.26 (0.62)	16.26 (0.62)	17.26 (0.62)	18.26 (0.62)	19.26 (0.62)
	Problem drinking	21.60 (3.79)	22.33 (4.89)	22.62 (4.75)	23.14 (5.16)	23.56 (5.93)
	Parental control	22.18 (2.14)	20.65 (2.71)	18.25 (3.50)	14.10 (3.69)	11.89 (3.74)

Analysis

On the basis of the research questions, it was chosen to perform a hierarchical multiple regression analysis. In this way, it was possible to look at the unique contributions of the factors involved. All the assumptions were met, except for the assumption of normality. However, because the current sample size was big, the analysis was performed nevertheless. The variable of parental behavioral control was standardized so that the variables could be better compared. Relations between gender, age and education were explored to decide which covariates should be taken into the model. The relation between genotype of the parents and their parental control were investigated as well, but appeared to have no correlation with each other. Three steps were involved in the regression analysis. In the first step, the confounders were entered, which were age, gender and education. From the second wave on, measurements in the first wave of alcohol problem drinking and parental control were added as covariates as well. In the second step, parental control and genotype were added to the model and in the third step the interaction term between these two variables was added. As was mentioned in the preliminary results, the analysis was also performed one time without extreme values. This model did not differ that much from the model where extreme values were adjusted. For

that reason, it was chosen to perform the model with these adjusted values, so that as many values as possible could be taken into the analysis.

Results

The results of the hierarchical multiple regression analysis are described in Table 3.

Covariates. The first step, which included the covariates, did not contribute significant to the explained variance of the model in the first wave. In the second wave, the first step explained a significant proportion of the variance of the model (R^2_{change} = .431, F = 28.5, p < .01), which continued into the third wave (R^2_{change} = .292, F = 15.5, p < .01), fourth wave (R^2_{change} = .241, F = 11.9, p < .01) and fifth wave (R^2_{change} = .249, F = 12.5, p < .01). As regards to gender, its effect on problem drinking was only significant in the second wave (β = -.112, t(191) = -1.99, p < .05), third wave (β = -.238, t(191) = -3.76, p < .01), fourth wave (β = -.184, t(191) = -2.83, p < .05) and fifth wave (β = -.209, t(191) = -3.22, p < .05). In none of the waves did age have a significant effect and education was only significant in the first wave (β = -.163, t(191) = -2.25, p < .05).

Main effects. In the second step of the model, the main effects of genotype and parental control were added. For the first wave and the fifth wave, this step contributed significantly to the model (first wave: R^2_{change} = .031, F = 3.11, p < .05 and fifth wave: R^2_{change} = .032, F = 4.21, p < .05). The standardized regression coefficients of parental control were significant in the first wave (β = -.179, t(192) = -2.49, p < .05), the fourth wave (β = -.172, t(192) = -2.42, p < .05) and in the fifth wave (β = -.191, t(192) = -2.84, p < .05). The standardized regression coefficients of genotype were not significant, which supplied for each wave.

Interaction effect. In the third step of the model, the interaction effect of the variable '5-HTTLPR' and the standardized variable 'parental control' was added. For each wave, this step did not contribute significantly to the regression model. None of the standardized regression coefficients of the interaction term were significant.

Table 3

Results of the hierarchical multiple regression analysis predicting Alcohol Problem Drinking from covariates, main effects and interaction

	Wave									
	1		2		3		4		5	
	ΔR^2	β	ΔR^2	β	ΔR^2	β	ΔR^2	β	ΔR^2	β
Step 1	.032		.431**		.292**		.241**		.249**	
Age		.019		-.005		-.114		-.049		-.083
Gender		-.053		-.112*		-.238**		-.184*		-.209*
Education		-.163*		.041		.103		.025		.097
Alcohol problem drinking wave 1				.636**		.476**		.435**		.445**
Parental control wave 1				-.036		.058		-.037		-.008
Step 2	.031*		.008		.005		.023		.032*	
Parental control		-.179*		-.114		-.057		-.172*		-.191*
5-HTTLPR		.023		-.025		-.058		.015		-.031
Step 3	.003		.001		.003		.003		.005	
5-HTTLPR x parental control		.062		-.046		.072		-.064		.090
Total R^2	.066		.441		.300		.267		.287	

Note. $n = 194$

$\Delta R^2 = R^2$ change

*$p < .05$ ** $p < .001$

Conclusion and Discussion

The present study examined the effect of parental behavioral control on alcohol problem drinking among youth. Also, we looked whether participants were more vulnerable for this environmental influence based on their *5-HTTLPR* genotype. Because evidence from the literature suggests that an oversupply of serotonin could interfere with one's ability to control impulses (Hallikainen et al., 1999), it was predicted that adolescents who experience a deficit in their serotonergic system would be in more need of behavioral control performed by their parents. Like the diathesis-stress view states that some children are more vulnerable for certain environments than other children, possibly for genetic reasons (Zuckerman, 1999). The present results indicate that parental behavioral control predicted alcohol problem drinking among youth in this study, although this effect was small. Further, 5-HTTLPR did not influence alcohol problem drinking and did not interact with parental behavioral control. Thereby, we can conclude that in our study parental behavioral control predicts, although a small effect, alcohol problem drinking among youth, regardless of the different alleles of *5-HTTLPR.*

These findings are not in line with our predictions. First, although parental behavioral control appeared to affect alcohol problem drinking, this did not supply for every age in our study. The finding is partly conformable to our hypothesis in which we predicted that the more behavioral control by parents, the less alcohol problem drinking among their children. In the begin of the study, when adolescents had reached the average age of 15.3 years, and in the end of the study, when adolescents had reached the average age of 18.3 and 19.3 years parents behavioral control predicted alcohol problem drinking among their children. It could be possible that at a certain stage in adolescence, parent's influence diminishes and other social influences become more important. According to developmental theory, a person's life is divided into different stages of development (Harris, 1998). Adolescence is characterized by developing one's own identity and becoming an autonomous human being. With becoming autonomous, the influence of parents' decreases and other social influences become more incorporated into the new identity. Behavior and attitude of peers gain a bigger influence on one's behavior, also as regards to alcohol consumption (Daw et al., 2013). This could explain our

observation where the influence of parental behavioral control is temporal absent. Perhaps, when the participants were aged between 15 and 18 years, most of them experienced the stage of adolescence, whereby the influence of peers became more important than the influence of parents. Next, when the participants reached the age of 19 years, most of the adolescents moved on to late adolescence. Multiple studies show that the influence of parental behavioral control even holds into late adolescence, which could explain why parental behavioral control was significant again in our study at the average age of 19 years (Daw et al., 2013).

Further, *5-HTTLPR* did not interact with parental behavioral control, as was predicted. Several explanations can be proposed for this contrasting finding. First, the cause could be situated in the relationship between *5-HTTLPR* and the system of impulse control. Our result could mean that *5-HTTLPR* does not affect someone's impulse control. In that case, impulse control of carriers of the short allele would be intact and they would not benefit extra from behavioral control performed by their parents. On the other hand, the explanation could be situated in the relationship between *5-HTTLPR* and the chosen social environmental factor. For example, there has been said that the short allele of *5-HTTLPR* could be more related to alcohol use associated with anxiety and depression (Laucht et al., 2009). This seems plausible, because *5-HTTLPR* has been linked to elevated levels of anxiety and exaggerated responses to stress (Barr et al., 2004). An environmental factor such as the degree of parental behavioral control might belong to another area and perhaps researchers should instead focus on the association of *5-HTTLPR* and environmental situations associated with anxiety and stress on alcohol use. Besides that, Kaufman and colleagues (2007) mention that gene-environment interactions are easiest found for 'extreme' environmental influences. Parental behavioral control can possibly not be seen as an 'extreme' environmental influence, such as child maltreatment or other traumatic influences. Moreover, most of the families were functioning normal and the levels of behavioral control performed by parents were thereby not extremely high or low. The fact that the current environmental factor was probably not an 'extreme' influence, could contribute partly as an explanation for the absence of our hypothesized effect. Not to mention, this does not mean that such an interaction does not exist.

This study has some limitations. It should be taken into account that alcohol consumption may be a socially stigmatized behavior for some people (Room, 2005). This could have caused participants to underreport their actual alcohol consumption and thereby possibly threaten the validity of the results. Moreover, questionnaires were based on self-report measures and were completed by one informant. This could have limited the reliability in some way. Also, we chose to investigate an interaction effect with a specific environmental factor such as behavioral control. Behavioral control can be seen as one form of parental behavior that is part of monitoring. Interaction effects are easier to detect with broader environmental factors (Vaske et al., 2012). In this respect, perhaps the environmental factor 'parental monitoring' could better have been investigated in our design. As last, readers should take into account that this study focused on a Dutch sample, with a specific family structure, and that findings are only representative for a similar Dutch population.

This study benefits from some strengths. For example, our results contribute to the current knowledge on the effect of the *5-HTTLPR* genotype on alcohol problem drinking, thanks to its specific gene-environment combination. As far as known, this is the first study that has investigated a possible interaction effect between parental behavioral control and *5-HTTLPR* on alcohol problem drinking among youth. Because findings on the effects of *5-HTTLPR* keep being heterogeneous, researchers should be encouraged to investigate different environmental situations to discover under which conditions this genetic polymorphism affects alcohol use among youth. Another strength is the longitudinal design that was used, whereby the influence of parenting could be investigated over a timespan. Thereby, we could reveal the specific course of the influence of parental behavioral control on alcohol consumption. Besides that, our big sample size was an asset. It is said that associations between genes and complex behaviors like alcohol behavior are small in magnitude (Kendler, 2005). Thereby, sample sizes in gene-environmental research should be large.

It is important that future research examines the current subject in more depth. As far as known, this is the first study that has investigated such a gene-environment interaction and replication studies are needed before definitive conclusions can be drawn. However, the results of gene-environment studies on alcohol consumption remain contradictory and thereby, two advices can be taken by the results of the present study. First, future researchers should take an example of this study

and dare to investigate different environments than the ones that have been examined multiple times already. In this way, it could be discovered under which circumstances *5-HTTLPR* does make a child more vulnerable to consume alcohol and inconsistency could be thereby ended. Second, since parental behavioral control seems to play a role in alcohol consumption among youth, regardless of *5-HTTLPR*, this environmental influence is worth investigating in combination with other genes. If we could discover more about the mechanisms of this environmental influence, a new potential source where interventions should focus on would be discovered.

Excessive alcohol use among youth remains a serious problem in our western society, with dramatic consequences. Because of the mixed findings among *5-HTTLPR* on alcohol consumption, future research on gene-environment interactions is needed to reveal the circumstances in which this polymorphism influences alcohol consumption among youth. Although we did not find support for an interaction effect between *5-HTTLPR* and parental behavioral control on alcohol consumption among youth, we did find an interesting effect of parental behavioral control on alcohol problem drinking. This is an important discovery that means that every child, despite of type of *5-HTTLPR* genotype, benefits of behavioral control performed by their parents in counteracting excessive alcohol use. Interventions that aim to decrease alcohol problem drinking among youth, should thereby not only focus on the adolescents themselves, but on their parents as well.

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