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Role of the Serotonin Transporter Gene and Family Function in Adolescent Alcohol Consumption

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Background: That the extent to which a particular individual will engage in problematic behaviors such as delinquency, violence, or drug abuse is determined by the way psychosocial, situational, and hereditary factors interact is widely accepted. However, only recently have researchers begun to investigate the interactions between specific genotypes and psychosocial factors in relation to behavior. The purpose of the present study was to investigate possible interactions between a polymorphism in the promoter region of the serotonin transporter (5-HTT) gene and family relations on adolescent alcohol consumption.

Methods: A cross-sectional study with a randomized sample from a total population of 16- and 19-year-old adolescents from a Swedish county was conducted. Eighty-one male and 119 female adolescents, who volunteered to participate after having answered a questionnaire, were randomly selected from quartiles of volunteers representing various degrees of psychosocial risk behavior.

Results: 5-HTT genotype ($p = 0.029$) and family relations ($p = 0.022$) predicted alcohol consumption independently as well as through an interaction with one another ($p = 0.05$). The model explained 11% of the variance in alcohol consumption. In a binary logistic model, we found that adolescents with the LS variant of the 5-HTT gene and with family relations being "neutral" or "bad" had a 12- to 14-fold increased risk for high intoxication frequency.

Conclusions: In sum, our results show that a functional polymorphism of the 5-HTT genotype, family relations, and interactions between these variables predict adolescent alcohol consumption in a randomized sample of adolescents.

Key Words: Alcohol, Adolescent, Genes, Environment.

THAT THE EXTENT to which a particular individual will engage in problematic behaviors such as delinquency, violence, or drug abuse is determined by the way psychosocial, situational, and hereditary factors interact is widely accepted (e.g., Freud, 1949; Lorenz, 1966; Rutter, 2002; Silberg et al., 2003). However, only recently have researchers begun to investigate the interactions between specific genotypes and psychosocial factors in relation to behavior (e.g., Caspi et al., 2002; Foley et al., 2004).

One area of behavior in which the need for a dual approach, involving both hereditary and psychosocial variables, has been demonstrated is that of adolescent drinking behavior. On one hand, evidence that hereditary factors are of great importance comes from twin studies that show greater concordance for alcohol dependence in monozygotic than in dizygotic twins (Kendler et al., 1992; McGue et al., 1992), as well as from adoption studies (Cloninger et al., 1981; Sigvardsson et al., 1996). On the other hand, a number of observations can be invoked in support of the idea that normative and deviant behaviors are learned social behaviors, (e.g., Jessor, 1987; Kandel and Andrews, 1987) suggesting that social behaviors as well as patterns of alcohol consumption are products of the interaction of social, psychological, and cultural characteristics (Lang and Stritzke, 1993).

According to these views, norms for social behavior, including drug use, are learned predominantly in the context of interactions with the primary socialization sources (Oetting and Donnermeyer, 1998). Several biological and psychosocial vulnerability models of alcoholism have been proposed (Hill et al., 1986), and in 1995, Cadoret et al. (1995a,b) showed a gene–environment interaction in aggression, conduct disorders, and drug abuse in adoption studies.

In studies of interactions between genotype and behavior, it is essential to identify specific genes that might be of relevance. Polymorphisms of the serotonin transporter (5-

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HTT) gene have been associated, in several studies, with personality and affective disorders. At least two 5-HTT polymorphisms have been identified: an insertion/deletion polymorphism in the upstream regulatory region (5-HTTLPR) (Heils et al., 1995) and a variable number of tandem repeats polymorphism in the second intron (Ogilvie et al., 1996). Functionality studies have shown that 5-HTT gene transcription is differentially modulated by the long and short variants of the 5-HTTLPR, where the short variant is associated with lower expression of 5-HTT and lower 5-HT reuptake activity (Collier et al., 1996).

The 5-HTTLPR polymorphism has been studied extensively in relation to personality and psychiatric disorders, and a number of studies have indicated that genotype of the 5-HTTLPR allele is associated with anxiety-related personality traits, such as neuroticism and affective disorders (Collier et al., 1996; Lesch et al., 1996; Mazzanti et al., 1998; Sen et al., 2004). Although not undisputed, the existence of such associations were recently supported by results from an overview (Van Gestel and Van Broeckhoven, 2003) and a meta-analysis (Schinka et al., 2004).

With regard to central serotonergic neurotransmission and alcohol dependence, there is a fairly consistent notion that especially alcohol dependence with a prominent genetic component and characterized by impaired impulse control, type 2 alcoholism, is associated with a central nervous system serotonin deficit (Cloninger, 1987). It in interesting that there is a nonhuman primate model of type 2 alcoholism that in many respects seems to mimic human conditions. This model has the advantage that a variety of genetic and environmental factors can be studied more effectively than in humans, and Barr et al. (2003b) showed that decreased serotonin turnover was associated with both lower initial sensitivity to alcohol and higher prospective alcohol consumption in rhesus macaques. Furthermore, it was shown that animals that were separated from their mothers and reared in peer-only groups were more likely to consume alcohol as adults (Barr et al., 2003a). Studies have also been performed on nonhuman primates with regard to a functional 5-HTT polymorphism, which seems to resemble closely the human 5-HTTLPR polymorphism, showing that monkeys with deleterious early rearing experiences were differentiated by 5-HTT genotype with regard to levels of cerebro spinal fluid (CSF) 5-HIAA. Thus, only animals with presence of the short 5-HTTLPR allele (low function) differed with significantly lower 5-HIAA levels indicating a 5-HTT gene–environment interaction (Bennett et al., 2002). In studies on humans, there are several reports on an association between alcoholism resembling the criteria of type 2 and presence of the short 5-HTTLPR allele (Hallikainen et al., 1999; Hammoumi et al., 1999; Ishiguro et al., 1999). There also are reports in which this association could not be verified (Stoltenberg et al., 2002). The purpose of the present study was to examine possible interactions between 5-HTTLPR genotype and family function in relation to adolescent alcohol consumption. It is, to our knowledge, the first report of a gene–environment interaction related to alcohol consumption among humans.

MATERIALS AND METHODS

Subjects

All 16-year-old (ninth-grade students, compulsory school) and 19-year-old third-grade students in secondary school in Västmanland, a medium-sized county of Sweden, i.e., 2987 ninth graders and 2186 third graders, comprised the target population. The students were asked to complete a mental and psychosocial health-screening questionnaire, Survey of Adolescent Life in Vestmanland (SALVe), in their classroom during a 1-hr session under the supervision of a specially trained research assistant. The present study is one of several ongoing studies in the SALVe project with the aim to investigate gene–environment interactions in relation to deviant behavior among adolescents. The alcohol consumption questions used in the form have been used widely in a European collaboration project, the ESPAD report (Hibell et al., 1997; Plant and Miller, 2001). In total, 2611 (mean age, 16.0 years) and 1649 (mean age, 19.2 years) students, 87 and 75% respectively, completed the questionnaire. All students had an opportunity to give their informed consent to participate in an in-depth interview and the drawing of a blood sample, by giving their full personal code number at the form front page. Informed consent was received from 785 students who could be traced with valid names. All students were classified with a risk index, depending on their risk behaviors (alcohol, narcotic, sexual, property offense, and violent offense) reported in the questionnaire, and divided into four groups according to their respective risk index. Randomized samples of 400 students, matched for age, sex, and risk behaviors, were drawn from the volunteers. The procedure with an initial risk survey was used to ensure that we would have enough participants from both ends of the deviant behavior continuum. There were no explicit exclusion criteria. Eighty-one of the boys and 119 of the girls agreed to give blood samples and to take part in an interview when asked for informed consent a second time (in line with recommendations from the human ethical committee of the Medical faculty at Uppsala University which approved of the study; Fig. 1). The risk index showed no significant differences between those who were interviewed and those who responded to the initial questionnaire.

5-HTT Gene Analysis

Venous blood was drawn from all interviewed students for molecular genetic analyses. Two students were excluded because of hepatitis infection. DNA was extracted from venous blood, and with regard to the 5-HTTLPR polymorphism, PCR-based genotyping was performed according to a modified protocol by Collier (Collier et al., 1996). For confirming that the correct regions of the 5-HTT gene were amplified, PCR products representing all genotypes were sequenced using BigDye Terminator chemistry (Applied Biosystems, Foster City, CA) and analyzed by an automated ABI PRISM (Perkin Elmer, Foster city, CA). The DNA fragments were analyzed using the Sequencer 3.1.1 software.

Interview Structure Design

Definitions of Alcohol Consumed and High Intoxication Frequency. To ensure that the qualitative data from each participant were complete and fulfilled the purpose for the study, we designed an interview guide. All participants were asked to leave three small hair samples from different areas of the scull and told that these hair samples would be analyzed for alcohol and other drug content [as a bogus pipeline procedure (Wagenaar et al., 1993)]. A total of 196 participants gave hair samples (4 had no hair on their head). The semistructured interview contained questions of defined quantifiable areas for measuring the alcohol consumption. The questions, however, were not definite in advance; the formulation, sequence, and extent were adaptable to the situation. The interview object reflected and resonated of his or her alcohol habits, a qualitative dimen-

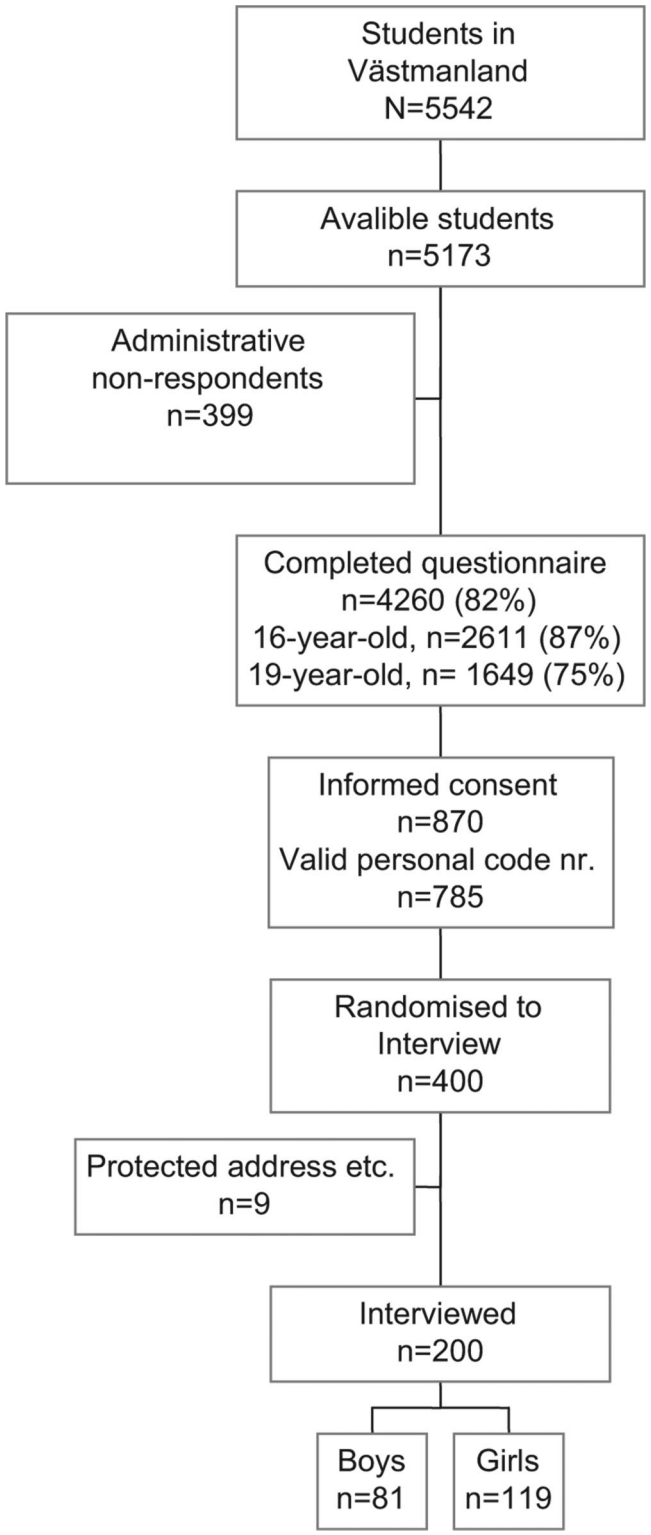

Fig. 1. A flowchart describing the study population.

sion. The goal of the interviews was to elicit the personal alcohol behavior, interaction with peers and alcohol etc.

Questions were asked about personal experience of alcohol; age at first occasion; first intoxication; with whom, where, what, and how much they drank; how they get alcohol; how they acted after drinking; and why they drank. Special attention was made to "capture" drinking history from first occasion all the way to the interview day. First, there was an exploration of when, how much, and where, then with whom. Then a reflective "mirror image" from the interviewer was made whereby the questions were time and place anchored, e.g., last summer vacation, on the beach, with your brother and his friends, or last weekend at your friend's house, with your hockey peers. All interviews were audiotaped, and these tapes were used to code the amount and frequency of different alcohol beverages and transform them to grams of alcohol per year. Adolescents who drank two times (or more) per month and always (or almost always) became drunk were coded as "high intoxication frequency." The interview (with hair samples taken) was more sensitive with regard to "high intoxication frequency" as compared with the initial questionnaire (104 individuals compared with 79 of 200).

Definitions of Family Functioning. The psychosocial variables were measured by the questions, "Could you describe your family," "What is good with your family, your mother/father and siblings," and, "What is not so good with your family," "How was it in your family when you were 7, . . . 13," "Have there ever been any tough or hard periods within your family . . .?" When the respondent described controversies within the family, they were followed up. The responses of the participants to these questions of family function then were brought together and transformed from a "qualitative" to a "quantitative" ordinal scale final stage, comprising the psychosocial variable "quality of relation within the family" (good/neutral/bad). Interrater reliability for two raters, who listened to a 10% sample of the audiotaped interviews, was a correlation of 0.998 (Crohnbach α) for alcohol consumption in grams per year, a Cohen's κ of 1.0 for high intoxication frequency, and 0.7 for quality of relation within the family.

Statistical Analysis

A general linear model (GLM) was used to investigate the 5-HTTLPR genotype and quality of family relation and their interaction (the biosocial model) in relation to the dependent variable alcohol consumption in grams per year. Because of the skewed distribution of the alcohol consumption in grams per year (a log transformation had no significant effect on the distribution of the dependent variable), a nonparametric test for interactions based on aligned ranks (program written in FORTRAN) was also applied. Briefly, this test is based on the joint ranking of all observations after removing the effect of the factors 5-HTTLPR gene and the quality of family relations. Suitably normalized, the weighted sum of squared differences between the subcategories mean rank (each combination of 5-HTTLPR gene and quality of family relations) and the total mean rank will be approximately F-distributed (Öhrvik, 2002). For ascertaining the validity of the test when the data were unbalanced, the actual significance level was calculated for the nominal levels 10, 5, and 1%, based on 10,000 replicates from the distribution under the null hypothesis using the actual sizes of the subcategories. The achieved significance levels were 10.0, 4.8, and 0.8%, respectively. When the null hypothesis was rejected, a Kruskal-Wallis test was run with each 5-HTTLPR gene and quality of family relations combination constituting a separate subcategory. For investigating the biosocial model i.e., the 5-HTTLPR gene and quality of family relation and their interaction to the binary dependent variable "high intoxication frequency," logistic regression was applied. A two-sided $p < 0.05$ was considered significant in all analyses of main effects and < 0.1 when testing for the presence of interactions.

RESULTS

A total of 104 (52%) adolescents had high intoxication frequency: 55% of the girls and 48% of the boys. The mean consumption among girls was 2010 g of alcohol per year, and the corresponding amount for boys was 2287 g.

The distribution of alcohol consumed according to our family and gene interaction model is shown in Fig. 2. It can be observed that two groups of adolescents differed from

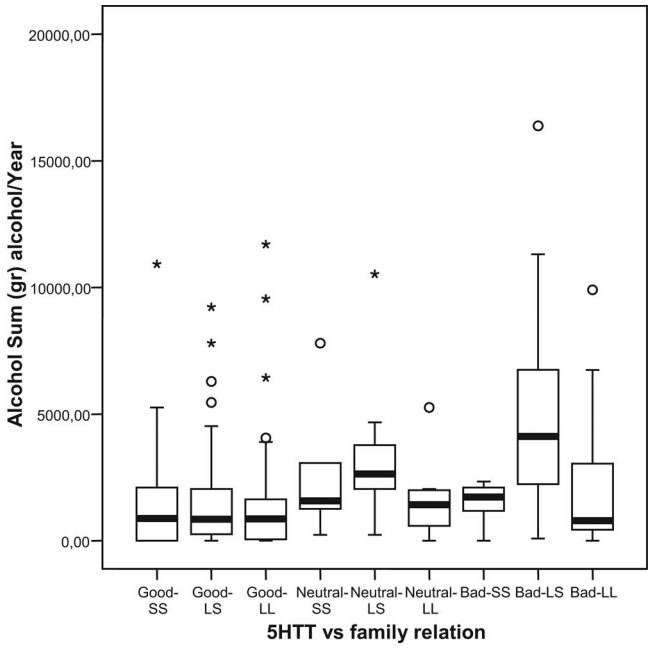

Fig. 2. Box plot of the independent variables 5-HTTLPR gene and the relations within the family in relation to the dependent variable total alcohol consumption per year.

Table 1. Biosocial Model: Dependent Variable: Alcohol Sum (g) Alcohol/Year

	df	*F*	*p*
1. 5-HTT	2	3.623	0.029
2. Family relations	2	3.909	0.022
1*2	4	2.421	0.050

Adj $R^2 = 0.112$.

the others, namely those from neutral families and those from bad families, provided that they had the long/short variant of the 5-HTTLPR polymorphism.

In our biosocial GLM of alcohol consumption, we found that the 5-HTTLPR polymorphism and family relations were related to alcohol consumption. Furthermore, 5-HTTLPR and family relations showed a significant interaction effect on alcohol consumption (Table 1). The model explained 11% of the variance in alcohol consumption. The *F* values indicate that the 5-HTTLPR genotype and relations within the family, i.e., long/short/5-HTT genotype and family relations not classified as good, contributed in a comparable order of magnitude to the variation of the amount of alcohol consumed.

The GLM was validated using two nonparametric tests. Interaction of the gene and environment after removing the effect of the factors 5-HTTLPR and quality of family relations showed significant difference ($F = 16.40$, $p < 0.001$). Mean rank of the subcategories of relations within the family and 5-HTTLPR genotype also differed significantly ($\chi^2 = 24.34$, $p = 0.002$; Table 2).

In the binary logistic model (Table 3), we found that adolescents who had the long/short variant of the 5-HTT gene and came from neutral or bad relation families had an 12- to 14-fold increased risk for high intoxication frequency. Adolescents who had long/short 5-HTTLPR and came from families with good family relations, conversely, had no elevated risk compared with the reference subcategory (5-HTT/short/short-good family relations). Adolescents with the two other variants of the 5-HTTLPR polymorphism, long/long or short/short showed no increased risk, despite deleterious family relations.

DISCUSSION

The results of the present biopsychosocial study show a gene–environment interaction that increased the risk 12 to 14 times for adolescents with regard to high alcohol intoxication frequency in the binary logistic model. The increased risk was associated with a combination of the long/short variant of the 5-HTTLPR polymorphism and poor relations within the family. Conversely, the results also suggest that a favorable family milieu protects against a genetic vulnerability and that the increased risk brought about by poor family relations may be counteracted by genetic factors.

Similar patterns were also apparent in the GLM with regard to the amount of alcohol consumed per year. Adolescents who had the long/short variant of the 5-HTTLPR polymorphism and had poor family relations consumed significantly more alcohol. Each variable was significantly related to the amount of alcohol consumed per year, and there was a significant interaction effect as well.

The dependent variable, alcohol consumption in grams per year, was severely skewed, and we had several outliers within each of the 5-HTTLPR and quality of family relation subcategories. To handle outliers by removing some of them can be seen as subjective actions that may bias the analysis. Another, more objective method is to transform the data, e.g., by a log or log-log transform. However, in the present study, neither the log nor the log-log transformation produced a symmetric distribution of the data. Instead, we used nonparametric methods to validate our findings from the GLM analysis. Both the test for interaction based on aligned ranks and the Kruskal Wallis test reached statistical significance and confirmed the results of the parametric GLM.

It was shown previously that the short variant of the 5-HTTLPR polymorphism is linked to a lower degree of expression of the gene (Collier et al., 1996). Furthermore, in previous studies in which interactions between this polymorphism and environment have been studied, presence of the short 5-HTTLPR allele has been associated with an increased risk for depression (Caspi et al., 2003; Eley et al., 2004). In this study, individuals who were homozygous for the short/short variant had higher alcohol consumption than those with long/long variants (Table 2), provided that family relations were not good. These results thus may be seen as consistent with previous findings.

Another possibility is that our results may reflect a molecular heterosis effect with regard to the 5-HTTLPR in-

Table 2. Percentages of High Intoxication Frequency, Means, Medians, Quartiles, and Mean Ranks Among Adolescents Within "Quality of Family Relation vs 5HTT Genotype Subcategories"

Quality of family relation vs. 5HTTLPR genotype	% of high intoxication frequency	Alcohol sum (g/year)			Mean rank ($p < 0.001$[a]; $p = 0.002$[b])
		Mean	Median	Quartiles (q1, q3)	
Good-SS ($n = 33$)	39	1649	878	0, 2106	86
Good-LS ($n = 61$)	46	1624	853	263, 2048	92
Good-LL ($n = 46$)	44	1574	865	59, 1638	85
Neutral-SS ($n = 5$)	60	2789	1580	1258, 3071	120
Neutral-LS ($n = 9$)	89	3282	2633	2048, 3773	134
Neutral-LL ($n = 7$)	57	1696	1424	593, 1999	98
Bad-SS ($n = 6$)	67	1514	1726	1185, 2106	103
Bad-LS ($n = 20$)	90	4985	4117	2238, 6752	148
Bad-LL ($n = 9$)	44	2471	790	439, 3042	94

SS, short/short variant; LS, long/short variant; LL, long/long variant.
[a] Nonparametric test for interaction (Öhrvik, 2002).
[b] Kruskal Wallis test.

Table 3. Biosocial Model of the 5-HTT Gene and Family Relations in Relation to "High Intoxication Frequency"

Family relations vs. 5-HTT	*n*	%	Odds ratio	Confidence interval
Good-SS	33	16.5	1.0	
Good-LS	58	29.6	1.3 NS	0.6–3.2
Good-LL	50	25.5	1.2 NS	0.5–3.0
Neutral-SS	4	2	1.5 NS	0.2–12.3
Neutral-LS	9	4.6	12.3[a]	1.4–110.3
Neutral-LL	7	3.6	2.0 NS	0.4–10.7
Bad-SS	6	3.1	3.0 NS	0.5–19.3
Bad-LS	20	10.2	13.8[b]	2.7–70.0
Bad-LL	9	4.6	1.2 NS	0.3–5.4
			R^2.15	

NS, nonsignificant. R^2 Nagelkerke.
[a] 0.05.
[b] 0.001.

teraction with family relations, because the strongest effects were found among individuals with the long/short genotype but not among individuals who were homozygous for this polymorphism. There is some evidence that molecular heterosis is common in humans and may occur in up to 50% of all associations between genotype and behavior (Comings and MacMurray, 2000). In a favorable family environment, there was no evidence for differences in alcohol consumption as a result of different genotypes, whereas in an unfavorable environment, the heterozygotic individuals showed the most divergent phenotypes. It may be speculated that a wider range of gene in 5-HTTLPR heterozygotic adolescents resulted in a higher alcohol consumption (or covariate of alcohol consumption) as a consequence of a wider range of adaptability to an unfavorable environment.

The lack of significant associations between 5-HTTLPR genotype and alcohol consumption, alcohol abuse, etc. in some previous studies (Edenberg et al., 1998; Parsian and Cloninger, 2001; Stoltenberg et al., 2002) are, in view of the present results, as has been pointed out by several authors, likely to be explained by a large psychosocial variation among the study participants. Another explanation of lack of significant associations in studies of gene-phenotypic expression is a too small sample size leading to low statistical power, as has been proposed by Sen et al. (2004), or failure to consider the possibility of heterosis (Comings and MacMurray, 2000).

The relations presented in this study are associative, which means that conclusions regarding the directions of cause and effect must be considered tentative. Another important limitation of our study is that it relies primarily on self-reports. Such reports regarding potentially ambiguous circumstances, which may be subject to subjective retrospective interpretations and reconstructions, such as whether a person has been subject to poor parenting, should be interpreted with caution (Offer et al., 2000; Sjoberg and Lindblad, 2002; Widom et al., 1999). Similarly, it is often assumed that a significant underreporting of socially stigmatized behavior as alcohol consumption may be common (Harrison, 1997; Harrison and Hughes, 1997; Midanik, 1982, 1988). Because of this, we used interview data collected simultaneously with hair samples taken and information that hair samples can be used as a biochemical marker for alcohol consumption. This can be described as a "bogus pipeline procedure" (Wagenaar et al., 1993). The use of the interview data was based on the assumption that these data were more sensitive than questionnaire data. This assumption is supported by the fact that reporting was higher for interview data. However, the utility of a bogus pipeline procedure is somewhat unclear (Campanelli et al., 1987; Wiers et al., 1998).

That the extent to which a particular individual will engage in problematic behaviors such as delinquency, violence, or drug abuse is determined by the way psychosocial, situational, and hereditary factors interact is widely accepted. In sum, our results show that a functional polymorphism of the 5-HTTLPR genotype, family relations, and interactions between these variables predict adolescent alcohol consumption in a randomized sample of adolescents. These results suggest that psychosocial and molecular aspects of human life all may eventually become appropriate targets for therapeutic interventions to reduce adolescent alcohol consumption and that such interventions when combined may even enhance the effect of one another.

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