

# The monoamine oxidase A (MAO-A) gene, family function and maltreatment as predictors of destructive behaviour during male adolescent alcohol consumption

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## ABSTRACT

**Aim** To investigate possible interactions between a polymorphism in the monoamine oxidase A (MAO-A) gene promoter, family relations and maltreatment/sexual abuse on adolescent alcohol-related problem behaviour among male adolescents. **Design, setting and participants** A cross-sectional study of a randomized sample of 66 male individuals from a total population of 16- and 19-year adolescents from a Swedish county. Boys, who volunteered to participate answering an alcohol-related problem/behaviour questionnaire, were investigated with regard to interactions between such problems, family function, maltreatment and MAO-A genotype. **Measurements** MAO-A genotype, family relations history, history of being maltreated or abused and alcohol-related problem behaviour. **Findings** Boys with the short (three-repeat) variant of the MAO-A gene, who had been maltreated/abused or came from families with poor relations, showed significantly higher scores of alcohol-related problems. We also found that maltreatment/abuse independently showed the strongest relation to alcohol-related problems among boys in our model. **Conclusions** The results suggest that both maltreatment and MAO-A genotype may be useful for the understanding of male adolescent alcohol-related problem behaviour.

**Keywords** Adolescent, alcohol drinking, behaviour, environment, genes, juvenile delinquency, risk-taking.

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## INTRODUCTION

High alcohol consumption is not always the same as destructive alcohol use or problem behaviour. However, there is a strong statistical association between alcohol use, antisocial problematic behaviour and violent crimes [1]. Some authors have concluded that alcohol intake 'causes aggression' in humans and mechanisms, which have been discussed, are reduced inhibitions, overall arousal and changes in social expectations [2].

However, while the majority of violent crimes are associated with alcohol consumption, alcohol intake among the majority of users is not associated with violence, or even aggressive behaviour. On the contrary, alcohol most often has an inhibiting effect on the central

nervous system (CNS) and on most behaviours, including aggression [3–5].

The exact manner in which genes and environmental living conditions predict the way in which an individual will react to alcohol is poorly understood. However, some of the best evidence available suggests that genes and environment interact to predict both high alcohol consumption (e.g. [6]) and aggression and conduct disorders [7,8].

One gene, which is of particular interest as far as male aggression and delinquency is concerned, is the monoamine oxidase A (MAO-A) gene. This gene is located on the X-chromosome (Xp11.23–11.4) [9] and codes for an enzyme (MAO-A; EC 1.4.3.4), which is involved in the metabolism of biogenic amines including dopamine, noradrenaline and serotonin [10]. Several observations

in animal studies, such as, for instance, MAO-A knockout mice, suggest that normal activity of this gene is essential for regulation of aggression [11]. The most striking observation demonstrating that MAO-A regulates human behaviour has been found in a Dutch family with a form of X-linked non-dysmorphic mild mental retardation. All affected males in this family have characteristic abnormal behaviour, in particular aggressive and sometimes violent behaviour and other types of impulsive behaviour, including arson, attempted rape and exhibitionism. Moreover, results of urine analysis of members of this family indicated a marked disturbance of monoamine metabolism [12,13]. With regard to polymorphisms of the human MAO-A gene, a variable number of tandem repeats (VNTR), located 1.2 kb upstream of the transcription initiation site, has been shown to affect transcriptional activity in transfected cells. In these the longer four-repeat allele was more active than the shorter three-repeat allele [14–16].

In 1999, Samochowiec and coworkers found significantly increased frequencies of the three-repeat allele of the MAO-A promoter polymorphism (low activity) in antisocial alcoholics than in controls. They suggested that the presence of this allele results in increased susceptibility to antisocial behaviour rather than to alcohol dependence *per se* [17]. Others have reported a minimal or non-significant association of the MAO-A genotype and antisocial alcoholism [18,19]. However, in a recent study it was found that the three-repeat allele was significantly more frequent among alcohol-dependent patients than controls, suggesting that the short three-repeat variant of the MAO-A allele could play a role in susceptibility to alcoholism [20].

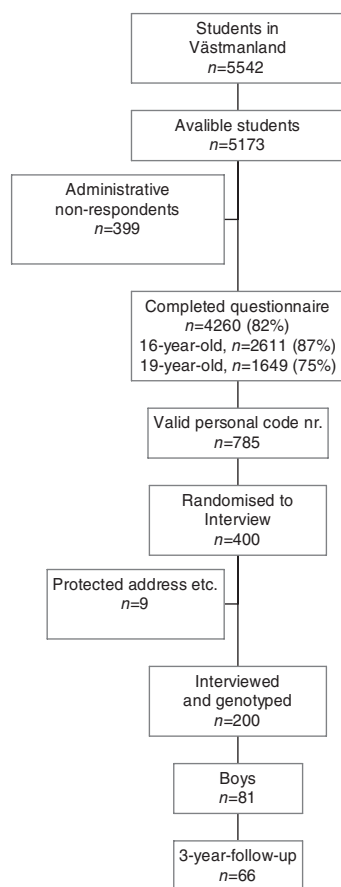
In a number of recent studies on humans it has, furthermore, been shown that the short, and thus presumably less active, variant of this MAO-A polymorphism seems to interact with environmental factors such as childhood maltreatment in explaining male adolescent delinquency [21–23]. An additional recent study on 744 males in a longitudinal North American sample, however, was unable to replicate these findings but found trends moving in a similar direction [24]. The findings of MAO-A genotype–environment interactions in humans are supported furthermore by parallel observations made on an orthologous MAO-A polymorphism and rearing conditions in non-human primates [25].

Against this background we decided to investigate possible gene–environment interactions related to alcohol-related behaviour problems among humans, with the hypothesis that the presence of the short (less active) MAO-A gene allele should interact with a poor psychosocial environment to precipitate such problems. Similar studies have not, to our knowledge, been described previously.

## 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-hour 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 of investigating gene–environment interactions in relation to deviant behaviour among adolescents. The alcohol consumption questions used in the form have been used widely in a European collaboration project, the ESPAD (European School Survey on Alcohol and Other Drugs) report [26,27]. In total there were 2611 (mean age 16.0 years) and 1649 (mean age 19.2 years) students, 87% and 75%, respectively, who 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 on the front page of the form. Informed consent was received from 785 students who could be traced with valid names. All students were classified with a risk index, describing their risk behaviours (alcohol, narcotic, sexual, property offence and violent offence) as reported in the questionnaire. They were divided subsequently into four groups according to their respective risk index. Randomized samples of 200 boys, matched for age and risk behaviours, were drawn from the volunteers. The procedure with an initial risk survey was to ensure that we would have enough participants from both ends of the deviant behaviour continuum. There were no explicit exclusion criteria. Eighty-one of the boys 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). The risk index showed no significant differences between the group interviewed and those responding to the initial questionnaire. For a further description of participants, selection and risk behaviour see Nilsson *et al.* [6]. A second questionnaire including questions concerning alcohol-related problem behaviour was mailed 3 years after the initial investigation. In this 3-year follow-up, 66 boys completed the questionnaire (Fig. 1). There were no significant differences between the 15 non-responders to the 3-year follow-up and the 66 responders with regard to amount and frequency of



**Figure 1** A flowchart describing the participants in the study

alcohol use (distributions of these variables in the whole group have been described previously elsewhere [6]).

### Interview structure design

#### *Definitions of family functioning and maltreatment/abuse experiences*

Psychosocial variables were measured by the question; '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 were things in your family when you were seven, . . . thirteen' and 'have there ever been any tough or hard periods within your family?'. If the respondent described controversies within the family they were followed-up with 'mirroring' statements/questions, e.g. 'your father hit your mother', 'your mother seemed depressed' or 'you said that your brother caused a lot of trouble'. The responses of the participants to these questions of family function were then brought together and transformed from a 'qualitative' to a 'quantitative' ordinal scale final stage, comprising the psychosocial variable 'quality of relations within the family' (good/intermediate/bad). This transformation of the answers from the research interview of the psychosocial

factor was made in order to mimic a clinical interview [Axis IV of psychosocial and environmental problems in the *Diagnostic and Statistical Manual of Mental Disorders* version IV (DSM-IV) manual, which is usually considered a useful instrument for organizing and communicating clinical information] [28]. If no significant maltreatment or abusive experiences were detected in the above-mentioned questions, a more direct question was asked: 'have you ever been exposed to any type of mean treatment from others?' followed by examples such as: been hit, shameful or disgraceful handling, sexually abusive activities by others? This topic was coded as 'experiences of maltreatment/abuse' (yes or no). Inter-rater reliability (measured as Cohen's  $\kappa$ ) for two raters, who listened to a 10% sample of the audiotaped interviews, was 1.0 for experiences of violent victimization and 0.7 for quality of family relations.

#### *Definitions of alcohol-related problem behaviour*

Three years after the initial questionnaire, interviews and blood samples, an additional questionnaire was mailed to the participants. Of the initial study population 66 of 81 boys answered, at that time being 19 and 22 years of age. The following question about alcohol-related problem behaviour was asked: 'have you during the latest 12 months, had any of the following problems due to alcohol intake: (1) quarrels; (2) been in fights; (3) been in an accident; (4) lost money or articles of value; (5) damaged clothes or objects; (6) taken money or other valuable things; (7) relations to parents; (8) relations to friends; (9) relations to other adults; (10) performance at school or at work; (11) unwanted sex; (12) unprotected sex; (13) driven a motorbike or car while intoxicated; (14) been robbed or mugged; and (15) been in trouble with the police?'. The response alternatives were 'never', 'once', 'twice' and 'three times or more'. The adolescents who had experienced problems in any of the above-mentioned problem areas fulfilled the requirement for alcohol-related problem behaviour. Furthermore, the participants also answered the structured Alcohol Use Disorders Identification Test (AUDIT) to measure risk-consumption. An AUDIT cut-off score of 6 or greater demonstrated a sensitivity of 91.0% and a specificity of 60.0% in the detection of high-risk drinkers in a same-age population [29]. Similar results have been found [30,31] with a sensitivity of 80–84% and a specificity of 71–78%. These studies support the use of the AUDIT in college student samples.

#### *MAO-A-VNTR analysis*

Genomic DNA was isolated from venous blood samples drawn from each subject. For genotyping, a 50  $\mu$ l reaction mixture was used containing 75 ng genomic DNA, polymerase chain reaction (PCR) buffer (200 mM

Tris-HCl, pH 8.4, 500 mM KCl), 1 mM MgCl<sub>2</sub>, 400 μM deoxyribonucleoside triphosphate (dNTP) [100 μM each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), thymidine triphosphate (dTTP), deoxyguanine triphosphate (dGTP)], 4 pmol of each primer, 0.03% W-1 buffer and 1.5 units *Taq*-DNA polymerase (Invitrogen™). As a forward primer, 5'-AC AGC CTG ACC GTG GAG AAG-3' was used, and as a reverse primer 5'-GAA CGG ACG CTC CAT TCG GA-3' was used. PCR reactions were performed on a GeneAmp 9700® with the following profile: 95°C for 60 seconds, 35 × (95°C for 60 seconds, 63.5°C for 60 seconds and 72°C for 90 seconds) and finally 72°C for 5 minutes. The PCR products were analysed by electrophoresis on a 2% agarose gel. The gel was run for 1 hour at 90 V and visualised under UV light by ethidium-bromide staining. The buffer used as running buffer was 0.5 × [Tris-borate-ethylendiamine tetraacetic acid (EDTA)] TBE buffer. DNA bands representing the different genotypes were read from the gel and validated by another person from the gel photographs.

When genotypes were not obvious to the readers (five cases), the PCR procedure was re-run. In order to validate the MAO-A VNTR genotypes, PCR products representing all genotypes were sequenced using BigDye® Terminator chemistry (Applied Biosystems, Foster City, CA, USA), and were analysed by an automated ABI PRISM™ version 310 Genetic Analyzer (ABI PRISM™, Perkin Elmer, Foster City, CA, USA). The DNA sequences were analysed using Sequencher™ version 3.1.1 software. No errors in the genotype analyses were detected.

### Statistical analysis

To compare the alcohol-related problem behaviour and AUDIT-scores Spearman's *r* was used.

A general linear model (GLM) and regression analysis were used to investigate the MAO-A genotype, quality of family relations, maltreatment/abuse and their interaction in relation to the dependent variable alcohol-related problem behaviour. Because of the non-normal distribution of the behaviour variable alcohol-related problem, a non-parametric test for interactions based on aligned ranks was also applied. Briefly, this test is based on the sum of all observations after a measure of the central tendency of the sample in each particular variable (main effects) has been subtracted from each individual observation. In this case, effects of the MAO-A gene factor and of psychosocial factors were removed from the values of each individual one at a time. The sum of squared differences between the levels displayed by the individuals in each subcategory (each combination of MAO-A gene factor and a psychosocial factor) and the total mean rank will be approximately

**Table 1** Percentage of adolescent boys who reported different alcohol-related problem behaviours during the latest 12 months.

<i>Once or more often</i>	<i>Boys, n = 66 (%)</i>
Quarrels	48
Been in fights	20
Been in an accident	18
Lost money or articles of value	26
Damaged clothes or objects	31
Taken money or other valuable things	3
Relations to parents	9
Relations to friends	15
Relations to other adults	17
Performance at school or at work	14
Unwanted sex	19
Unprotected sex	42
Driven a motorbike or car while intoxicated	20
Been robbed or mugged	5
Been in trouble with the police	9
Sum. any alcohol-related problem	70

*F*-distributed [32]. This proposed test has been applied in simulation studies by Correa & Bellavance [33] using multivariate normal and gamma distributions with substantially higher power than a modified *F*-test, also reviewed by Putt & Chincilli [34]. As a measure of central tendency the Hodges-Lehmann estimator (H/L estimator), which is the median of the pairwise means of the observations, was used. It is known to have very good robustness properties, e.g. it is neither sensitive to extremes nor is it sensitive to gaps in the middle of the data set [35,36]. If the null hypothesis was rejected a Kruskal-Wallis test was run with each MAO-A gene and psychosocial risk combination, constituting a separate subcategory.

A two-sided *P*-value less than 0.05 was considered significant in all analyses of main effects and less than 0.1, when testing for the presence of interactions [37].

### RESULTS

Three boys (4.5%) did not drink alcohol at all. According to the AUDIT scale, 75% of the boys were risk-consumers of alcohol. The overall correlation between the alcohol-related problem behaviour scores and AUDIT scores was 0.619, *P* < 0.001.

The most common alcohol-related risk behaviour was quarrels (48%), followed by unprotected sex (42%), damaged clothes or objects (31%) and lost money or articles of value (26%) (Table 1).

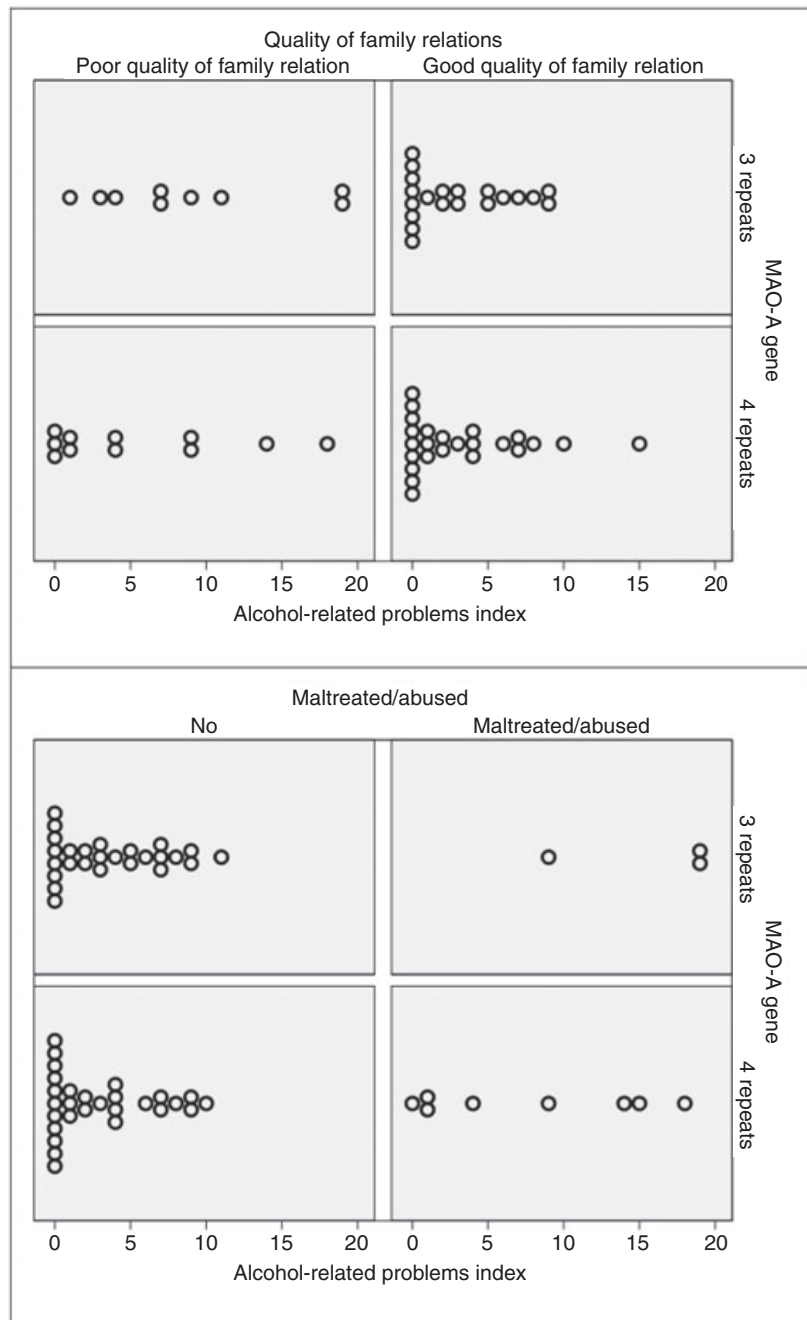
The MAO-A genotype frequencies in the series were as follows: 41% displayed three repeats, 57% displayed four repeats and one boy displayed 2.5 repeats (excluded).

**Table 2** Quality of family relations and maltreatment/abuse separated by the MAO-A gene.

Boys	MAO-A gene		
	3 repeats	4 repeats	Total
Quality of family relations			
Good	20 (69%)	25 (69%)	45 (70%)
Intermediate	5 (17%)	5 (14%)	10 (15%)
Poor	4 (14%)	6 (17%)	10 (15%)
Maltreated/abused			
No maltreatment/abuse	26 (90%)	28 (78%)	54 (83%)
Maltreated/abused	3 (10%)	8 (22%)	11 (17%)

There was no association of the different MAO-A genotypes in relation to quality of family relations ( $\chi^2 = 0.979$ ,  $df = 2$ ,  $P = 0.613$ ) or maltreatment ( $\chi^2 = 0.404$ ,  $df = 1$ ,  $P = 0.525$ ). Due to the small sample size the variable quality of family relations was merged into a dichotomous variable (good versus intermediate/bad) (Table 2).

In Fig. 2, the raw data for each individual are plotted, first the MAO-A genotype, depending on quality of family relations and secondly, MAO-A genotype depending on maltreatment/abuse. There were small group sizes in the psychosocial risk groups, poor quality of family relations and maltreated/abused.



**Figure 2** Two dot-plots describing the alcohol-related problem behaviour in the subgroups of MAO-A genotype and the two psychosocial variables, quality of family relations and maltreated/abused

**Table 3** The regression model of direct- and interaction effects of MAO-A genotype, family relations and maltreatment/abuse in relation to alcohol-related problem behaviour with B, actual significance level, *t*-statistics and 95% confidence interval for B (model A). The independent effects of each variable; MAO-A (model B), quality of family relations (model c) and maltreatment/abuse (model D) in relation to alcohol-related problem behaviour are also shown.

Alcohol-related problem behaviour					
<b>Model A: Full model No interaction</b>	B	Sig.	t	95% CI for B lower	95% CI for B upper
1. MAO-A	-0.878	0.105	-1.646	-1.945	0.189
2. Family relations	1.867	0.135	1.515	-0.600	4.352
3. Maltreatment/abuse	6.220	<0.001	4.034	3.136	9.304
Adj R <sup>2</sup> = 0.295					
<b>Model B: Interaction Model</b>	B	Sig.	t	95% CI for B lower	95% CI for B upper
1*2	-2.045	0.085	-1.749	-4.383	0.295
1*3	-2.709	0.086	-1.749	-5.808	0.391
2*3	2.638	0.389	0.868	-3.449	8.724
Adj R <sup>2</sup> = 0.374					
<b>Model C: MAO-A gene</b>	B	Sig.	t	95% CI for B lower	95% CI for B upper
MAO-A	-0.414	0.509	-0.664	-1.659	0.832
Adj R <sup>2</sup> = -0.009					
<b>Model D: Quality of family relations</b>	B	Sig.	t	95% CI for B lower	95% CI for B upper
Family relations	3.911	0.003	-3.144	1.425	6.397
Adj R <sup>2</sup> = 0.122					
<b>Model E: Maltreatment/abuse</b>	B	Sig.	t	95% CI for B lower	95% CI for B upper
Maltreatment/abuse	6.655	<.001	4.697	3.825	9.485
Adj R <sup>2</sup> = 0.245					

To analyse alcohol-related behaviour problems as a function of the MAO-A genotype, quality of family relations, maltreatment/abuse and interactions between those variables we first used general linear models (GLM) and regression analysis. Because the MAO-A gene is located on the X-chromosome boys have a single gene, short (S) or long (L), with regard to the polymorphism investigated. In our first model (Table 3, model A) we analysed boys (S or L in the MAO-A gene; good, intermediate/poor in quality in family relations; and maltreated/abused, yes or no) without the two-way interactions. The first model showed no direct effects of MAO-A and family relations, whereas maltreatment/abuse were related to alcohol-related problem behaviour (model A). Furthermore, the MAO-A genotype showed trends of interaction with both the quality of family relations and maltreatment/abuse, with the model explaining 37% (adjusted R<sup>2</sup>) of the variance in alcohol-related

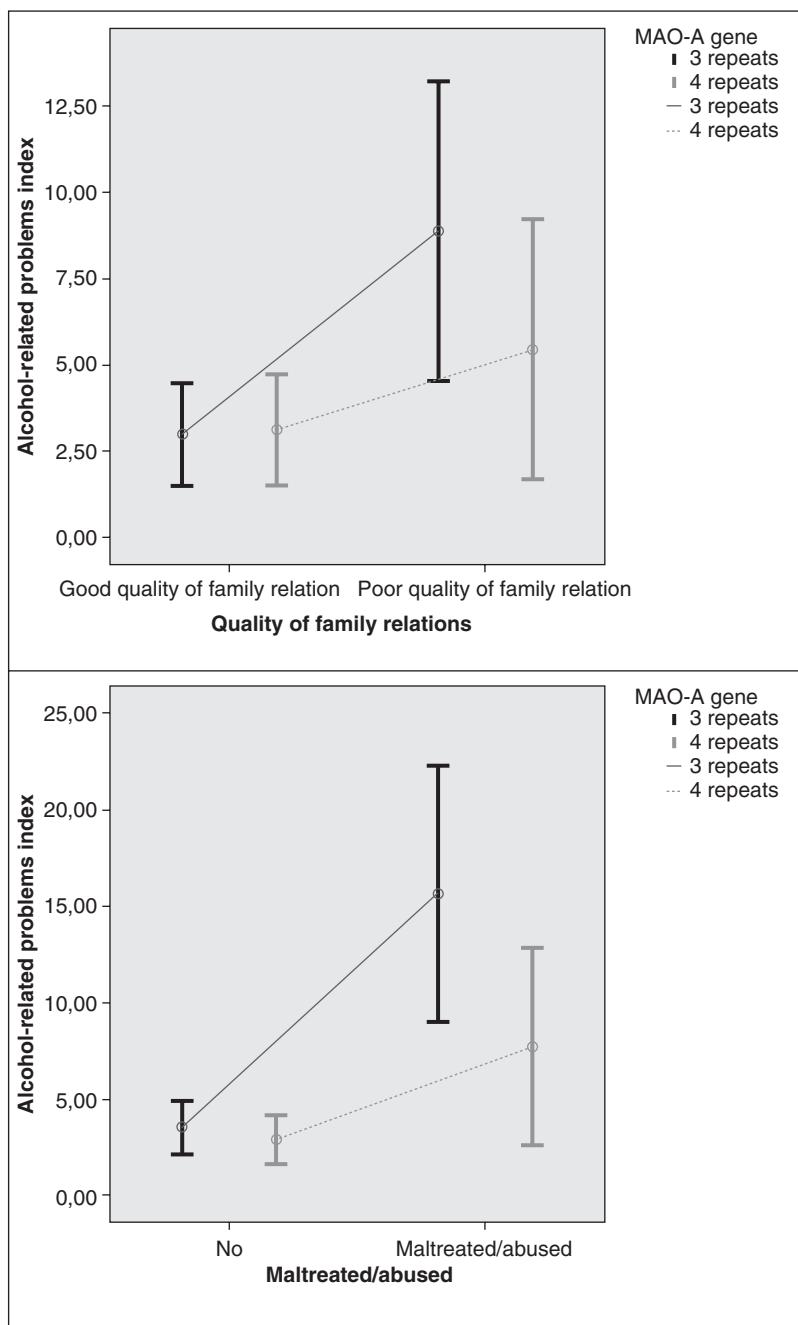
problem behaviour (model B). There was no significant interaction effect between quality of family relations and maltreatment/abuse.

If MAO-A was entered in the model (model C) without the two psychosocial variables there was no relation to alcohol-related problem behaviour. We also performed a model of the two psychosocial variables. If both variables were entered into the same model, only maltreatment/abuse showed a significant effect and there were no interaction effects (not shown in Table 3). In separate models (models D–E), both showed independently a relation to alcohol-related problem behaviour. Quality of family relations had an explained variance of 12%, whereas the corresponding figure for maltreatment was 24.5%.

The relation between dependent variables and interactions with alcohol-related problem behaviour is visualized in Fig. 3. Boys from families reported as having a good quality of family relations had fewer alcohol-related problem behaviours, whereas boys from families with poor relations had more problems. Similarly, boys who had been maltreated/abused had more alcohol-related problem behaviour than other boys. However, the alcohol-related problem behaviour among boys from families with poor family relations and maltreated or abused boys was higher among boys with the short, less active, three-repeat variant MAO-A genotype compared with boys with the long, more active, four-repeat variant of the gene.

To validate the parametric models we used two non-parametric tests (Ohrvik aligned rank test of interaction and Kruskal–Wallis test) to investigate the differences in mean rank of alcohol-related problem behaviour among subgroups defined by: MAO-A genotype (three versus four repeats), quality of family relations (good versus intermediate + poor), maltreated/abused (yes or no) and in gene–environment interaction. We found no significant interactions based on aligned ranks of the H/L estimator for MAO-A and quality of family relations ( $F = 2.47$ ,  $df_1 = 1$ ,  $df_2 = 65$ ,  $P = 0.121$ ). However, MAO-A and maltreatment/abuse had significant interaction effects ( $F = 4.52$ ,  $df_1 = 1$ ,  $df_2 = 65$ ,  $P = 0.037$ ) see Table 4. Furthermore, we found significant patterns in the distribution of the dependent variable in subcategories for MAO-A-quality of family relations ( $\chi^2 = 8.81$ ,  $df = 3$ ,  $P = 0.032$ ) and MAO-A-maltreatment/abuse ( $\chi^2 = 10.86$ ,  $df = 3$ ,  $P = 0.013$ ) with the Kruskal–Wallis test.

To enlarge the 'psychosocial at-risk' subgroups we merged the psychosocial variables to a variable with psychosocial risk, any or both. There were no differences between three- and four-repeat individuals of the MAO-A gene, if at no psychosocial risk. However, individuals at psychosocial risk showed elevated alcohol-related problem behaviour, especially the individuals with the



**Figure 3** Two error-bar-chart describing the alcohol-related problem behaviour in the subgroups of MAO-A genotype and the two psychosocial variables, quality of family relations and maltreated/abused, with trends  $\pm 2$  standard deviations

short, less active three-repeat variant, and there was a significant interaction between the merged psychosocial variable and the MAO-A gene ( $\chi^2 = 11.197$ ,  $df = 3$ ,  $P = 0.011$ ) with the Kruskal–Wallis test. The non-parametric tests showed the most robust overall gene–environment interaction in relation to alcohol related problem behaviour.

## DISCUSSION

In the present study we tested the hypothesis that there is a gene–environment interaction in male adolescent

alcohol-related problem behaviour with regard to a polymorphism in the MAO-A gene promoter. The results show that boys with the short variant of the MAO-A gene, who had been either maltreated/abused or came from families with poor-quality relations, showed significantly higher scores of alcohol-related problems than those who did not meet these criteria. These results are reminiscent of previous findings of interactions between environment and the MAO-A genotype investigated in the present study for male adolescent delinquency [21–23].

Maltreatment/abuse showed independently the strongest overall relation to alcohol-related problems among

**Table 4** Number, means, medians, H/L-estimators, quartiles, mean ranks and actual significance levels within MAO-A genotype, quality of family relations and maltreatment/abuse sub-categories.

	Mean	Median	H/L-estimator (a) ( $P = 0.121$ )	Quartiles ( $q1-q3$ )	Mean rank (b) ( $P = 0.032$ )
MAO-A versus quality of family relations					
3 repeats: good, $n = 20$	3.8	2.0	3.0	0–5.5	28.65
3 repeats: poor, $n = 9$	8.9	7.0	8.0	3.5–15	48.11
4 repeats: good, $n = 24$	3.1	1.5	2.5	0–7	28.56
4 repeats: poor, $n = 11$	5.4	4.0	4.5	0–9	35.32
MAO-A versus maltreatment/abuse					
			(a) ( $P = 0.037$ )	( $q1-q3$ )	(b) ( $P = 0.013$ )
3-repeats: no abuse, $n = 26$	3.6	3.0	3.5	0–7	31.77
3 repeats: abuse, $n = 3$	15.7	19.0	16.5	14–19	61.50
4 repeats: no abuse, $n = 28$	2.9	1.5	2.5	0–5	28.43
4 repeats: abuse, $n = 8$	7.8	6.5	7.8	1–14.5	42.31

(a) Non-parametric test for interaction [32]; (b) Kruskal–Wallis test.

boys in our model. However, maltreatment showed indications of higher impact among individuals with the short (three-repeat variant) of the MAO-A gene, which has been shown previously to be less active in transfected cells [14]. Furthermore, we found no evidence of association of MAO-A genotype with alcohol-related problem behaviour when psychosocial factors were not controlled for, whereas the biosocial model exceeds the social model with 14% explained variance.

The distribution of the different alleles in the present sample [41% three repeats, 57% four repeats and one 2.5 repeat (excluded)] differs somewhat from other studies, where three repeats have usually been less common, e.g. 38% in Caspi *et al.* [21] and 34% in a Swedish study by Jonsson *et al.* [38]. This was not, however, a result of the stratified selection of probands. Thus, there were no significant differences in the frequency of the three-repeats allele in the three risk groups from which the probands were drawn randomly (no risk,  $n = 29$ , 41%, medium risk,  $n = 22$ , 54.5% and high risk  $n = 28$ , 35.5%).

The results of the present study support the notion that MAO-A genotype and psychosocial risk factors interact to predict male adolescent risk behaviour. However, the question of whether these differences occur as a result of differences in alcohol intake, if they are related to different pharmacological effects of alcohol, or whether they are mediated by situational and personality factors, cannot be answered on the basis of this type of study. In order to study this question, controlled experimental animal and/or human studies would ideally be needed.

Alcohol consumption among these now 19- and 22-year-old adolescents was frequent. Seventy-five per cent of the boys were risk-consumers according to the AUDIT scale. Only 4% were teetotalers. As many as 70% have had some kind of alcohol-related problem

behaviour during the latest 12-month period, and the most frequent problems were quarrels, unprotected sex and damaging clothes or objects. We also found that the alcohol-related problem behaviour index had a reasonable high correlation with AUDIT scores.

The questions used in the initial form have been used extensively in ESPAD report studies, and has some relation to DSM measures. The ESPAD report measures alcohol use: life-time, latest 12 months and last 30 days. Furthermore, it measures: alcohol debut age, heavy consumption last 30 days, intoxication, life-time, latest 12 months and last 30 days, perceived consequences from drinking alcohol, experiences of problems because of alcohol, disapproval of different behaviours and perceived risk of use. Those questions were used as the base for the qualitative semistructured interview, and therefore replication both within and outside Europe has population-based reference estimates. Even though that there is no direct overlapping between ESPAD and DSM, several of the ESPAD measures could be 'translated' to the different DSM criteria of alcohol intoxication, dependence and abuse.

The dependent variable, alcohol-related problem behaviour, was severely skewed and we had outliers within each of the MAO-A and quality of family relations subcategories. One way of dealing with this type of problem in the data would be by removing outliers. However, such actions may be seen as a subjective source of potential bias of the analysis. Another more objective method is to transform the data, e.g. by a log or log–log transformation. However, in the present study neither the log- nor the log–log transformation produced a symmetric distribution of the data. Furthermore, the sample size was small, not optimal for parametric modelling methods. Instead, therefore, we used non-parametric methods to validate our findings from the parametric

analysis. The procedure with complementary statistical approaches can help to eliminate scaling artefact, one of the most ubiquitous sources of artefact in interaction research. Both Ohrvik's test for interaction based on aligned ranks and the Kruskal–Wallis test reached statistical significance and confirmed the results of the parametric model. However, as the Kruskal–Wallis test tests jointly both main and interactions effects the results should be interpreted with caution.

One potential limitation of the present study is that it relies primarily on self-reports. Such reports, regarding potentially ambiguous circumstances, may be subject to retrospective reinterpretations and should thus be interpreted with caution [39–41].

Another limitation of the present study is that our data are based on associations, which means that conclusions regarding the directions of cause and effect must be considered tentative for maltreatment, which was a significant predictor. Therefore, for instance, even though we found that experiences of being a victim of violence (maltreatment/abuse) or poor quality of family relations may predict alcohol-related problem behaviour, it may be objected that what we have actually measured might be an outcome of an 'innate tendency' towards deviant behaviour. Such a tendency may have led an individual to situations which will sometimes result in 'alcohol-related problem behaviour', as quarrels or fights, by people defending themselves from him or as a punishment by parents for hazardous drinking. This might have led some of the individuals to rate their quality of family relations in a more negative way, or even have led them to report maltreatment from family members.

A third limitation in this study is the small sample size and the question of representativeness and generalizability of the result. We compared our study sample with the target population concerning the initial risk index and found that there were no differences. Nevertheless, the results of this study need replication before a conclusive declaration can be made about the gene–environmental interaction in relation to destructive alcohol consumption.

The lack of significant results in studies on genotype in relation to phenotypic expression can often be explained by small sample size. This study demonstrates that even with a relatively small sample, genetic contributions to behavioural phenotypic expressions can be observed if models are adjusted for environmental factors. However, the significant direct effect of the MAO-A gene, when controlled for the two psychosocial variables and the interaction effects, is probably an artefact of the model containing interaction effects.

Moreover, even though this is a small sample, very few genetic study samples of alcohol-related problems

originate from the general population, which gives this study some advantages.

The results suggest that both psychosocial and molecular perspectives may be useful for the understanding of male adolescent alcohol-related problem behaviour. They suggest furthermore that both biological and environmental factors might be targets for therapeutic interventions in order to reduce adolescent alcohol-related problem behaviour and that such interventions, when combined, might even enhance the effect of one another.

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