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Evidence for interplay between genes and parenting on infant temperament in the first year of life: monoamine oxidase A polymorphism moderates effects of maternal sensitivity on infant anger proneness

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Background: The low expression polymorphism of the MAOA gene in interaction with adverse environments (G × E) is associated with antisocial behaviour disorders. These have their origins in early life, but it is not known whether MAOA G × E occurs in infants. We therefore examined whether MAOA G × E predicts infant anger proneness, a temperamental dimension associated with later antisocial behaviour disorders. In contrast to previous studies, we examined MAOA G × E prospectively using an observational measure of a key aspect of the infant environment, maternal sensitivity, at a specified developmental time point. **Methods:** In a stratified epidemiological cohort recruited during pregnancy, we ascertained MAOA status (low vs. high expression alleles) from the saliva of 193 infants, and examined specific predictions to maternal report of infant temperament at 14 months from maternal sensitivity assessed at 29 weeks of age. **Results:** Analyses, weighted to provide general population estimates, indicated a robust interaction between MAOA status and maternal sensitivity in the prediction of infant anger proneness (p = .003) which became stronger once possible confounders for maternal sensitivity were included in the model (p = .0001). The interaction terms were similar in males (p = .010) and females (p = .016), but the effects were different as a consequence of an additional sex of infant by maternal sensitivity interaction. **Conclusions:** This prospective study provides the first evidence of moderation by the MAOA gene of effects of parenting on infant anger proneness, an important early risk for the development of disruptive and aggressive behaviour disorders. **Keywords:** Monoamine oxidase A, promoter polymorphism, maternal sensitivity, infant temperament, anger proneness, gene by environment interaction.

Introduction

Our understanding of the neurobiology of violence, childhood conduct disorders and antisocial personality in adulthood has been substantially increased by the identification of interactions between genetic and environmental variations. A genotype by environment interaction (G × E) occurs when a person's genotype moderates the effect of environmental experience on physical or mental health outcomes or when environmental experience moderates a genetic effect (Moffitt, Caspi, & Rutter, 2005).

One of the most widely studied G × E interactions involve polymorphisms of the monoamine oxidase (MAOA) gene 30-bp Variable Number Tandem Repeat located in the upstream promoter region (the uVNTR). Possession of 3.5 and 4 copies of the repeat is associated with higher in vitro expression (MAOA-H) than possession of 3 or 5 repeat (MAOA-L) variants (Sabol, Hu, & Hamer, 1998). The MAOA-L variant in interaction with childhood maltreatment has been found to be associated with externalizing childhood behaviours and adult antisocial outcomes in many (Beach et al., 2010; Caspi et al., 2002; Fergusson, Boden, Horwood, Miller, & Kennedy, 2011, 2012), although not all (Huizingaa et al., 2006; Prichard, Mackinnon, Jorm, & Easteal, 2008), studies. Furthermore, both Kim-Cohen et al. (2006) and Beach et al. (2010) found that MAOA G × E was associated with attention deficit and hyperactivity problems, but not with conduct problems, in children. In most cases, the interaction has arisen from associations of environmental variations with outcomes in the low expression, but not the high expression variant of the MAOA gene.

In spite of the extensive study of associations of MAOA variants with antisocial behaviours, there are no published studies examining the early developmental processes that may link MAOA G × E to disorder. Violence in adults is almost always preceded by problems of aggression and oppositionality in early childhood (Odgers et al., 2008), so the study of processes preceding the appearance of childhood symptoms needs to start in infancy. There are probably several pathways from infancy to conduct disorders in young children; however, evidence converges

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on the centrality of negative emotionality, either as a main effect or in interaction with environmental factors (Hill, 2002; Lahey et al., 2008). In particular, indices of proneness to irritability or fussiness, or of distress to limitations taken as an index of anger proneness, have been shown to predict later externalizing symptoms, either as main effects or in interaction with environmental factors (e.g. Lahey et al., 2008; Smeekens, Riksen-Walraven, & van Bakel, 2007). In a study of 1863 children, elevated infant fussing rated by mothers predicted conduct problems assessed by parental report on at least two occasions between 4 and 13 years (Lahey et al., 2008). A negative emotionality by environment interaction was reported by Belsky, Hsieh, and Crnic (1998) who found that infants who scored high in negative emotionality at 12 months of age, and who experienced the least supportive mothering and fathering across their second and third years of life, scored highest on conduct problems at 36 months of age. Similarly, Smeekens et al. (2007) reported an interaction between toddler anger proneness rated by parents and quality of observed parent–child interactions at 15 and 28 months in the prediction of conduct problems rated by parents and teachers at the age of 5 years. Lack of firm warm guidance from parents predicted conduct problems only in infants with high anger proneness (Smeekens et al., 2007).

Studies of rare individuals with point mutations of the MAOA gene, resulting in deficiency of MAOA enzymatic activity, have found associations with impulsive aggression consistent with there being developmental consequences of lower MAOA enzyme levels specifically on anger proneness (Brunner, Nelen, Breakefield, Ropers, & van Oost, 1993). This is also supported by structural and functional imaging studies of adults. For example, Buckholtz and Meyer-Lindenberg (2008) found that MAOA-L adults have lower volumes throughout the limbic system, and patterns of activation suggestive of reduced connectivity between the medial prefrontal cortex and the amygdala, consistent with impaired prefrontal regulation of limbic systems associated with impulsive aggression. Meyer-Lindenberg et al. (2006) have proposed that the low expression MAOA variant may specifically contribute to anger-related impulsive aggression, but not to instrumental aggression, which is more likely to be associated with lack of remorse and low fear or anxiety.

Genetic influences on temperamental variations are well established from behavioural and molecular genetic studies (e.g. Sheese, Voelker, Posner, & Rothbart, 2009). There is also some evidence consistent with effects of the environment moderated by genotype. In a cross-sectional study of 45 children aged 18–21 months, there was an association between observed quality of parenting and parental report of infant temperament which was modified by DRD4 genotype (Sheese, Voelker, Rothbart, & Posner, 2007). Support for G × E on infant temperament arising from the foetal environment comes from a study in which a serotonin transporter polymorphism moderated associations between prenatal maternal anxiety and infant negative emotionality at 6 months (Pluess et al., 2011). Studies of the interplay between MAOA polymorphisms, environmental variations and infant temperament have not previously been reported.

Most G × E studies of MAOA have shown interactions with traumatic or stressful environmental exposures. These findings could be interpreted to mean that individuals possessing MAOA-L are vulnerable to adversity, while those with MAOA-H are resilient. An alternative hypothesis is that the MAOA-uVNTR influences an individual's responsivity to variations in the social environment irrespective of whether they entail adversity (Belsky & Pluess, 2009). This is supported by functional imaging studies of adults indicating that MAOA influences social responsivity over the normal range (Eisenberger, Way, Taylor, Welch, & Lieberman, 2007). In line with this, if MAOA-L infants are more responsive to social processes of acceptance or rejection, then they will be more greatly affected by mothers' sensitivity to their cues for playful participation, support or comfort in comparison with MAOA-H infants. In this study, we aimed to test this hypothesis in the context of a longitudinal investigation of the earliest origins of conduct problems.

As the MAOA-uVNTR locus is located in the X chromosome, many studies of MAOA G × E only report on males because of uncertainties regarding the expression status of female heterozygotes (Caspi et al., 2002; Fergusson et al., 2011; Kim-Cohen et al., 2006). Some studies that have included females have found sex differences (e.g. Aslund et al., 2010), while others have not (e.g. Nikulina, Widom, & Brzustowicz, 2012).

Set against the background of previous studies of MAOA by child maltreatment interactions in the prediction of antisocial behaviour problems, this study is novel in five respects: first, it examines MAOA G × E using an observational measure of the environment rather than self or parent reports; second, the environmental variation is assessed at a specified developmental time point with prospective follow-up to the outcome; third, it investigates the contribution of MAOA G × E to temperamental variations likely to contribute to risk of later conduct disorders; fourth, these are examined in infancy; and fifth, it evaluates the role of normal variations in maternal caregiving.

Here, we report findings from a longitudinal study in which we made two predictions. First, MAOA-uVNTR variants will interact with variations in maternal care over the normal range, assessed as maternal sensitivity, in the prediction of infant anger proneness. Second, the interaction will arise from an association of lower maternal sensitivity with anger proneness in the MAOA-L, but not the MAOA-H group.

Sex differences were not hypothesized, but in the light of some previous studies, we check for evidence that the interactions differed between males and females. In testing the robustness of findings for this prespecified interaction, other explanations, including interactions of MAOA with a number of alternative measures of the environment, and additional G × E effects are examined.

Methods

Design

The participants were members of the Wirral Child Health and Development Study, a prospective epidemiological longitudinal study of prenatal and infancy origins of conduct disorders. This uses a two-stage stratified design in which a larger general population sample of first-time mothers was recruited during pregnancy (extensive sample) and from which a subsample was drawn for more intensive assessment (intensive sample). All families in the extensive sample follow a brief assessment protocol while those in the intensive subsample receive more time-consuming detailed assessments such as the observations of mother–infant interactions described in this paper. The design allows general population estimates of means and associations to be derived for all extensive or intensive sample measures.

Approval for the procedures was obtained from the Cheshire North and West Research Ethics Committee (UK). The extensive sample was identified from consecutive first-time mothers who booked for antenatal care at 12 weeks gestation between 12/02/2007 and 29/10/2008. The booking clinic was administered by the Wirral University Teaching Hospital which is the sole provider of universal prenatal care on the Wirral Peninsula. Socioeconomic conditions on the Wirral range between the deprived inner city and affluent suburbs, but with few from ethnic minorities. The study was introduced to the women by clinic midwives who asked for their agreement to be approached by study research midwives when they attended for ultrasound scanning at 20 weeks gestation. After complete description of the study to the women, written informed consent was obtained by the study midwives, who then administered questionnaires and an interview in the clinic.

Participants

Of those approached by study midwives, 68.4% gave consent and completed the measures, yielding an extensive sample of 1,233 mothers with surviving singleton babies. The sampling flow chart has been published previously (Sharp et al., 2012). The mean age at recruitment of extensive sample participants was 26.8 years (*SD* 5.8, range 18–51). Using the UK Index of Multiple Deprivation (IMD) (Noble et al., 2004) based on data collected from the UK Census in 2001, 41.8% of the extensive sample reported socioeconomic profiles found in the most deprived UK quintile, consistent with the high levels of deprivation in some parts of the Wirral. Forty-eight women (3.9%) described themselves as other than White British. Demographic and antenatal stratification measures were administered at 20 weeks gestation with all extensive sample participants. Reports of infant temperament were available from 722 mothers at 14 months of age.

A stratified random subsample of 316 mothers was recruited to the intensive sample at 32 weeks gestation on the basis of their prior responses to a measure of partner psychological abuse on entry into the extensive sample at 20 weeks gestation (Sharp et al., 2012). Of these, 272 were observed in interaction with their infants as part of a comprehensive assessment lasting around 3 hr, from which maternal sensitivity measures were derived at mean age of 29.1 (*SD* 3.1) weeks ('29 weeks'). At 14 months, when saliva was collected for DNA, 268 mothers and infants came to the laboratory for a further 3-hr assessment. Seven parents declined consent for DNA collection, three samples were spoilt and 25 assessments were curtailed before saliva collection because of time constraints. Saliva was therefore available from 233 infants, and genotyping was successful for 216, giving a call rate of 92.7%. Infant Behaviour Questionnaires on a further 23 infants were not returned at 14 months, so 193 cases had 29 weeks maternal sensitivity, DNA data and 14 months infant temperament measures available for analysis.

Measures

Infant temperament at 29 weeks and 14 months. Anger proneness was assessed twice by maternal report using the Infant Behavioral Questionnaire – Revised (IBQ – R) (Gartstein & Rothbart, 2003), first at the same time as maternal sensitivity and second as an outcome measure at 14 months of age. Anger proneness was assessed using the 'Distress to Limitations' scale which has 16 items reflecting fussing, crying or showing distress while frustrated. The IBQ-R has established reliability and validity and has been widely used in developmental studies (Gagne & Goldsmith, 2011; Parade & Leerkes, 2008).

Maternal sensitivity at 29 weeks post natal. Maternal sensitivity was assessed with a 15-min standard laboratory-based procedure (NICHD Early Child Care Research Network, 1999). Mothers were asked to play with their infants as they would at home, for 7 min with toys supplied by the mother, and for 8 min with a standard set of toys provided by the experimenter. Maternal sensitivity

was rated from video recordings on a global 5-point scale, ranging from 1 (*not at all characteristic*) to 5 (*highly characteristic*) reflecting mothers' appropriate, supportive, warm responding to infant communications, playful bids or distress. Training on the sensitivity measure was provided by an investigator from the NICHD Network. Three raters, blind to the other measures, coded sensitivity from video recordings. Each rater achieved good inter-rater reliability for maternal sensitivity on a subset of 30 assessments (ICCs .85–.91).

Confounders and stratification variable. At recruitment into the extensive sample, mothers completed the stratification measure which was a report of partner psychological abuse. This was assessed using a 20-item questionnaire covering humiliating, demeaning or threatening utterances in the partner relationship during pregnancy over the previous year (Moffitt et al., 1997). The scale comprised the total from 20 no-yes (coded as 0 absent, 1 present) items. Participants first rated these items about their own behaviour towards their partner, and then about their partner's behaviour towards them. The measure has been shown to yield large correlations between self and partner informant reports (Moffitt et al., 1997). As this variable was used to select women who were eligible for intensive sample membership, it was included in the analyses reported here within the weights. The psychological abuse variable used in the analyses was the highest of the partner-to-participant and participant-to-partner scores for each family.

Age of mother, cohabiting status and years of education, were each recorded at recruitment. In the intensive sample, maternal negative temperament was assessed at 32 weeks gestation using a 28-item version of the Negative Temperament scale of the Schedule for Nonadaptive and Adaptive Personality (SNAP) (Morse et al., 2009) administered to the mothers. Adult negative temperament scores assessed in this way are highly correlated with measures of neuroticism (Clark, 2005).

DNA extraction and genotyping. DNA was extracted from saliva pads using methods from Oragene (http://www.dnagenotek.com/DNA_Genotek_Product_OG250_Lit.html) and using cheek swabs via a standard protocol (Freeman et al., 1997). Quantification was performed using a nanodrop 1000 Nanodrop (Fisher Scientific, Loughborough, UK). MAOA VNTR genotyping was performed on an ABI 3130 Life Technologies (Paisley, UK). capillary electrophoresis machine. DNA (~20 ng) was amplified using FAM-labelled primers (IDT, UK) and a PCR reaction and thermocycling protocol taken from Sabol et al. (1998). Genotypes were called using Genemapper V4.0 (Life Technologies) by laboratory staff and were independently rechecked by GB. Duplicate samples were tested and both positive and negative controls were included on each 384-well plate (Abgene, Cambridge, UK). Genotyping was performed blind to phenotypic data. The average DNA yield was 36 μg (*SD* 26 μg) which is comparable to yields reported in other studies using infant saliva (Lehmann, Haas, McCormick, Skaar, & Renbarger, 2011). Genotypes were recalled by two investigators and in the 2% where there were different calls due to weak bands on the gels, the samples were excluded. Overall, the genotyping success rate was 92.7%.

Statistical analysis

The two-phase stratified sample design allows estimates to be reported for the general population by applying weights. The combination of extensive sample data from pregnancy together with genetic, mother–child interactional and infant temperament data at intervening times on the stratified subsample allowed us to construct an overall sampling probability and inverse probability weight to account for loss-to-follow-up to the 14-month extensive sample assessment (weights based on cohabiting status, mothers age and years of education, neighbourhood deprivation, prenatal reported partner psychological abuse and infant's sex). Test statistics for weighted means, correlations and regression estimates are based on survey-adjusted Wald tests (*t*-tests if single degrees of freedom (*df*) or *F*-tests if multiple *df*), using the robust 'sandwich' estimator of the parameter covariance matrix (Binder, 1983), except for testing Hardy–Weinberg equilibrium where a weighted bootstrap p-value was constructed for the D statistic (Hartl & Clark, 2006). For key findings, we provide significance level estimates for both weighted and unweighted analyses. For simplicity of interpretation, all continuous variables have been standardized. Analyses were undertaken in Stata 11 (StataCorp., 2009).

Results

Allele frequencies

Four alleles of the 30-bp uVNTR were observed: 3- (36.1%), 3.5- (1.4%), 4- (59.6%) and 5-repeats (2.9%) in agreement with frequencies in other studies (Kim-Cohen et al., 2006). All studies agree on the functional classification of the two most common alleles, that is, the 3-repeat as low activity and the 4-repeat as high activity. Of rare alleles, both Sabol et al. (1998) and Deckert et al. (1999) assayed the 3.5-repeat with the same result (high activity). We used the classification of Sabol et al. (i.e., 5-repeat equals low MAOA activity) in line with other studies (Kim-Cohen et al., 2006). Hardy–Weinberg equilibrium (HWE) was observed for genotypes in the females ($N = 101$), D statistic unweighted $p = .323$; population weighted $p = .382$, while allele frequencies in males were also consistent with HWE

Table 1 Estimated population means and proportions by MAOA status

		MAOA variant						Group differences *p*-values	
		Low/Low $N_f = 17$ $N_m = 38$		Low/High $N_f = 43$		High/High $N_f = 41$ $N_m = 54$			
Measure	*N*	Mean	*SD*	Mean	*SD*	Mean	*SD*	3 group 2df test	2 group (LL vs. LH+HH) controlling for sex
Maternal age at consent (years)	193	26.6	5.32	25.7	5.44	27.7	5.99	.234	.619
Psychological abuse (std)	193	0.27	1.24	−0.02	0.87	−0.13	0.89	.086	.049
Partner status (%) non-cohabiting	193	28.7		23.0		16.7		.389	.303
Most deprived quintile (%)	193	40.0		51.5		32.2		.202	.823
Left school LE 18 years (%)	187	61.1		66.9		53.0		.376	.372
Prenatal maternal negative temperament (std)	185	0.03	1.12	−0.15	0.98	0.06	0.95	.571	.931
Maternal sensitivity (std) during observation at 29 weeks	193	−0.24	1.13	0.10	1.04	0.08	0.90	.314	.152
29 Weeks IBQ Distress to limitations (std)	181	−0.16	0.95	−0.08	0.74	0.18	1.09	.077	.154
14 Months IBQ Distress to limitations (std)	193	0.04	1.07	−0.19	0.91	0.06	1.00	.435	.973

Population weighted estimates and tests of group difference. N_m and N_f are male and female frequencies.

Table 2 Estimates of population correlations among maternal sensitivity, infant temperament and potential confounders[a]

	Maternal Age	Psychological abuse	Marital status	Deprivation	Education	Maternal negative temperament	Maternal sensitivity	27 weeks distress to limitations
Psychological abuse	−.14							
Cohabiting status	**−.27**	**0.27**						
Deprivation	−.11	.11	.12					
Education	−.13	.12	.15	**.22**				
Maternal negative temperament	−.13	**.43**	**.38**	−.01	−.03			
Maternal sensitivity	**.27**	**−.20**	−.10	−.09	**−.26**	−.15		
27 weeks distress to limitations	**−.29**	.01	**.18**	−.02	.01	**.25**	−.02	
14 months distress to limitations	.06	.05	.12	.10	−.06	.16	.09	**.42**

[a]Bold figures are significant at $p < .05$. Cohabiting status is the binary variable for not being married or cohabiting; psychological abuse is the highest of the partner to participant and participant to partner scores; deprivation is the binary variable for being in the most deprived quintile; education is the binary variable for no schooling after the age of 18: all other variables as defined in methods section.

($p = .61$). In view of the uncertainty of expression for the heterozygous females, we omitted these ($N = 43$) in the analyses pooling males and females, and treated them as a separate group in analyses of females. Table 1 shows the simple associations of MAOA with the target variables and confounders of this paper. There was little evidence of associations of MAOA status with these variables, and in particular there was not an MAOA–maternal sensitivity (GE) association.

MAOA variant, maternal sensitivity and infant distress to limitations (anger proneness)

In Table 1, MAOA variant showed little association with study variables, only the association with psychological abuse reaching nominal significance. By contrast, Table 2 shows that maternal sensitivity was significantly associated with several potential confounders, being correlated with mother's age ($r = .27$, $p < .001$), partner psychological abuse ($r = -.20$, $p = .013$) and maternal education ($r = -.26$, $p = < .001$).

To test whether infant MAOA variants significantly modify infant temperamental response to maternal sensitivity, we estimated the regression models of Table 3, first without confounders (column 1) then with possible confounders (column 2), pooling the MAOA-LL and MAOA-HH girls with the corresponding MAOA-L and MAOA-H groups of boys. The interaction between MAOA status and 29-week maternal sensitivity on the infants' distress to limitations at 14 months was highly significant without confounders (weighted standardized coefficient = 0.52, 95% CI 0.18, 0.86; $F(1,143) = 9.19$; $p = .003$, and unweighted = 0.54, 95% CI 0.17, 0.90; $F(1,143) = 8.27$; $p = .005$). Adding the main effects of the six confounders left, the G × E effect largely unchanged at 0.50 (CI 0.09, 0.79; $p = .015$). The addition of the interaction of MAOA status with each of the six potential confounders (column 2) increased the estimated size and significance of the MAOA ×

Table 3 Regression of 14 months distress to limitations (anger proneness) on MAOA status, maternal sensitivity and infant sex

	Without adjustment for confounders		With adjustment for confounders[a]	
	Estimate(*SE*)	*p*-value[b]	Estimate(*SE*)	*p*-value[b]
Female infant	.045 (.297)	.879	.336 (.356)	.347
Maternal sensitivity	−.330 (.155)	.032	−.498 (.140)	.001
Maternal sensitivity × female	.347 (.172)	.046	.376 (.173)	.033
MAOA-H	.184 (.240)	.443	0.207 (.253)	.415
MAOA-H × female	−.285 (.370)	.442	−.449 (.418)	.285
MAOA-H × maternal sensitivity	.522 (.172)	.003	.702 (.172)	.0001

Analyses are weighted to provide estimates for the general population by accounting for sample attrition and sample stratification. All covariates are standardized, and MAOA low activity and the male infants are the reference categories of binary dummy variables.
[a]Confounder effects included for partner psychological abuse, maternal negative temperament, mother's age at consent, maternal age leaving education (>18 vs. rest) cohabiting status (single vs. rest), neighbourhood deprivation (most deprived UK quintile vs. rest) as main effects and in interaction with MAOA variant.
[b]Derived from survey adjusted *F*-tests.

maternal sensitivity interaction. The variance explained by the MAOA by maternal sensitivity interaction was 6.1% before, and was 7.9% after the inclusion of interactions with potential confounders. The interaction could therefore not be explained by the identified potential confounders.

The MAOA-confounder interaction effects were introduced into the analysis as controls to help confirm the interpretation of the effect of the interaction with maternal sensitivity. As they were not hypothesized, we would not wish to make claims concerning effects for these confounders. It is nonetheless noteworthy that the test over these six interactions was significant [$F(6,123) = 2.62$, $p = .020$], with interactions with marital status, maternal negative temperament and mother's age each being individually nominally significant.

To assess whether the interaction effect with maternal sensitivity continued to act later in the infant's development, we added the 29-week IBQ-R infant anger proneness as an additional covariate to the model with all confounders and their interactions with MAOA status. The estimated MAOA × maternal sensitivity interaction was reduced, but remained significant (0.50, CI 0.18, 0.86; $p = .003$). The interaction effect thus seemed to be ongoing beyond 29 weeks of age. Figure 1 displays this relationship taking all other covariates at their mean values and dummy variables as their reference category.

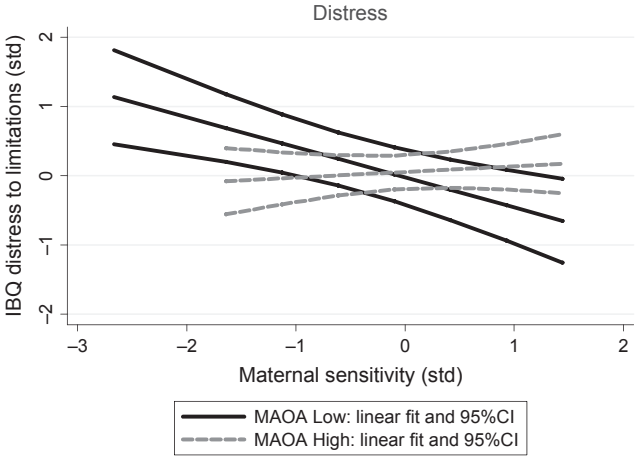

Figure 1 Population weighted infant distress to limitations (anger proneness) and maternal sensitivity by MAOA genotype. Regression lines are shown after covarying for confounder interactions and 29 weeks maternal report of infant distress to limitations. In females only homozygous individuals are included

Sex differences

When estimated in males and females separately, the interaction coefficients were similar, 0.60 (95% CI 0.15, 1.06; $p = .010$) in males and 0.87 (95% CI 0.17, 1.57; $p = .016$) in females. Pooling the data from both sexes, the test of the three-way interaction of MAOA by maternal sensitivity by sex was nonsignificant ($p = .104$). However, Table 3 shows that in addition to the MAOA by maternal sensitivity there was a further significant two-way interaction of sex and maternal sensitivity. Figure 2 shows the predicted values from the model of column 1. While there was a common MAOA by sensitivity interaction, it is clear that the interpretation of this interaction differed by sex. The simultaneous presence of the sex by maternal sensitivity interaction rotated the graph. The *differences in the slopes* of the regression lines in the MAOA-L and MAOA-H groups were not sex dependent, but there were *differences in the values* of the slopes. The combined effects of the G × E and sex × E interactions were such that the slope coefficients for maternal sensitivity on anger proneness (and approximate implied estimate of explained variance) were as follows: −0.330 (11%) for MAOA-L boys, −0.146 (2%) for MAOA-H boys, 0.017 (0%) for MAOA-LL girls and 0.723 (50%) for MAOA-HH girls. Thus, MAOA-H boys were like MAOA-LL girls, both showed little response to the environment. MAOA-L boys and MAOA-HH showed greater response but in opposite directions.

Although not shown in Figure 2, when the heterozygote MAOA-HL girls were added to the model of

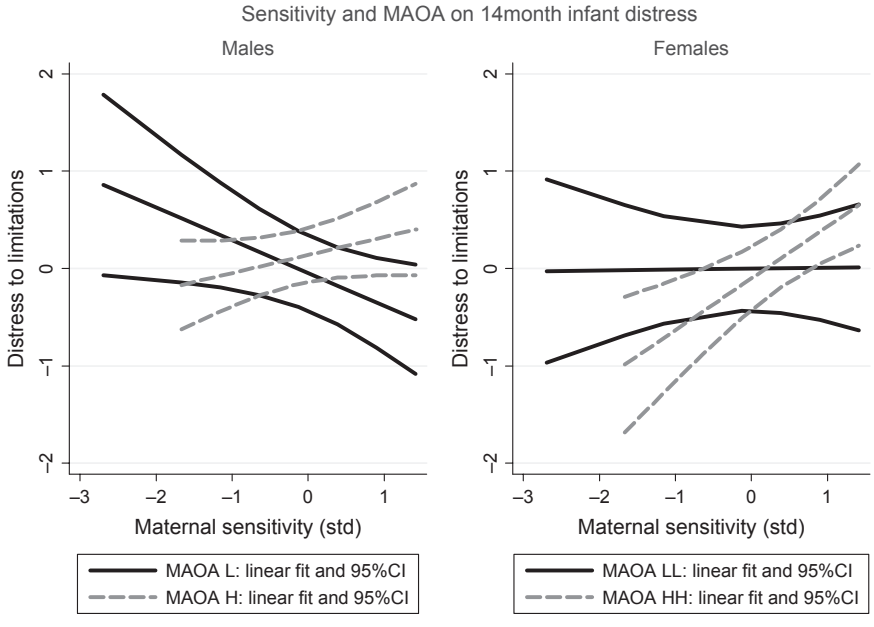

Figure 2 Population weighted relationship of maternal sensitivity to distress to limitations (anger proneness) by MAOA genotype in males and females. Regression lines are shown after covarying for confounder interactions and 29 weeks maternal report of infant distress to limitations. In females only homozygous individuals are included

Table 3, the estimated pattern of response lay between that of the MAOA-LL and MAOA-HH girls.

Discussion

Using a prospective design, with a general population sample, we showed that the effect of observed maternal sensitivity assessed at 29 weeks on anger proneness rated by mothers at 14 months was modified by MAOA expression status. This is consistent with findings on MAOA G × E in children and adults and suggests that the interaction contributes to early developmental pathways to later antisocial behaviour problems. We did not find evidence that the interaction term differed between males and females. Strengths of this study include that the sampling method ensured that findings pertain to the general population, and that the measure of the infant environment was based on direct observation rather than parental report. In addition, the G × E effect was examined prospectively, plausible confounding variables were examined including their own potential action via interaction with MAOA, and multiple testing was minimized by targeted candidate gene selection based on available findings on the early development of antisocial and violent behaviours.

Limitations include that although there were consistent effects across males and females, analyses of females were limited by the small number of female infants homozygous for MAOA-L ($N = 17$), and statistical power for the three-way interaction was limited by the overall sample size. Infant temperament was assessed by maternal report and it is possible that the use of observational measurement may not yield the same findings. Maternal sensitivity increased with maternal age and was lower in single mothers, consistent with the possibility that sensitivity was merely a marker for other maternal characteristics. The reverse, however, was the case. After inclusion of these and other possible confounder interactions, the MAOA by maternal sensitivity interaction was undiminished. Some or all of the interactions may have reflected an interaction between infant genotype and maternal genotype in the prediction of infant anger proneness, and in the absence of maternal genotype this cannot be excluded.

The interaction was identified in both males and females, and there was no indication of a difference in its size or direction as found in some previous studies (e.g. Aslund et al., 2010). However, there was a sex by maternal sensitivity interaction that rotated the slopes of the female regression lines anticlockwise in comparison to the males. This is consistent with studies that have found sex differences in the consequences of early social interaction with mothers (Warren & Simmens, 2005; Weinberg, Tronick, Cohn, & Olson, 1999). As a consequence, in males, decreasing maternal sensitivity was associated with increasing anger proneness, but only in the MAOA-L group, while in females the environmental effect was found in the MAOA-HH group where decreasing maternal sensitivity was associated with decreasing anger proneness. The finding highlights the need to examine interaction effects in each sex to confirm a common or different interpretation, even when formal test suggests no difference in the interaction term itself.

Developmentally, the interpretation is not straightforward and needs further investigation. However, sex differences in which female infants appear to show superior performance in the face of adversity have been reported. For example, Murray, Fiori-Cowley, Hooper, and Cooper (1996) found a sex by postnatal

depression interaction in the prediction of cognitive levels at the age of 18 months which arose from lower cognitive abilities in the exposed males, but higher in the females. Furthermore, the developmental implications of high anger proneness identified in MAOA-L males and MAOA-H females in this study may not be the same. According to Belsky's differential susceptibility theory (Belsky & Pluess, 2009), anger proneness in association with low maternal sensitivity, which was identified in the MAOA-L males in our study, will increase the risk of poor outcomes. By contrast, in the MAOA-H girls, where elevated anger proneness was associated with high maternal sensitivity, outcomes are predicted to be superior. Consistent with this hypothesis, Pluess and Belsky (2009) reported that infants with high negative emotionality exposed to low maternal care had the highest levels of behaviour problems and the lowest social competence at the age of 4.5 years, while those who experienced high care had the least behaviour problems and the highest social competence, when compared with low negative emotionality infants.

We report one of the largest genetic studies of infancy that include observational measurement of parenting. Multiple testing and reporting biases have made some readers sceptical of findings from all genetic studies except those involving tens of thousands of participants. We addressed this concern by adopting a very conservative testing approach that allowed no exploration of multiple candidate genes or environments. The finding that three of the six confounders also showed nominally significant interaction effects with MAOA is also remarkable. While our conservative approach restrains interpretation of the individual effects, the evidence from this study that MAOA is highly active in infant development would seem unavoidable.

In the context of previous studies showing G × E for the MAOA gene in child and adult antisocial disorders, these findings, together with results recently published from the same study on MAOA G × E during pregnancy (Hill et al., 2013), are the first to show how MAOA-environment interplay may contribute to the earliest processes in pathways to child and adult antisocial disorders. While previous studies have identified MAOA by adversity interactions during childhood (Caspi et al., 2002; Enoch, Steer, Newman, Gibson, & Goldman, 2010; Kim-Cohen et al., 2006), none has reported on prospective associations during infancy. Furthermore, the interaction was associated with anger proneness consistent with the role of infant fussing and distress to limitation in pathways to conduct disorders. We were also able to demonstrate that MAOA status interacts with maternal sensitivity to predict future anger proneness even after accounting for anger proneness at the time of the sensitivity assessment. This would support an effect of maternal sensitivity on subsequent development.

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Key points

- MAOA genotype by environment interactions have been implicated in child externalizing and adult antisocial behaviour problems, but their role in developmental pathways to conduct problems remain unknown.
- This is the first report of MAOA G by E in relation to temperamental variations in infancy likely to increase the risk of later conduct problems, and using a timed observational measure of the environment, maternal sensitivity.
- Decreasing maternal sensitivity was associated with increasing infant anger proneness only in infants with the low expression MAOA variant.
- The contribution of maternal sensitivity was further modified by sex of the infant.
- Genotype and sex of infant may be important sources of heterogeneity in planning early prevention programmes for antisocial behaviour problems.

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