Evidence for interplay between genes and maternal stress in utero: monoamine oxidase A polymorphism moderates effects of life events during pregnancy on infant negative emotionality at 5 weeks

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The low activity variant of the monoamine oxidase A (MAOA) functional promoter polymorphism, MAOA-LPR, in interaction with adverse environments (G×E) is associated with child and adult antisocial behaviour disorders. MAOA is expressed during foetal development so in utero G×E may influence early neurodevelopment. We tested the hypothesis that MAOA G×E during pregnancy predicts infant negative emotionality soon after birth. In an epidemiological longitudinal study starting in pregnancy, using a two stage stratified design, we ascertained MAOA-LPR status (low vs. high activity variants) from the saliva of 209 infants (104 boys and 105 girls), and examined predictions to observed infant negative emotionality at 5 weeks post-partum from life events during pregnancy. In analyses weighted to provide estimates for the general population, and including possible confounders for life events, there was an MAOA status by life events interaction (P = 0.017). There was also an interaction between MAOA status and neighbourhood deprivation (P = 0.028). Both interactions arose from a greater effect of increasing life events on negative emotionality in the MAOA-LPR low activity, compared with MAOA-LPR high activity infants. The study provides the first evidence of moderation by MAOA-LPR of the effect of the social environment in pregnancy on negative emotionality in infancy, an early risk for the development of child and adult antisocial behaviour disorders.

Keywords: Infancy, life events, MAOA polymorphism, negative emotionality, pregnancy

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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 et al. 2005). One of the most widely studied G×E interactions involves polymorphisms of the monoamine oxidase (MAOA) gene which encodes the structure of the enzyme monoamine oxidase A. Monoamine oxidase A has a central role in the catabolism, and hence regulation, of key neurotransmitters, notably 5 hydroxytryptamine (5HT) and norepinephrine (NE). Possession of 3.5 and 4 copies of a 30 bp variable number tandem repeat in the upstream promoter region of the gene (the uVNTR), the MAOA-LPR high activity variant, is associated with higher in vitro expression compared with 3 or 5 repeat, low activity, variants (Sabol et al. 1998). The MAOA-LPR low activity variant in interaction with childhood maltreatment has been found to be associated with child behavioural and adult antisocial outcomes in many (Beach et al. 2010; Caspi et al. 2002; Enoch et al. 2010; Ferguson et al. 2011; Ferguson et al. 2012; Kim-Cohen et al. 2006) studies. The plausibility of a causal role for the MAOA-LPR low activity variant is supported by findings that MAOA knockout mice display marked aggression and have elevated 5HT and NE levels, and in humans a rare functional knockout is associated with severe aggression (Buckholtz & Meyer-Lindenberg 2008). Furthermore MAOA is expressed in the brain during foetal development, and in animal studies prenatal inhibition of MAOA results in elevated aggression similar to that seen in MAOA knockout (Baler et al. 2008; Mejia et al. 2002). Modification of an in utero effect of MAOA genotype status by social stressors has not previously been reported.

Many studies of MAOA G×E only report on males because the gene is on the X chromosome, and there are uncertainties regarding the activity status of female heterozygotes (Caspi et al. 2002; Ferguson et al. 2011; Kim-Cohen et al. 2006). Some studies that have included females have found sex differences (Aslund et al. 2011), while others have not (Nikulina et al. 2012). The possibility of sex differences therefore needs to be examined in studies of MAOA G×E.

Repeated antisocial behaviours in adults are almost always preceded by conduct problems of aggression and oppositionality in early childhood (Baillargeon et al. 2007;
Hill et al.

Ogders et al. (2008), so the study of processes preceding the appearance of childhood symptoms needs to start in infancy. High negative emotionality, a core component of infant temperament, confers early vulnerability to childhood conduct problems (Caspí & Silva 1995, Smeekens et al. 2007). In animal studies prenatal stress is associated with behavioural markers of increased emotional reactivity in the offspring (Weinstock 2008), and in humans, maternal cortisol levels during pregnancy predict infant emotional reactivity over the first days of life (Davis et al. 2011). We therefore examined whether MAOA-LPR genotype modifies the effects of social stressors during pregnancy on negative emotionality at 5 weeks of age.

Materials and methods

Design

The participants were members of the Wirral Child Health and Development Study, a UK prospective epidemiological longitudinal study of prenatal and infancy origins of conduct disorders (http://www.liv.ac.uk/psychology-health-and-society/research/first-steps/about/). This uses a two stage stratified design in which a larger general population sample of first-time mothers was recruited in pregnancy (extensive sample) and from which a sub-sample was drawn for more detailed assessment (intensive sample). All families in the extensive sample follow a brief assessment protocol while those in the intensive subsample receive an additional battery of labour intensive measurement, such as that used in this study to assess infant negative emotionality. 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 antenatal care on the Wirral Peninsula, a geographical area bounded on three sides by water. 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 midwife who then administered questionnaires and an interview in the clinic.

Sample

The extensive sample of 1233 mothers with surviving babies had a mean age at recruitment of 26.8 years (SD 5.8, range 18–51). Measures for this study were obtained from the extensive sample (from which the intensive sample was drawn) at 20 weeks gestation and from the intensive sample at mean 32.1 (SD 2.0) weeks of pregnancy (‘32 weeks gestation’) and when the infants were mean 5 weeks 2 days (SD 9 days). Of the 316 mothers recruited to the intensive subsample during pregnancy, 282 were assessed using the Neonatal Behavioural Assessment (Brazelton & Nugent 1995) from which ratings of infant negative emotionality were derived. Adequate DNA for MAOA analyses was obtained from 209 of these infants, 164 boys, 165 girls and they comprise the sample for this report.

Socioeconomic status was determined using the revised English Index of Multiple Deprivation (IMD; Noble et al. 2004) based on data collected from the UK Census in 2001. According to this system, postcode areas in England are ranked from most deprived (i.e. IMD of 1) to least deprived (i.e. IMD of 32 482) based on neighbourhood deprivation in seven domains: income, employment, health, education and training, barriers to housing and services, living environment and crime. All mothers were given IMD ranks according to the postcode of the area where they lived and assigned to a quintile based on the UK distribution of deprivation; 41.8% of the extensive sample had 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.

Measures

DNA extraction and genotyping

DNA was extracted from saliva pads using methods from Oragene (http://www.dnaingenotek.com/DNA_Genotek_Product_0280_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. The PCR was amplified using FAM labelled primers (IDT, Integrated DNA Technologies, Leuven, Belgium) and a polymerase chain reaction (PCR) and thermocycling protocol taken from Sabol et al. (1999). The PCR to amplify the region of the uVNTR was performed in non-skirted 96 well plates (ThermoFisher, Loughborough, UK) using an unlabelled forward primer: 5'-GAA CGG AGC CTC CAT TCG GA-3' and an 5' FAM labelled reverse primer 5'-FAM-ACA GCG TGG AGA AG-3' (IDT). The PCR conditions included 1.5mM MgCl₂ and 1μM concentrations of each primer in a 10μl reaction. The products were diluted at 1:20 and subject to capillary electrophoresis using manufacturers’ protocols on an Applied Biosystems (Life Technologies) 3130 system. Sizing was performed vs. a labelled standard using the GeneMapper software supplied (ABI Ltd, Applied Biosystems, Life Technologies, Paisley, UK). Genotypes were called using Genemapper V4.0 (Life Technologies) and were independently rechecked. 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 sex, measures of pregnancy life events and infancy negative emotionality.

Infant negative emotionality

The Neonatal Behavioural Assessment (NBAS) was administered to the extensive sample at 5 weeks after birth. The NBAS is a standardized measure designed to assess orienting, motor and emotion regulatory processes during the first weeks of life (Brazelton & Nugent 1995, Lester et al. 1976). It is conducted by a trained administrator who carries out, in a prescribed sequence, a range of manoeuvres, designed to elicit the infant’s optimal orienting and motor performance, and emotional responses to mild aversive procedures such as undressing or being brought from lying to sitting. Ratings are generally made by the administrator from memory immediately after the assessment. This limits the scope for determining interrater reliability so in this study the assessments were video recorded using four cameras placed to obtain a comprehensive picture of infant responses. The NBAS coding method yields a total of 27 scores which have been previously reduced in factor analytic studies, yielding variously three to nine factors (McCollam et al. 1997). We used only the ‘irritability’ scale which is a count of the number of occasions on which the infant shows a change of state from calm, of at least three seconds, to fussing or crying in response to seven standard challenges. The count of fuss/cry episodes provided a specific measure of reactivity parallelling maternal report and observational measures of temperament later in infancy, where responses to challenges such as restraint or the unpredictable noises are assessed (Gartstein & Rothbart 2003, Rothbart et al. 2001). Three assessors were trained by Dr Joanna Hawthorne, director of the UK Brazelton Centre. Pair-wise agreement (ICC) between independent ratings made from memory and video recordings on 220 infants ranged between 0.81 and 0.89. Video based scores were used in analyses.

Life events

The Life History Calendar (LHC) (Caspí et al. 1996) was administered to participants in the extensive sample at 32 weeks gestation.
The LHC is a calendar-based response sheet, which is marked with key events in the respondent’s life, such as birthdays. It is administered as a structured interview, and begins with questions regarding life trajectories, for example, where the respondent has lived and worked. This information, serves as memory cues for later questions about the occurrence of specific life events. The LHC has been shown to generate reliable recall of events over periods of up to five years (Caspi et al. 1996, Ehrensaft et al. 2004) and has been used previously in studies of G × E (Caspi et al. 2003). The total number of life events during pregnancy, with values of 0, 1, 2, 3 and ‘4 or more’, was used in analyses.

**Demographic variables, potential confounders and stratification variable**

At recruitment into the extensive sample, mothers’ ages, cohabiting status and years of education were obtained, together with the stratification measure which was 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 for eligibility 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.

Indices of social stress that might be confounded with life events were the UK Index of Multiple Deprivation (Noble et al. 2004) and marital satisfaction assessed with the Kansas Marital Satisfaction Scale (Schumm et al. 1985) from the extensive sample. Maternal anxiety was assessed at 32 weeks gestation in the intensive sample using the State Anxiety Scale (Spielberger et al. 1983) because of its reported associations, respectively, with adolescent depression (Costello et al. 2007) and externalizing disorders in infancy (Murray et al. 1996).

**Statistical analysis**

The two-phase stratified sample design allows estimates to be reported for the general population by applying weights. Inverse probability weights were constructed using the sample stratification factor, mother’s cohabiting status, age and years of education, the deprivation index for her home neighbourhood, and the infant’s sex, birth weight and 1 min Apgar score. Test statistics for weighted means, correlations and regression estimates were 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). Models used for inference analysed the count of fuss/cry episodes using Poisson regression, with inverse probability weights and robust parameter covariance matrix estimator for valid inference from probability weighted data, and to account for possible overdispersion in the count. For models with covariates, a form of stabilized weight was used that removed weight variability associated with the conditioning covariates. Analyses were undertaken in Stata 11 (2009).

**Results**

**Allele frequencies**

Four alleles of the 30 bp u-VNTR 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 = 105, D-statistic unweighted P = 0.527; population weighted P = 0.523) while allele frequencies in males were also consistent with HWE (P = 0.618).

**MAOA genotype, prenatal risk factors and infant negative emotionality**

Table 1 gives means and percentages by MAOA-LPR variant for the target, confounder and possible mediator variables of this study. All variables were standardized except ages, binary variables and the variables of primary focus, namely the counts of life events (treating 4 or more as 4) and of fuss/cry episodes. There was little evidence of direct effects, the only nominally significant effect being the association with relationship satisfaction. In particular, there was a non-significant trend for the MAOA-LPR low activity variant to be associated with more life events in pregnancy.

All analyses included as background factors the sex and age of the infant and maternal depression score at the time of the NBAS assessment. We considered life-events in pregnancy and potential confounding effects of neighbourhood deprivation, single cohabiting status, and, at 32 weeks of pregnancy, maternal anxiety score and poor marital satisfaction as our set of prenatal risk factors that were external to the baby. Associations among these measures are shown in Table 2.

Since MAOA is an X linked gene, and in view of the uncertainty regarding the activity status of heterozygous females (Kim-Cohen et al. 2006), primary analyses were performed in males and female homozygotes combined (n = 163). We first tested whether the effects of the risk set as a whole were moderated by infant genotype. When all risk factors and their interactions with MAOA-LPR variants were included, the overall test for the interaction terms was significant, \( \chi^2(5) = 12.30, P = 0.031 \), with individual interactions being life-events \( P = 0.017 \), deprivation \( P = 0.028 \), marital satisfaction \( P = 0.577 \), prenatal anxiety \( P = 0.682 \) and marital status \( P = 0.648 \). Retaining the life-events and deprivation interaction effects and the main effects of the other risk factors gave the model displayed in Table 3. Coefficients reflect the proportional change in the number of NBAS manoeuvres which evoked a fuss/cry response, with coefficients above one implying a proportional increase and those below a decrease for each unit change in the covariate. MAOA-LPR low activity individuals comprised the reference group for the interaction so that the table shows the main effect of life events in the MAOA-LPR low activity
Hill et al.

Table 1: Estimated population means and proportions of life events, neighbourhood deprivation and potential confounders by MAOA status

<table>
<thead>
<tr>
<th>Measure</th>
<th>MAOA variant</th>
<th>Group differences P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>Maternal age at consent (years)</td>
<td>209</td>
<td>27.5</td>
</tr>
<tr>
<td>Most deprived quintile (%)</td>
<td>209</td>
<td>41.9</td>
</tr>
<tr>
<td>Single non-cohabiting (%)</td>
<td>209</td>
<td>27.3</td>
</tr>
<tr>
<td>Partner psychological abuse (std)</td>
<td>209</td>
<td>0.20</td>
</tr>
<tr>
<td>Maternal prenatal (32 week) anxiety (std)</td>
<td>209</td>
<td>0.25</td>
</tr>
<tr>
<td>Prenatal (32 week) marital satisfaction (std)</td>
<td>191</td>
<td>-0.30</td>
</tr>
<tr>
<td>Life-events in pregnancy (0,1,2,3,4+)</td>
<td>209</td>
<td>1.91</td>
</tr>
<tr>
<td>Birthweight by gestation (std)</td>
<td>209</td>
<td>-0.02</td>
</tr>
<tr>
<td>Low APGAR at 1 min (%)</td>
<td>209</td>
<td>8.2</td>
</tr>
<tr>
<td>Maternal depression at 4 weeks (std)</td>
<td>207</td>
<td>-0.08</td>
</tr>
<tr>
<td>Childs age at NBAS (days)</td>
<td>209</td>
<td>38.9</td>
</tr>
<tr>
<td>Count of NBAS cry/fuss episodes</td>
<td>209</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Population weighted estimates and ordinary/logistic regression t and F-tests of group difference. Nm and Nf are male and female frequencies.

Table 2: Estimates of population correlations among prenatal life-events and observed infant negative emotionality at 5 weeks, and potential confounders and mediators

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td>.15</td>
<td>.09</td>
<td>.33</td>
<td>.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological abuse</td>
<td>.09</td>
<td>.26</td>
<td>.29</td>
<td>.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal anxiety</td>
<td>-.08</td>
<td>.33</td>
<td>-.57</td>
<td>-.26</td>
<td>-.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relationship satisfaction</td>
<td>-.17</td>
<td>-.57</td>
<td>-.26</td>
<td>-.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life-events in pregnancy</td>
<td>.00</td>
<td>.27</td>
<td>-.20</td>
<td>.21</td>
<td>-.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight/gestational age</td>
<td>-.08</td>
<td>-.05</td>
<td>-.07</td>
<td>.06</td>
<td>.02</td>
<td>-.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apgar (1 min)</td>
<td>.01</td>
<td>-.01</td>
<td>.02</td>
<td>.02</td>
<td>-.06</td>
<td>-.17</td>
<td>.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal depression</td>
<td>.00</td>
<td>.19</td>
<td>.16</td>
<td>.40</td>
<td>-.32</td>
<td>.28</td>
<td>.03</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td>Infant negative emotionality</td>
<td>-.08</td>
<td>.24</td>
<td>.06</td>
<td>.17</td>
<td>-.19</td>
<td>.17</td>
<td>.01</td>
<td>-.08</td>
<td>-.01</td>
</tr>
</tbody>
</table>

Bold values are significant at nominal P < 0.05. Marital 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 left education before age 19; all other variables as defined in methods section.

The coefficient of 1.34 reflects that among those with the MAOA-LPR low activity variant, each increase of one life event was associated with a 1.34 proportional increase in the number of times that infants responded with fussing or crying. Thus, compared with infants whose mothers experienced zero life events during pregnancy, exposure to four or more was associated with a 1.34² = 3.22, proportional increase in the number of NBAS fuss/cry episodes. The association between pregnancy life events and infant fuss/cry in the MAOA-LPR high activity group is given by the product of the MAOA-LPR low activity and the interaction term coefficients, i.e. 1.34 × 0.77 = 1.03, a small and non-significant effect. Figures 1 and 2 display simple sub-population means illustrating the two interactions identified. Infants with MAOA-LPR low activity variant display almost no change to the estimated interaction coefficients.
Table 3: Summary of Poisson regression estimates of MAOA by prenatal environment interactions predicting observed infant negative emotionality at 5 weeks

<table>
<thead>
<tr>
<th></th>
<th>Relative risk</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female infant</td>
<td>0.87</td>
<td>0.394</td>
<td>0.63, 1.20</td>
</tr>
<tr>
<td>Infant age at NBAS (weeks)</td>
<td>0.74</td>
<td>0.001</td>
<td>0.63, 0.88</td>
</tr>
<tr>
<td>Maternal depression at NBAS</td>
<td>1.00</td>
<td>0.963</td>
<td>0.96, 1.04</td>
</tr>
<tr>
<td>Prenatal maternal anxiety</td>
<td>1.16</td>
<td>0.065</td>
<td>0.99, 1.37</td>
</tr>
<tr>
<td>Cohabitating status</td>
<td>1.01</td>
<td>0.950</td>
<td>0.62, 1.68</td>
</tr>
<tr>
<td>Partnership satisfaction</td>
<td>1.01</td>
<td>0.897</td>
<td>0.83, 1.23</td>
</tr>
<tr>
<td>Deprivation</td>
<td>1.55</td>
<td>0.102</td>
<td>0.91, 2.62</td>
</tr>
<tr>
<td>Prenatal LEs</td>
<td>1.34</td>
<td>&lt;0.001</td>
<td>1.18, 1.53</td>
</tr>
<tr>
<td>MAOA-LPR high activity</td>
<td>2.26</td>
<td>0.004</td>
<td>1.30, 3.95</td>
</tr>
<tr>
<td>MAOA-LPR high activity × deprivation</td>
<td>0.44</td>
<td>0.030</td>
<td>0.21, 0.92</td>
</tr>
<tr>
<td>MAOA-LPR high activity × prenatal LEs</td>
<td>0.77</td>
<td>0.016</td>
<td>0.63, 0.95</td>
</tr>
</tbody>
</table>

Analyses are weighted to provide estimates for the general population by accounting for sample attrition and sample stratification. All covariates except ages and categorical variables are standardized, and MAOA-LPR low activity, male infants and non-deprived neighbourhood are the reference categories of binary dummy variables.

LEs, life events.

and both remained significant (life events \(P = 0.019\), deprivation \(P = 0.033\)). Thus the G × E effects identified did not appear to be mediated by these variables.

**Sex differences**

We examined evidence for sex differences firstly by examining each 3-way interaction (sex by risk factor by MAOA variant) in the presence of the 2-way interactions. In neither case was the 3-way interaction significant (deprivation \(P = 0.561\), life-events \(P = 0.563\)). Also in both cases, the two 2-way interactions involving sex of infant were also non-significant (\(P = 0.928\) life events, \(P = 0.062\) deprivation).

Since the power of the test of the 3-way interaction was not large, we made further checks of the homogeneity of effects across boys and girls, fitting the model with just the basic control variables at the time of infant assessment, that is, infant age and mother’s depression score. In boys, for the G × E with life events in pregnancy, the relative risk was 0.79, very similar to that for the sample as a whole (0.77), although non-significant \((P = 0.065)\), and for deprivation it was 0.47 \((P = 0.096)\) compared with 0.44 for the whole sample. We fitted the same model to girls, but allowing 3-categories for MAOA to include the heterozygous participants previously excluded from the analysis. Compared to the MAOA-LPR low activity variant, the heterozygous variant showed less sensitivity to life events (relative risk \(0.65\); CI 0.49, 0.87; \(P = 0.004\)) and similar to that for the homozygous MAOA-LPR high activity variant (relative risk compared to low 0.72; CI 0.52, 1.00; \(P = 0.053\)). The same pattern was seen for deprivation with the heterozygous group showing less sensitivity (relative risk 0.32; CI 0.12, 0.89; \(P = 0.028\)) and
similar to the homozygous high activity variant (relative risk compared to low 0.44; CI 0.15, 1.26; $P = 0.125$). Pooling the heterozygous girls with the homozygous high gave relative risks for girls of 0.68 (CI 0.52, 0.89; $P = 0.004$) for life events and 0.37 (CI 0.14, 0.96; $P = 0.041$) for deprivation. Thus heterozygous girls behaved much like those with the homozygous-high variant and the effect of the genetic variant was similar among boys and girls.

Discussion

This is the first study to report that social adversity during pregnancy modifies the association between MAOA-LPR activity variants and early infant negative emotionality. Using a prospective design with a general population sample, we showed that in the presence of the low activity, but not the high activity, variant of the MAOA gene, increasing life events were strongly associated with higher infant negative emotionality. Among infants possessing the MAOA-LPR low activity variant, reporting four or more life events in pregnancy, compared with none, was associated with a more than three times increase in the likelihood of infants reacting to a low key challenge with fussing or crying at 5 weeks of age. This association was absent in the presence of the MAOA-LPR high activity variant. There was a similar interaction with neighbourhood deprivation.

The findings are consistent with previous studies that have found a wide range of adverse environments to be associated with antisocial outcomes only in individuals with the MAOA-LPR low activity genotype. Many studies have reported only on males because of uncertainties regarding MAOA activity in female heterozygotes (Casi et al. 2002; Fergusson et al. 2012; Kim-Cohen et al. 2006). We report the findings first omitting female heterozygotes to deal with this problem, but we also found that the outcome associated with heterozygous MAOA-LPR was indistinguishable from that of homozygous MAOA-LPR high activity variant. Some studies of females have found that, as in males, the MAOA-LPR low activity genotype confers vulnerability to maltreatment (Ducci et al. 2008; Widom & Brzustowicz 2006), while in others it has been the MAOA-LPR high activity variant that is associated with antisocial outcomes in interaction with the environment (Aslund et al. 2011). In this study the direction and size of effect in males and females were the same.

Strengths of this study include that the sampling method ensured that findings pertain to the general population, the G × E effect was examined prospectively, plausible confounding variables were examined, and multiple testing was minimized by targeted candidate gene selection. The outcome variable was developmentally relevant, based on available findings on early vulnerability to antisocial and violent behaviours. In contrast to the standard method for rating the NBAS by the assessor from memory, we used video recordings so that inter-rater reliability could be checked. As far as we are aware we are the first to report on agreement between memory and video based ratings.

Although the sample size was adequate to detect two G × E interactions, others of smaller effect may have been missed. We showed that there were consistent effects across males and females, however, analyses of females were limited by the small number of female infants homozygous for MAOA-LPR low activity ($N = 16$). Life events in pregnancy were associated with other measured indices of social stress, such as marital dissatisfaction and single parent status, and so may also have been associated with unmeasured indices. It is therefore possible that life events were a proxy for other environmental risks and do not
themselves have a causal role. Furthermore, there are genetic contributions to life events (Vinkhuyzen et al., 2010), so we cannot rule out the possibility that the apparent $G \times E$ in fact represents an interaction between maternal and infant genetic variations. The finding of an effect on early infant negative emotionality does not yet constitute direct evidence that MAOA $G \times E$ in utero influences behavioural or antisocial outcomes in childhood or adult life. Whether this is the case requires longitudinal study over many years.

A complex interplay of etiological, maintaining, and ameliorating factors give rise to antisocial disorders in early childhood and contribute to their persistence or desistance (Hill, 2002). Against this background, current evidence on MAOA $G \times E$ effects, while impressive, shows risk for disorder without yet elucidating how they may influence specific developmental pathways. This requires study of potential biological or psychological mediators of vulnerability or resilience. There are numerous pathways from infancy to conduct disorders in young children, however, evidence converges 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 (Lahey et al., 2008; Smeekens et al., 2007).

The study reported here is the first to show effects on infant negative emotionality in utero MAOA $G \times E$. The findings have the potential to inform two key connected questions, how do $G \times E$ processes operate over time, and what might be the mechanisms? Little is known about timing and developmental sequences of $G \times E$. Most studies finding effects of maltreatment during childhood do not provide evidence regarding timing because the maltreatment variables cover the whole of childhood. They may reflect effects of sustained maltreatment over time, or more discrete episodes occurring at different times, or specifically timed effects. The report by Kim-Cohen et al. (2006) of MAOA $G \times E$ with physical abuse in seven year olds suggests there are effects in early childhood, as do findings from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) in which life events experienced during infancy and up to 3.5 years, in interaction with MAOA-LPR, predicted hyperactivity at age 7 years (Enoch et al., 2010).

A possibility opened up by this study is that early $G \times E$ contributes to a temperamental vulnerability which in interaction with subsequent adverse environments, for example of physical abuse or partner violence, develops into disorder. Equally, there could be successive $G \times E$ processes in which MAOA-LPR low activity individuals either accumulate risk as they are exposed to a series of adverse environments, which through passive, active or evocative GE correlations, may be disproportionately adverse; or reduce risk by taking advantage of supportive environments in which they may be particularly well adapted (Pickles & Hill, 2006). We have recently reported MAOA-LPR $G \times E$ at 7 months consistent with this possibility. In the presence of the MAOA-LPR low activity variant, decreasing maternal sensitivity at 7 months was associated with increasing anger proneness at 14 months (Pickles et al., In Press).

Mechanisms of $G \times E$ remain speculative. After birth they may for example occur from the interplay between genetically influenced behavioural traits and the environment. In the case of the MAOA gene, MAOA-LPR low activity individuals show greater threat sensitivity, poor emotion control and enhanced fear memory, which are likely to confer vulnerability in unfavourable environments. Thus the interaction is thought to arise because the individual’s MAOA influenced socioaffective scaffold is more or less adaptive depending on the characteristics of the environment (Buckholz & Meyer-Lindenberg, 2008). In utero the interaction may arise from neurotransmitter, hormonal or gene expression effects. In animal studies effects of prenatal stressors on post-natal behavioural outcomes are mediated via effects of maternal glucocorticoids on foetal physiology (Weinstock, 2008). Specifically, the offspring of prenatally stressed rats show elevated anxiety and stress induced hypothalamic-pituitary axis (HPA) reactivity which is abolished by adrenalectomy of their mothers. Glucocorticoid-mediated mechanisms in the prediction of infant temperament (Davis et al., 2007) and associations between prenatal stress and later antisocial behaviour disorders (O’Connor et al., 2003) have also been described in humans. The MAOA-LPR low activity variant has been shown to be associated with increased HPA reactivity to a stressor (Bouma et al., 2012), and hence the combination of MAOA-LPR low activity status and prenatal exposure to life events could account for increased emotional reactivity found in this study mediated via altered HPA axis reactivity. Alternatively, the interaction could occur at the level of MAOA gene expression. Mechanisms for glucocorticoid regulation of MAOA expression have been identified (Ou et al., 2006), so that the effect of life events could arise from altered MAOA expression resulting from effects of glucocorticoid exposure. In order for this to explain the interaction, the behavioural consequences of the gene expression must differ between the MAOA-LPR low activity and MAOA-LPR high activity individuals. Genotype dependent effects of environments on gene expression have rarely been investigated although they have been predicted using bioinformatic approaches (Shumay & Fowler, 2010), and empirical support for the phenomenon in the MAOA gene has recently been described (Philibert et al., 2011).

Several studies have reported associations between prenatal stress and antisocial behaviour problems and this study adds to that work by showing that extent of the effect may be modified in utero by genetic variations. If the finding is replicated, and effects on antisocial behaviour disorders in childhood and adult life can be showed, this would provide a basis for the development of prenatal prevention programs targeted at mothers with MAOA-LPR low activity foetuses.

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