Serotonin Transporter Polymorphism Moderates Effects of Prenatal Maternal Anxiety on Infant Negative Emotionality

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Background: Consistent with the fetal programming hypothesis, effects of maternal prenatal anxiety have been found to predict various measures of infant temperament in the early postnatal period. In recent years, a polymorphism in the serotonin transporter gene (5-HTTLPR) emerged as a moderator of diverse environmental influences on different outcomes, with individuals carrying the short allele being generally more vulnerable to adversity.

Methods: We tested whether the association between self-reported maternal anxiety at 20 weeks gestation (Brief Symptom Inventory) and mother-rated infant negative emotionality at 6 months after birth (Infant Behavior Questionnaire-Revised) would be moderated by the 5-HTTLPR in a large Dutch cohort sample ($n = 1513$). We hypothesized that infants carrying the 5-HTTLPR short allele would be more susceptible and therefore more affected by both low and high prenatal maternal anxiety vis-à-vis negative emotionality than other genotypes.

Results: Findings of a significant gene × environment interaction ($B = .65$, $p = .01$) were supportive of a vulnerability model, with infants carrying the short allele being more negatively emotional when mothers reported anxiety during pregnancy, whereas there was no difference between genotypes on negative emotionality when maternal anxiety was low.

Conclusions: The association between maternal anxiety during pregnancy and negative emotionality in early infancy was significant in infants carrying one or more copies of the short allele but not in those homozygous for the long allele. The 5-HTTLPR short allele might increase vulnerability to adverse environmental influences as early as the fetal period.

Key Words: 5-HTTLPR, differential susceptibility, gene × environment (GXE) interaction, infant temperament, prenatal programming, serotonin transporter

Early experiences and environmental influences have been found to shape human development as early as the fetal period. This observation has been interpreted in terms of the fetal programming hypothesis (1,2), which stipulates that the fetus adjusts its phenotype (e.g., metabolism and stress reactivity) in utero—on the basis of placental transferred maternal nutritional and hormonal cues about the "outside" world—as a means of optimally adapting to the (anticipated) conditions of the postnatal environment.

Findings consistent with the fetal programming hypothesis have been reported repeatedly (3), along with perhaps related evidence linking prenatal maternal anxiety and mother-reported infant temperament. For example, higher levels of maternal anxiety during pregnancy has been found to predict: 1) greater infant temperament reactivity at 8 weeks after birth (4); 2) greater infant negative behavioral reactivity at 4 months after birth (5); 3) greater infant difficult temperament at 4 and 6 months after birth (6); and 4) decreased infant attention regulation at 3 and 8 months after birth (7). The fact that all the just-cited investigations controlled for postnatal maternal anxiety clearly suggests that the predicted differences in infant temperament are a function of prenatal maternal anxiety rather than just of postnatal maternal psychological state.

What the available research has yet to address is whether the putative effects of maternal anxiety on infant temperament vary across fetuses due to their genetic make-up. That this might be the case is certainly suggested by recent studies of gene × environment (GXE) interaction. Most prominently, perhaps, a polymorphism in the serotonin transporter promoter gene area (SLC6A4), the 5-HTTLPR, has been found to moderate the apparent effect of adverse early environmental influences on a variety of phenotypic outcomes. For example, severe childhood maltreatment has been associated with more depression symptoms in adulthood in individuals that carried one or two copies of the short 5-HTTLPR allele but not in individuals homozygous for the long allele (8). Similarly, low maternal sensitivity at 7 months predicted insecure attachment at 15 months exclusively for infants carrying 5-HTTLPR short alleles, whereas attachment quality of infants homozygous for the long allele was independent of observed levels of maternal sensitivity (9).

Most such GXE results have been interpreted in terms of diathesis-stress thinking (10), with the 5-HTTLPR short allele regarded as a vulnerability factor (or diathesis) predisposing individuals toward problematic functioning (e.g., depression) in the face of contextual adversity (e.g., child maltreatment). But as noted by Taylor *et al.* (11) in their study of a GXE interaction involving 5-HTTLPR and quality of the early family environment in the prediction of adult depression as well as by Belsky *et al.* (12,13) in their analysis of many other GXE findings, the short allele might perhaps be better conceptualized as a "plasticity gene" rather than a "vulnerability gene." This is because individuals with the short allele seem in some research to be not only more likely than others to succumb to the negative effects of adverse environments but also more likely than others to benefit from positive supportive ones (14). This proves true even in work in which environmental support is operationalized as merely the ab-

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sence of negative contextual conditions (e.g., no childhood maltreatment).

Evidence of this kind is consistent with Belsky's (13,15–17) "differential-susceptibility hypothesis," which posits that some individuals—including those with the short allele of the 5-HTTLPR—are more affected by both negative and positive environmental conditions than others (i.e., for better and for worse) rather than just disproportionately and negatively affected by contextual adversity than others (11,18). A recent reanalysis of data from a GXE study by Neuman *et al.* (19) of effects of maternal smoking during pregnancy on ADHD in childhood (20) provided first evidence of genetically related differential susceptibility to effects of prenatal experiences. The study, however, investigated the moderating effect of DRD4 and not 5-HTTLPR. Children carrying the dopamine DRD4 7-repeat allele—an allele repeatedly associated with differential susceptibility (12,13,21)—tended to be most and least likely to develop ADHD, depending, respectively, on whether their mothers did or did not smoke during pregnancy. It remains to be determined whether the effect of stressful prenatal experiences is moderated in a manner consistent with differential susceptibility when the moderator is 5-HTTLPR.

In light of evidence that prenatal maternal anxiety predicts infant temperament and that the short allele of 5-HTTLPR might function as a plasticity gene, moderating environmental influences in a for-better-and-for-worse manner (17), the current study tested whether the temperaments of infants with one or two short alleles would be more affected by maternal prenatal anxiety than those homozygous for long alleles and whether this moderation would be more consistent with a differential susceptibility than diathesis-stress model (i.e., whether they would prove to be less negatively emotional than others under conditions of low maternal prenatal anxiety, yet more negatively emotional than others under conditions of high maternal prenatal anxiety).

Methods and Materials

Design

This research was embedded in the Generation R Study, a population-based cohort study investigating growth, development, and health from fetal life into young adulthood in Rotterdam, the Netherlands. The Generation R Study has previously been described in detail (22). Briefly, all pregnant women living in the study area with a delivery date between April 2002 and January 2006 were informed about the research project by community midwives and obstetricians. Inclusion criteria were: 1) residency in study area at delivery date; 2) delivery date between April 2002 and January 2006; and 3) informed consent. Importantly, mothers with psychiatric disorders were not identified or excluded from study participation. Written informed consent and genetic data were available for 4345 study families.

The Generation R study has been approved by the Medical Ethics Committee (MEC) of the Erasmus Medical Center, Rotterdam (numbers: prenatal, MEC 198.782/2001/31, and postnatal, MEC 217.595/2002/202).

Participants

Only infants with at least one parent of self-reported Dutch ethnicity were included in the present study to avoid confounding effects of ethnic differences in gene frequency. Of the 3639 qualifying Dutch families 1513 had data on 5-HTTLPR, infant negative emotionality, and prenatal maternal anxiety and were consequently included in the study; see Table 1 for sample characteristics (when analyses were restricted to the 1136 infants with two parents of Dutch ethnicity, results remained the same). Comparisons between included and excluded families revealed no significant differences with regard to 5-HTTLPR, infant negative emotionality, infant gender, and postnatal maternal anxiety or depression. Significant differences emerged, however, for prenatal maternal anxiety, which was significantly greater for excluded than included mothers (mean = .22, SD = .38 vs. mean = .18, SD = .31, $p < .01$, $D = .14$), and for some variables which are not reported here because of restricted space (and are available on request).

Table 1. Demographic Characteristics of the Sample

Variables	*n* (%)
Age at First Contact (yrs)	mean = 31.81, SD = 4.03 (range: 17–43)
Educational Level	
No education	20 (1.3%)
Low (12 yrs or less)	129 (8.5%)
Mid-low (13–15 yrs)	369 (24.4%)
Mid-high (16–17 yrs)	399 (26.4%)
High (18 yrs or more)	596 (39.4%)
Living Situation	
Living with partner	1443 (95.3%)
Living without partner	70 (4.6%)
Income	
< €1200	69 (4.6%)
€1200–2200	261 (17.3%)
> €2200	1183 (78.2%)
Smoking During Pregnancy	174 (11.5%)
Alcohol During Pregnancy	861 (56.9%)
Anxiety During Pregnancy	mean = .18, SD = .31
Anxiety at 6 Months Postnatal	mean = .22, SD = .36
Depression at 6 Months Postnatal	mean = .16, SD = .35
Child Gender	
Boy	761 (50.3%)
Girl	752 (49.7%)
Child Gestational Age at Birth (weeks)	mean = 40.16, SD = 1.44
Child Birth Weight (g)	mean = 3552.40, SD = 508.28
Child 5-HTTLPR	
l/l	497 (32.8%)
s/l	738 (48.8%)
s/s	278 (18.4%)
Child Negative Emotionality at 6 Months	
Fear	mean = .33, SD = .27
Distress to limitations	mean = .62, SD = .30
Recovery of distress	mean = 1.56, SD = .28
Negative emotionality composite (standardized)	mean = .00, SD = 2.18

N = 1513.
5-HTTLPR, serotonin transporter polymorphism.

Measures

Maternal Prenatal and Postnatal Psychopathology Maternal psychopathology was assessed at 20 weeks of pregnancy and at 6 months after birth with the Brief Symptom Inventory, a validated self-report questionnaire with 53 items answered on a 5-point scale ranging from 0 = "not at all" to 4 = "extremely" (23–25). The Brief Symptom Inventory is a short version of the Symptom Checklist 90 (26) and defines a broad spectrum of psychiatric symptoms over the preceding 7 days. For this study, the prenatal and postnatal anxiety and the postnatal depression subscales were used.

Infant Negative Emotionality Infant temperament was assessed at 6 months after birth with an abbreviated version of the Infant Behavior Questionnaire—Revised (IBQ-R) (27). This measure