"Do as I say!" : parenting and the biology of child self-regulation

Kok, R.

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Variations in maternal 5-HTTLPR affect observed parenting

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Abstract

Little is known about the genetic determinants of sensitive parenting. We examined whether the serotonin transporter polymorphism (5-HTTLPR) is an independent predictor of observed maternal sensitivity, and whether observed child social fearfulness moderates the effect of 5-HTTLPR on maternal sensitivity. The population-based sample consisted of 767 mother-child dyads. Maternal sensitivity was repeatedly observed and coded with the Ainsworth's rating scales for Sensitivity and Cooperation and the revised Erickson rating scales for Supportive Presence and Intrusiveness over a three year period. At 3 years, child social fearfulness was observed using the Stranger Approach episode of the Laboratory Temperament Assessment Battery. Maternal 5-HTTLPR significantly predicted sensitivity; mothers carrying the *S*-allele were more sensitive towards their children ($p = .004$). Also, we found some evidence that child social fearfulness moderated the effect of 5-HTTLPR on sensitivity ($p = .059$). Mothers carrying the *S*-allele were more sensitive than mothers without *S*-alleles when parenting children with the lowest fear scores. However, no difference in sensitivity between mothers with different genotypes was observed if they parented more fearful children. Our study showed that variations in maternal 5-HTTLPR genotype appear to be involved in the etiology of parenting behavior.

Introduction

Parental support, guidance and structure are important for children to achieve developmental milestones, and they contribute to long term health (Sroufe et al., 2005a). Sensitive parenting, defined as the ability to accurately perceive children's signals and to respond to them in an adequate and prompt way (Ainsworth et al., 1978), is an important predictor of children's attachment security (Bakermans-Kranenburg, Van IJzendoorn, & Juffer, 2003). Secure attachment is, in turn, related to growth of self-reliance, social competence, and emotional regulation (Sroufe et al., 2005b). Furthermore, sensitive parenting has shown to be predictive of children's social problem solving (Raikes & Thompson, 2008), executive functioning (Bernier, Carlson, & Whipple, 2010), and relationships with siblings and peers (McFarlane et al., 2010; Volling & Belsky, 1992).

Against the background of the critical role of sensitive parenting in children's healthy development, research has investigated the determinants of sensitive parenting. According to Belsky's (1984) widely cited process model of parenting there are three main groups of determinants of parenting. The model presumes that parenting is influenced by parental characteristics including psychopathology, for example depression, anxiety disorder, and ADHD (Chronis-Tuscano et al., 2008; Dix et al., 2004; Newman et al., 2007; Nicol-Harper, Harvey, & Stein, 2007) and personality traits, such as neuroticism and agreeableness (Bornstein, Hahn, & Haynes, 2011; Clark, Kochanska, & Ready, 2000). Another important group of determinants is constituted by child characteristics. For example, sensitive parenting may be challenged by child negativity or difficult temperament of the child (Mills-Koonce et al., 2007; Van den Boom, 1994; Vaughn, Bost, & Van IJzendoorn, 2008). The third group of determinants identified in the process model are contextual sources of stress and

support in which the parent-child relationship is embedded, including social support (Kivijarvi et al., 2004), and work-related stress (Repetti & Wood, 1997).

Although a wide variety of determinants of parenting have been investigated, molecular genetic determinants have been studied to a far lesser extent (Swain et al., 2007). However, substantial genetic influences may be involved in parenting (Collins et al., 2000; Neiderhiser et al., 2004; Plomin et al., 1994). In terms of Belsky's process model (1984), genetic factors may impact on parenting by their effects on parental and child characteristics. Furthermore, they may interact with various other determinants of parenting. The first studies on the molecular genetic basis of parenting using the candidate genes approach targeting dopamine-, oxytocin-, and serotonin-related genes yielded promising results (Bakermans-Kranenburg & Van IJzendoorn, 2008; Van IJzendoorn, Bakermans-Kranenburg, & Mesman, 2008).

Three earlier studies focusing on a repeat polymorphism of the dopamine gene, the DRD4 7-repeat, consistently reported no direct effect of the DRD4 7-repeat on sensitive parenting (Fortuna et al., 2011; Kaitz et al., 2010; Van IJzendoorn et al., 2008) However, all investigators did report an effect of the DRD4 7-repeat in interaction with various stressors on sensitive parenting. Van IJzendoorn and colleagues (2008) reported this gene-environment interaction (GxE) for the combination of the DRD4 7-repeat and the COMT polymorphism, another polymorphism involved in the dopamine system. All three studies assessed very different stressors which were all previously related to sensitive parenting (i.e. infant difficult temperament and infant risk at birth, infant difficult temperament, and stressful life events). As the DRD4 7-repeat moderated the effect of all stressors, this may suggest that this polymorphism moderates the effect of stress in general, rather than the effect of specific stressors on maternal sensitivity. In line with these findings, Lee and colleagues (2010) reported that another polymorphism of the dopamine system, DAT1, interacted with the child's disruptive behavior to predict maternal negative parenting. However, in contrast to the previous studies, they also found a main effect of the DAT1 polymorphism on negative maternal parenting (e.g., critical and negative statements) while no main effect on positive parenting (e.g., praise, positive affect) was detected. Next to polymorphisms involved in the dopamine system, one study also investigated the effect of an oxytocin polymorphism on observed parenting for which a main effect was reported as well (Bakermans-Kranenburg & Van IJzendoorn, 2008).

In the current study we focus on the serotonin transporter polymorphism (5-HTTLPR). This polymorphism has been investigated by two previous studies (Bakermans-Kranenburg & Van IJzendoorn, 2008; Mileva-Seitz et al., 2011). Both studies reported a main effect of 5-HTTLPR on maternal sensitivity. The serotonin transporter gene encodes the serotonin transporter, a key receptor for regulating serotonin levels in the synaptic cleft. In humans, the 5-HTTLPR repeat polymorphism in the promoter-region of the gene has two alleles; the short (*S*) allele and the long

(*L*) allele. The alleles account for differences in transcription efficiency of the serotonin transporter gene; the short allele of the 5-HTTLPR is found to be less active than the long allele, resulting in decreased transcription of the serotonin transporter gene (Murphy & Lesch, 2008). Decreased transcription of the gene reduces serotonin transporter levels and consequently increases the levels of serotonin in the synaptic cleft. In humans, the *S*-allele of 5-HTTLPR is associated with an increased risk of depressive disorders in the presence of environmental stress (Karg et al., 2011), with higher levels of trait anxiety (Schinka, Busch, & Robichaux-Keene, 2004; Sen, Burmeister, & Ghosh, 2004), and with selective attention to negative, threat-related stimuli (Pergamin-Hight et al., 2012). Consistent with these findings, the *S*-allele has also been associated with relatively increased amygdala activation to negative stimuli, a key structure mediating emotional arousal (Munafo, Brown, & Hariri, 2008). In contrast, there is also evidence that the *S*-allele is related to better cognitive functioning including improved decision making and cognitive flexibility (Borg et al., 2009; Homberg & Lesch, 2011), and to social cognition (Canli & Lesch, 2007) which are fundamental components of parenting (Atkinson et al., 2009; Barrett & Fleming, 2011).

While the two previous studies focusing on 5-HTTLPR found a direct effect of the polymorphism on sensitive parenting (Bakermans-Kranenburg & Van IJzendoorn, 2008; Mileva-Seitz et al., 2011), they reported opposite effects: In a sample of mothers with toddlers at high risk for behavioral problems, mothers carrying the short allele of 5-HTTLPR had lower levels of observed sensitive parenting towards their toddlers (Bakermans-Kranenburg & Van IJzendoorn, 2008). In contrast, a general population-based study reported that mothers carrying the short allele had higher levels of observed sensitive parenting (Mileva-Seitz et al., 2011). Mileva-Seitz and colleagues (2011) also tested the hypothesis that early care quality (as experienced by the mother) moderated the relation between 5-HTTLPR and sensitive parenting. They found no evidence for an interaction effect on maternal sensitivity, but they did find a significant interaction effect for mother's orienting away from the baby during free play: early care quality moderated the association between 5-HTTLPR and orienting away from the baby, which was to a certain extent negatively associated with maternal sensitivity. Mothers with no *S* alleles oriented away more frequently from their babies if they reported more negative early care quality.

It is well recognized that for complex traits, such as maternal sensitivity, many genetic associations are not consistently replicated. Much attention has been paid to the attribution of population stratification (i.e. allele frequencies and disease risks differ between subpopulations leading to false-positive associations), misclassification of genotype and outcome, and to underlying gene-environment interaction to this inconsistency (Colhoun, McKeigue, & Davey Smith, 2003; Hirschhorn et al., 2002; Ioannidis et al., 2001). Other important reasons that also contribute to a high chance for initial false-positive findings are publication bias, variation of power between

studies, and failure to ascribe findings of positive association to chance (Colhoun et al., 2003; Wacholder et al., 2004).

In the current study we aimed to further examine the association between 5-HTTLPR and observed sensitive parenting while taking notice of the rectifiable problems attributing to inconsistent and false-positive findings: First, the current study is performed within an ethnically homogeneous cohort, thereby minimalizing the risk of population stratification. Second, we used a four times larger sample (n = 767 mother-child dyads) than in previous studies to increase the power to detect any effect of 5-HTTLPR. Precision of the findings was further improved by assessing observed maternal sensitivity repeatedly, at 14 months, at 3 years, and at 4 years. Furthermore, we assessed whether observed child social fearfulness moderated the effect of 5-HTTLPR on maternal sensitivity. Previous research has demonstrated that child characteristics such as shyness and approach withdrawal are associated with maternal intrusiveness and less maternal warmth (Bates & Pettit, 2007; Brunk & Henggeler, 1984). This association was especially observed in anxious mothers, most likely due to shared genetic factors (Moore, Whaley, & Sigman, 2004). It has also been proposed that shy children are cognitively more challenged in new situations, eliciting maternal overinvolvement (Bates & Pettit, 2007). Because social fear is implicated in maternal sensitivity and in the same neurobiological systems as 5-HTTLPR, social fearfulness is a good candidate environmental factor (Moffitt, Caspi, & Rutter, 2005). Because it is well-recognized that maternal sensitivity includes reciprocal interactions between mother and child (Shin et al., 2008), we also examined whether any associations with maternal 5-HTTLPR and sensitivity were independent of the child's 5-HTTLPR genotype. Last, to test the specificity of any association between 5-HTTLPR and maternal sensitivity, we repeated all analyses with two other polymorphisms available in this cohort and previously examined in relation to sensitivity: the Val158Met polymorphism in the Catechol-O-Methyltransferase gene (COMT) and rs53576, a polymorphism in the oxytocin-receptor gene (OXTR).

We hypothesized that in this large homogeneous cohort with repeated measurements of observed sensitive parenting genetic main effects of 5-HTTLPR can be detected.

Method

Setting

The study was embedded within the Generation R Study, a population-based prospective cohort from fetal life onwards in Rotterdam, the Netherlands, which has been described in detail elsewhere (Jaddoe et al., 2012).

In a randomly assigned subgroup of Dutch pregnant women and their children, detailed assessments were conducted including observations of maternal sensitivity and child temperament. This subgroup is ethnically homogeneous to exclude confounding or effect modification by ethnicity. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth-cohort. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam (numbers: prenatal, MEC 198.782/2001/31 and postnatal, MEC 217.595/2002/202). Written informed consent was obtained from all participants.

Study population

Mothers were considered eligible for the current study if they had singleton pregnancies and gave full consent for postnatal follow-up ($n = 1079$). Of these, data on 5-HTTLPR genotype was available for $n = 919$ mothers. Within this group, information on observed maternal sensitivity was available for $n = 780$ (85%) mothers. Data of 13 mother-child dyads were randomly excluded because they participated with multiple children (e.g., older or younger siblings). Thus, the cohort for analysis compromised $n = 767$ mothers. Of these mothers, the majority ($n = 584$, 76%) participated in 2 or 3 assessments of sensitivity.

To study the main effect, information on all 767 mother-child dyads were included in the analyses. As for the GxE effect, data on 604 mother-child dyads with assessments of child fearful temperament was available.

Non-response

Non-response (i.e. mothers without any data on maternal sensitivity, $n = 139$) did not differ on the distributions of 5-HTTLPR genotypes, parity, or level of family stress compared to mothers included in the study. Non-respondents were however lower educated than mothers included in the study (43.6% vs 34.4%, $\chi^2 = 4.22$, $p = .04$). The children of non-respondents did not differ on social fearfulness compared to children of mothers included in the study.

5-HTTLPR genotyping

Maternal DNA was derived from blood samples at enrolment and child DNA was derived from cord blood samples at birth. The 43-base pair insertion/deletion in the promoter region of the 5-HTT gene was genotyped using Taqman allelic discrimination. Primer sequences were taken from Hu and colleagues (2006). Reactions were performed in a 384-wells format in a total volume of 5 ul containing 2 ng DNA, 120 nM FAM-probe, 80 nM VIC-probe, PCR primers (100 nM each), dimethyl sulfoxide (DMSO) (4% by volume), and 1 x genotyping master mix (Applied Biosystems Inc.).

PCR cycling consisted of initial denaturation for 10 minutes at 95° C, and 40 cycles with denaturation of 15 seconds at 96° C and annealing and extension for 90 seconds at 62.5° C. Signals were read with the Taqman 7900HT (Applied Biosystems Inc.) and analyzed using the sequence detection system 2.3 software (Applied Biosystems Inc.). To evaluate genotyping accuracy of 5-HTTLPR, 225 random child samples were genotyped a second time. No discrepancies were found. Two additional maternal polymorphisms were genotyped using Taqman allelic discrimination: the Val158Met polymorphism, a functional variant in the Catechol-O-methyltransferase gene (COMT), and a polymorphism in the oxytocin receptor gene OXTR, rs53576).

Maternal sensitivity

During the lab visit at the child's age of 14 months, maternal sensitivity was observed during 5 minutes free play ($SD = 2.0$). Maternal sensitivity was coded from DVD recordings with the Ainsworth's 9-point rating scales for Sensitivity and Cooperation (Ainsworth, Bell, & Stayton, 1974). The intraclass correlation (ICC) for intercoder agreement was .79 for sensitivity and .69 for cooperation ($n = 24$). Sensitivity and Cooperation correlated strongly ($r = .84$). An overall 14-month sensitivity score was created by standardizing the two scores and computing the average.

During the lab visit at the child's age of 3 years and the home visit at age 4 years, maternal sensitivity was observed during two tasks that were too difficult for the child, considering his or her age: building a tower and etch-a-sketch. Mothers were instructed to help their child as usual. Maternal sensitivity was coded from DVD recordings with the revised Erickson 7-point rating scales for Supportive Presence and Intrusiveness (Egeland et al., 1990). An overall sensitivity score was created by reversing the Intrusiveness scale, standardizing the scores, and computing the average across both scales and both tasks. The two tasks were independently coded by 13 and 10 extensively trained coders, respectively. At 3 years, average ICCs for the subscales were .75 for the tower task ($n = 53$) and .79 for the etch-a-sketch task ($n = 55$). At 4 years, average ICCs for the subscales were .85 for the tower task ($n = 40$) and .79 for the etch-a-sketch task ($n = 40$).

Overall, coders were trained in approximately 7 sessions and regularly supervised during the coding process; interreliability between coders was not only assessed directly after the training, but also monitored during the coding process to avoid rater drift. Coders were unaware which of their DVDs would be assigned to a second coder. Based on the guidelines as described by Cicchetti and colleagues (2006) the ICCs for our sensitivity assessments, ranging from 0.69 to 0.85, are good to excellent.

Child social fearfulness

Child social fearfulness was measured using the Stranger Approach (SA) episode of the Laboratory Temperament Assessment Battery Preschool Version (Lab-TAB) during the lab visit at 3 years of age (Goldsmith et al., 1999). The Lab-TAB is a widely used, standardized instrument for observational assessment of early temperament. During the SA episode the child has to deal with social fear when a novel, slightly threatening stranger approaches. The episode was modeled after real-life events: The child was left alone in a room. After 10 seconds a stranger entered the room and asked the child standard questions in a neutral tone of voice.

Episodes were coded from DVD recordings according to the coding system described in the Lab-TAB manual. Coders were extensively trained and reliability was established before data were coded. Coders were blind to all other measures. Each episode was divided into nine epochs. Eight parameters were scored in each epoch: Intensity of fear expressions, distress vocalizations, activity decrease, approach, avoidance, gaze aversion, verbal hesitancy, and nervous fidgeting. For each parameter, average scores were calculated by dividing the child's overall score for that parameter across the 9 epochs. The mean intercoder agreement ICC for these average scores was .84 ($n = 25$). Then each average score was divided by the maximum attainable score for that parameter per epoch. This was done to standardize parameters along the same scale to range between 0 and 1. Finally, an overall 'fearfulness' score was created by taking the mean of the standardized average scores of the different parameters. This fearfulness score ranged from 0 to 1 with higher scores indicating a more social fearfulness.

Social fear was also assessed by questionnaire. When the child was three years old, parents reported on the following questions: 'my child is afraid of other children', 'my child is afraid of adults other than his/her parents', 'my child is afraid of places crowded with people, like a shopping mall or playground'. Parents responded on a 3-point-Likert scale (0 'not at all', 1 'sometimes', 2 'often'). The sum-scores of both parents were summed and the average was taken.

Other covariates

Maternal age, educational level, marital status, and parity were assessed using questionnaires at enrolment. Educational level (highest education finished) was dichotomized into 'lower education' (until secondary school) and 'higher education'. At 20 weeks of pregnancy, family stress was assessed by a subscale, General Functioning, of the Family Assessment Device (FAD), which is a validated self-report measure of health or psychopathology of the family (Byles et al., 1988). A score > 2.17 (cut-off) denotes unhealthy family functioning. Family stress was defined on the basis of the General Functioning cut-off score as either 'family stress present' or 'no family stress'.

Amount of non-parental care was assessed using a questionnaire at the child's age of one year. Mothers were asked 'for how many hours per week is your child been taken care of by 1) a babysitter, 2) an au-pair, 3) a host-parent, 4) neighbors or family members, 5) daycare, or 6) some-one else ?'. The total hours of non-parental care per week was computed by summing the answers to the different items.

Lifetime depressive and anxiety disorders of mother were assessed using the Composite International Diagnostic Interview (CIDI) Version 2.1. The CIDI is a structured interview based on DSM-IV criteria. A home interview was conducted at 30 weeks during pregnancy by research assistants trained in an official training centre for the CIDI. Good interrater reliability and validity have been reported (Andrews & Peters, 1998).

Statistical analyses

An additive model was used in the analyses with the 5-HTTLPR genotype, with *LL*=0, *LS*=1, and *SS*=2. Using this model an r-fold increased effect was assumed for *LS*, and a 2r-increased effect for *SS*. The 5-HTTLPR genotype was also analyzed by a general genetic model with the *LL* genotype as the reference group. Using this model 5-HTTLPR was analyzed per genotype.

Data were analyzed in three steps. We first assessed the main effect of maternal 5-HTTLPR on maternal sensitivity. To analyze the associations between the repeatedly measured sensitivity scores and 5HTTLPR we used unbalanced repeated-measurements regression analysis assuming random effects for intercept and slope. These regression models enable studies of repeatedly measured outcomes taking into account the correlation between measurements, and allowing for incomplete outcome data (Twisk, 2003). The covariance parameters were estimated using Restricted Maximum Likelihood (REML). We used unstructured covariance structures. These structures estimate every covariance individually and therefore offer the best fit. As simple models are preferred over more complex models including fractional polynomials (Royston & Sauerbrei, 2005) and a scatter plot of the raw data did not give evidence of non-linearity, a linear model was fit. The model fitted can be written as:

$$\text{Maternal sensitivity} = \beta_0 + \beta_1 * \text{5-HTTLPR} + \beta_2 * \text{age} + \beta_x * \text{covariates}.$$

In this model, 'β_0' reflects the intercept and 'β_1*5-HTTLPR' tests the difference in intercept between mothers with different alleles of 5-HTTLPR. The term 'β_2*age' reflects the linear slope of the model with age defined as the child's age in months at the sensitivity assessment. It was also tested whether 5-HTTLPR interacts with age, i.e. whether the development of maternal sensitivity over time differs between mothers with different alleles of 5-HTTLPR. However, as this term was not significant ($p = 0.54$) it was not further included in the models.

To test whether any effect of 5-HTTLPR on maternal sensitivity was driven by a specific time-point, we examined the per time-point associations between 5-HTTLPR and maternal sensitivity using multivariate linear regression analyses. Second, we tested whether the interaction between child social fearfulness and maternal 5-HTTLPR predicted maternal sensitivity. To this end, the fearfulness score was standardized. Again, unbalanced repeated-measurement regression analysis was used to test the repeated associations and multivariate linear regression analyses were performed to examine the per time-point associations. Third, we reran all analyses in the mothers, now adjusting for the child's genotype. This enabled us to test whether the results found for the maternal genotype were independent of the child's genotype. At the same time, it allowed us to test whether there was also an effect of the child's genotype on sensitivity.

Bivariate correlations between the determinants, outcome, and possible confounding covariates were assessed using Pearson's correlations for continuous variables and Spearman's rho for categorical variables. Based on the bivariate correlations, all analyses were additionally adjusted for family stress, maternal educational level, and parity, as these covariates were significantly correlated with 5-HTTLPR and maternal sensitivity (e.g., parity) or with maternal sensitivity alone (e.g., family stress and maternal educational level) (see Supplementary material, Table S1). Adjusting for covariates significantly associated with a quantitative outcome may improve the efficiency without biasing the associations between the predictors and the outcome (Schisterman, Cole, & Platt, 2009). Maternal lifetime depressive disorder, maternal lifetime anxious disorder, maternal age at intake, and gender of the child were also tested as possible covariates but were not significantly correlated with either the predictors or the outcome and were therefore not included in the analyses. To exclude gene-environment correlations, we assessed whether maternal or child 5-HTTLPR were associated with child social fearfulness. To test the specificity of our findings for 5-HTTLPR, the analyses testing the main effect of 5-HTTLPR and the interaction effect with social fearfulness were repeated using COMT and OXTR.

We used Multiple Imputation in SPSS 17 to impute the missing data on covariates (family stress 6.9%, educational level 0.8%, parity 0.1%, lifetime diagnoses of depression or anxiety disorder 12% each). All test statistics and regression coefficients were averaged over 5 imputed datasets. We used an alpha of .05 to indicate statistical significance. All repeated measurements analyses were carried out using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Gary NC, USA), including the PROC MIXED procedure for unbalanced repeated measurements. All per time-point analyses and correlations were carried out using the Statistical Package for the Social Sciences, version 17.0 for Windows (SPSS, Inc. Chicago, Illinois).

Results

Descriptive statistics of the mothers and children are presented in Table 1. Maternal and child 5-HTTLPR genotype distribution were both in Hardy Weinberg equilibrium ($p = .6$ and $p = .6$, respectively). Approximately 15% of the mothers met the criteria for a lifetime depressive disorder. Likewise, 14% of the mothers met the criteria for a lifetime anxious disorder.

Correlations between predictor variables, maternal sensitivity, and covariates are presented in Supplementary material, Table S1. Maternal 5-HTTLPR genotype was not correlated with either a lifetime depressive disorder ($\rho = -.03$) or a lifetime anxiety disorder ($\rho = -.02$). The correlations between the measurements of maternal sensitivity at different time points were low to modest (14 months and 3 years $r = .16$, 14 months and 4 years $r = .07$, 3 years and 4 years $r = .32$).

The repeated measurement analyses showed that, overall, with each additional *S*-allele of the mother she was more sensitive towards her child ($B = 0.11$ (95% C.I. = 0.03, 0.18), $p = .005$) taking into account family stress, educational level and parity (see Table 2). Using a general genetic model we found that mothers carrying the *SL* and *SS* genotypes were more sensitive towards their children than mothers with the *LL* genotype.

The results of the individual per time-point analyses are summarized in Table 2. Maternal 5-HTTLPR was associated with maternal sensitivity at 14 months and with maternal sensitivity at 4 years. These associations remained significant after adjusting for family stress, maternal educational level, and parity. Although 5-HTTLPR did not predict maternal sensitivity at 3 years, the association was in the same direction as the associations observed at 14 months and 4 years, and was not significantly different from those associations.

Table 1. *Sample descriptives (N=767).*

	Mean*	(*SD*)*
Mothers		
5-HTTLPR (%)		
LL (*n*=257)	33.5	
LS (*n* =371)	48.4	
SS (*n*=139)	18.1	
Sensitivity at 14 months, mean (range)[a]	0.0	(-4.16, 2.58)
Sensitivity at 3 years, mean (range)[b]	0.0	(-2.75, 2.86)
Sensitivity at 4 years, mean (range)[c]	0.0	(-2.56, 2.42)
Lifetime depressive disorder (%)	14.8	
Lifetime anxiety disorder (%)	14.4	
Family stress (%)	4.5	
Educational level (% lower)	34.6	
Parity (% nulli)	63.5	
Age at intake	31.8	(3.74)
Non-parental care, hours per week	16.0	(9.85)
Children		
5-HTTLPR (%)[d]		
LL (*n*=205)	26.7	
LS (*n*=295)	38.5	
SS (*n*=124)	16.2	
Child's social fearfulness, mean (range)[e]	0.0	(-2.72, 3.67)
Child's gender (% boys)	50.1	
Age at 14mo visit, months, median (95% range)	14.5	(13.4, 17.1)
Age at 3 years visit, months, median (95% range)	37.3	(35.5, 41.4)
Age at 4 years visit, months, median (95% range)	51.1	(49.8, 55.1)

* Unless otherwise indicated
[a] $n = 537$, [b] $n = 574$, [c] $n = 524$, [d] $n = 624$, [e] $n = 624$

Table 2. *Associations between 5HTTLPR and maternal sensitivity.*

	Maternal sensitivity (per *SD*)			
	Unadjusted Model		Adjusted Model	
	B (95% C.I.)	*p*	*B* (95% C.I.)	*p*
Repeated measurements analyses				
5-HTTLPR	0.11 (0.04, 0.19)	.004	0.11 (0.03, 0.18)	.005
5-HTTLPR (general model)				
LL	0.00 (ref)	-	0.00 (ref)	-
LS	0.17 (0.04, 0.29)	.008	0.18 (0.06, 0.29)	.01
SS	0.21 (0.05, 0.37)	.009	0.19 (0.04, 0.35)	.004
Per time-point analyses				
Sensitivity at 14 months (n=537)				
5-HTTLPR	0.13 (0.01, 0.25)	.04	0.12 (0.00, 0.24)	.049
Sensitivity at 3 years (n=574)				
5-HTTLPR	0.08 (-0.04, 0.19)	.2	0.06 (-0.05, 0.18)	.3
Sensitivity at 4 years (n=524)				
5-HTTLPR	0.16 (0.04, 0.28)	.008	0.17 (0.05, 0.28)	.006

Note. The adjusted model was adjusted for family stress, maternal educational level, and parity. Unless otherwise specified, additive models were used.

The repeated measurements analysis showed a trend for an interaction between 5-HTTLPR and child temperament in predicting maternal sensitivity; $B = -0.09$ (95% C.I. = -0.18, 0.00), $p = .059$ (see Table 3). Figure 1 shows that mothers carrying the *SS* or *SL* genotype were more sensitive than mothers carrying the *LL* genotype when parenting children with the lowest fear scores. In contrast, no difference in sensitivity between mothers with different genotypes was observed if they parented more socially fearful children. The per time-point analyses showed that the effects of an interaction between 5-HTTLPR and child temperament on sensitivity were essentially the same at 3 and 4 years (see Table 3). To test the robustness of these findings we also tested the effect of the interaction between 5-HTTLPR and social fear on sensitivity with social fear reported by both parents. The correlation between the observed and reported measurement of social fear was low ($r = .08$, $p = 0.07$, $n = 552$), but the effect of the interaction was similar ($B = -0.19$ [95% C.I. = -0.42, 0.57], $p = .13$), data not shown.

Table 3. *The moderating effects of social fearfulness on the association between 5-HTTLPR and maternal sensitivity.*

	Maternal sensitivity (per *SD*)			
	Unadjusted Model		Adjusted Model	
	B (95% C.I.)	*p*		
Repeated measurements analyses				
Social Fearfulness x 5-HTTLPR	-0.08 (-0.18, 0.02)	.099	-0.09 (-0.18, 0.00)	.059
Per time-point analyses				
Sensitivity at 36 months (n=532)				
Social Fearfulness x 5-HTTLPR	-0.08 (-0.20, 0.04)	.2	-0.09 (-0.20, 0.03)	.1
Sensitivity at 48 months (n=453)				
Social fearful x 5-HTTLPR	-0.07 (-0.19, 0.06)	.3	-0.08 (-0.20, 0.04)	.2

Note. The adjusted model was adjusted for family stress, maternal educational level, and parity. Furthermore, all models included the main effects of social fearfulness and 5-HTTLPR. Unless otherwise specified, additive models were used.

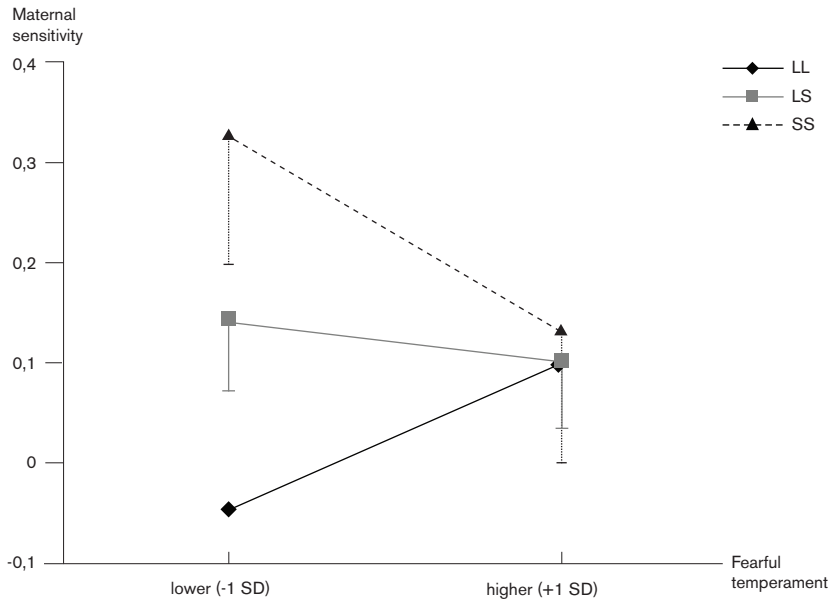

Figure 1. Mean maternal sensitivity per *SD* (with 95% C.I.'s) based on 5-HTTLPR genotype and child social fearfulness (per *SD*). Figure based on an additive genetic model (*LL* = reference), adjusted for family stress, maternal educational level, and parity.

To test whether our results were due to an independent effect of the maternal 5-HTTLPR genotype and could not be explained by the child's 5-HTTLPR genotype, we reran all analyses in mothers also including the child's 5-HTTLPR genotype. The results are illustrated in Figure 2, which shows the effect of the child's 5-HTTLPR genotype on maternal sensitivity adjusted for maternal genotype. Within the strata of maternal genotype, child 5-HTTLPR genotype did not affect maternal sensitivity ($B = -0.01$ [95% C.I. = -0.10, 0.08], $p = .9$), while the effect of maternal genotype on sensitivity remained essentially the same ($B = 0.12$ [95% C.I. = 0.03, 0.21], $p = .01$). Also, when tested separately, the child's genotype did not predict maternal sensitivity ($B = 0.05$ [95% C.I. = -0.03, 0.13], $p = .2$). Likewise, in strata of maternal genotype, there was no interaction between child 5-HTTLPR genotype and social fearfulness in the prediction of maternal sensitivity (data not shown).

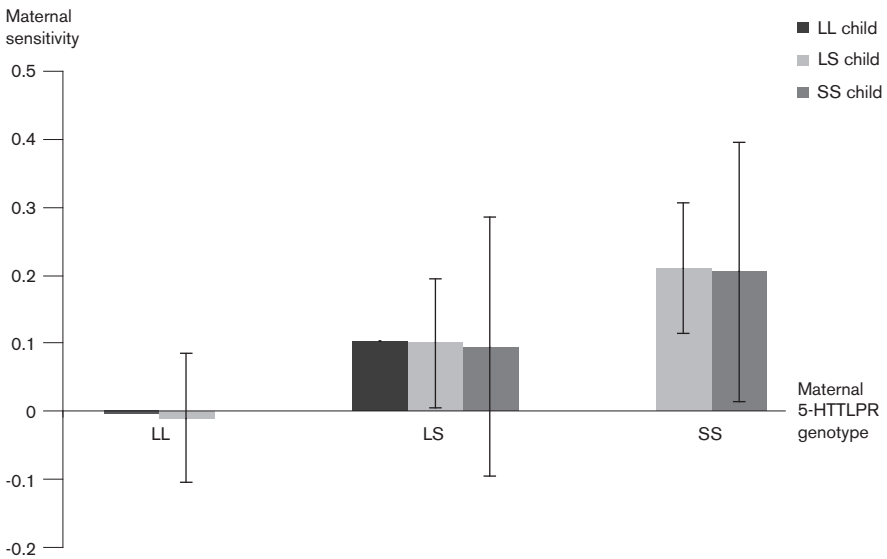

Figure 2. The effect of child 5-HTTLPR genotype on maternal sensitivity. In the different strata of maternal genotype, no effect of the child's 5-HTTLPR genotype on maternal sensitivity was observed. Figure based on an additive genetic model adjusted for family stress, maternal educational level, and parity.

To exclude the possibility that the reported GxE result was due to gene-environment correlation (Rutter, Moffitt, & Caspi, 2006), we assessed whether maternal or child 5-HTTLPR genotype was correlated with child social fearfulness. No significant correlations between maternal or child 5-HTTLPR genotype and child social fearfulness were observed (see Supplementary Table S1).

To test the specificity of the findings for 5-HTTLPR, the analyses were repeated using COMT and OXTR. No main effects or interaction effects with social fear on maternal sensitivity were found (see Supplementary Table S2).

Discussion

The present study investigated the effect of 5-HTTLPR on maternal sensitivity in a large population-based sample of mother-child dyads, using repeated measurements of sensitivity at different ages of the child. Mothers carrying *S*-alleles showed more sensitive behavior towards their children than mothers carrying *L*-alleles. Furthermore, we found some evidence that child social fearfulness may moderate the effect of 5-HTTLPR on maternal sensitivity. Mothers carrying the *SS* or *LS* genotype were more sensitive than mothers carrying the *LL* genotype when parenting children with the lowest fear scores. In contrast, no difference in sensitivity between mothers with different genotypes was observed when they parented more fearful children.

The findings of a direct effect of 5-HTTLPR on maternal sensitivity are in line with the observations of Mileva-Seitz and colleagues (2011) who also found that the *S*-allele was associated with more sensitive parenting. The 5-HTTLPR polymorphism may exert its influence on parenting through its associations with maternal characteristics because the 5-HTTLPR polymorphism is associated with various aspects of cognitive functioning. Both rodent and human studies have suggested that *S*-allele carriers show improved cognitive functioning on a variety of tasks including cognitive flexibility, reversal learning, attention, and inhibition (Brigman et al., 2010; Homberg & Lesch, 2011; Jedema et al., 2010). Especially cognitive flexibility and attention are important components of parenting behavior as sensitive parenting depends on the ability to accurately perceive children's signals and to respond to them in an adequate and prompt way (Ainsworth et al., 1978). For example, it has been shown that maternal attention deficit/hyperactivity disorder (ADHD) negatively impacts on maternal parenting practices (Chronis-Tuscano et al., 2008; Murray & Johnston, 2006). Also, poor working memory is predictive of observed reactive parenting (Deater-Deckard et al., 2010).

Besides an effect on parenting via maternal characteristics the 5-HTTLPR polymorphism may also exert a direct influence on parenting through underlying neural and hormonal influences. Both oxytocin and vasopressin appear to be of major importance for understanding differences in parenting behavior across species (Galbally et al., 2011; Swain et al., 2007). The two hormones are secreted by the hypothalamic paraventricular nucleus (PVN) which is innervated by serotonergic fibers (Skuse & Gallagher, 2011). Furthermore, serotonin receptors are present in the PVN. Studies have indicated that through its receptors, serotonin influences the release of

oxytocin and vasopressin (Jorgensen et al., 2003). Therefore, through its associations with the oxytocin and vasopressin systems, 5-HTTLPR may influence maternal sensitive parenting.

When we discuss our GxE finding, it should be noted that the finding was only marginally significant ($p = .059$) and must therefore be interpreted with caution. While the sample size in the current study was larger than previous reports on molecular genetics in relation to observed parenting, power was still small (e.g., < 20%) to detect a significant GxE in a fairly homogeneous sample (Duncan & Keller, 2011). We found that mothers with the *SS* or *LS* genotypes were more sensitive than mothers with the *LL* genotype when parenting low fearful children. In contrast, when parenting more fearful children, no differences in sensitive parenting between mothers with different genotypes was observed. The present observations of social fearfulness were obtained in a relatively healthy, general population sample of mother-child dyads. We cannot rule out the possibility that if the fear scores had included more extremes of social fearfulness (e.g., clinical levels of social fear) we might have observed a different picture. GxE effects depend on the distribution of the environmental exposure in the sample (Aiken & West, 1991; Belsky, Bakermans-Kranenburg, & Van IJzendoorn, 2007).

Also, if risk exposure differs among samples and there is an underlying GxE, findings for candidate genes may be inconsistent (Caspi et al., 2003). This offers a possible explanation for the divergent findings reported by Bakermans-Kranenburg & Van IJzendoorn (2008) who found that the *S*-allele was related to less sensitive parenting. Their study involved a sample of mothers with children with externalizing behavioral problems. In the absence of a reference group of children without behavioral problems, an underlying GxE (i.e. the 5-HTTLPR genotype in interaction with the stress of parenting a problematic child) could even have resulted in this seemingly reversed effect. On the other hand, children at high risk for externalizing behaviors may well be a different parenting challenge than children who are socially fearful.

In the current study we aimed to rule out artifact sources of GxE findings. First, no correlations were observed between the maternal or child's 5-HTTLPR and social fearfulness of the child. If the psychosocial environmental variable (here: social fearfulness of the child) is not genetically independent of the outcome variable (here: sensitive parenting), then any GxE would reflect Gene Environment correlation (rGE): Children inherit the genes of the mother associated with sensitive parenting which then predispose them to social fearfulness (passive rGE), or these inherited genetic variants may evoke certain parenting behaviors (evocative rGE) (Rutter & Silberg, 2002). Second, in the current study child social fearfulness was observed rather than reported by the mother. This is important as the 5-HTTLPR genotype has been associated with anxious and neurotic personality traits (Karg et al., 2011), and there is some evidence that maternal personality traits influence their reports of the child's

temperamental traits (Hayden et al., 2010; Kiel & Buss, 2006). In theory, mothers, predisposed by their genetic make-up, could ascribe their children certain temperamental characteristics (rGE). In the present study child fearfulness was observed, excluding maternal reporting bias.

Moreover, we showed that, in strata of maternal genotype, no effect of child genotype on sensitive parenting was observed. In other words, the effect of 5-HTTLPR genotype on maternal sensitivity was driven by the maternal genotype, thereby confirming the independent effect of the maternal genotype on maternal sensitivity.

Both the direct, indirect genetic effects, and GxE effects may be seen as an extension of Belsky's model. However, not only structural genetic variants account for the transmission of parenting effects. For example, animal research has shown that early maternal parenting alters the DNA structure of the offspring (i.e. DNA methylation) which may persist into adulthood. This altered DNA structure of the offspring subsequently affects the offspring's parenting as adults (Kappeler & Meaney, 2010; Meaney, 2001). Therefore, future research on the determinants of parenting would not only benefit from including genetic factors, but also from epigenetic research.

Our study has strengths and limitations, and these are worth mentioning as well. First, our results may be somewhat biased due to the overrepresentation of higher educated mothers. Second, the Generation R Focus Study is a relatively homogenous population-based cohort that mainly consists of low risk families. While the homogeneity of the sample is advocated for validly testing genetic effects, results may be less generalizable to samples including high-risk families. Furthermore, we did not differentiate between L and Lg although Lg is considered a low expressing genotypic variant of the 5-HTTLPR polymorphism (Hu et al., 2006). However, in Caucasian samples the percentages of Lg have been found to be rather low (Zalsman et al., 2006). Also, our GxE finding was only marginally significant. Clearly, independent replication of this finding is needed. Last, no more than 10% (varying from $n = 24$ to $n = 55$) of the sensitivity assessments were re-evaluated for rater agreement.

In conclusion, we showed that the maternal 5-HTTLPR polymorphism most likely is associated with maternal sensitive parenting. Furthermore, we showed that the association between maternal 5-HTTLPR and maternal sensitivity may differ depending on fearful temperamental traits of the child. These findings contribute to growing knowledge that parental behavior is a multifactorial concept. As noted by Swain and colleagues (2007), parenting can be viewed as an interaction among genes, past parenting, current experience, psychological state, neurobiological systems, and environmental constraints. Acknowledging and providing further insights into the multifactorial processes underlying parenting will provide a better understanding of parenting. In particular, investigation of possible mediators of the association between 5-HTTLPR and maternal sensitivity, such as cognitive flexibility and attention, may provide valuable insights into underlying biological pathways and provide

further evidence for an association between 5-HTTLPR and parenting. Moreover, as for many complex traits it remains challenging to find and recognize true genetic associations. Therefore, replication of the currents association between 5-HTTLPR and sensitive parenting remains warranted. In the future, all efforts to provide insight into processes underlying parenting may lead to early identification of mother-child dyads who are candidates for early (parenting) interventions.

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Supplementary material

Table S1. *Correlations among the variables.*

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Maternal 5-HTTLPR	-													
2 Child's 5-HTTLPR	.51***	-												
3 Sensitivity at 14mo	.08*	-.02	-											
4 Sensitivity at 3 year	.05	.07	.16**	-										
5 Sensitivity at 4 year	.11**	.07	.07	.32***	-									
6 Social fearfulness	-.03	-.01	.04	.01	-.01	-								
7 Lifetime depressive disorder	-.03	.05	-.03	.02	-.05	-.01	-							
8 Lifetime anxiety disorder	-.02	-.03	-.04	-.01	-.04	-.01	.24***	-						
9 Family stress	.02	.04	-.09**	-.04	-.06	-.00	.05	.04	-					
10 Educational level (ref=higher)	-.03	-.03	-.06	-.18***	-.20***	.02	.05	.02	-.01	-				
11 Parity (ref=0)	.04*	-.01	.02	.07**	.04	.05	.00	.00	.00	.02	-			
12 Age at intake	.03	-.07*	.05	.03	.00	.02	.04	.02	.00	-.20***	.40***	-		
13 Non-parental care	.03	.04	-.00	.06	.03	.01	-.10*	.00	-.04	-.30**	-.03	.18**	-	
14 Child's gender (ref=girl)	.03	.04	-.07	-.05	.00	.08**	-.01	.04	-.01	.05	.00	-.08**	.05	-

*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$

Table S2. *Associations between COMT, OXTR and maternal sensitivity.*

	Maternal sensitivity (per *SD*)			
	Unadjusted Model		Adjusted Model	
	B (95% C.I.)	*p*	*B* (95% C.I.)	*p*
COMT	0.02 (-0.06, 0.10)	.6	0.03 (-0.05, 0.11)	.5
COMT (general model)				
ValVal	0.00 (ref)	-	0.00 (ref)	
ValMet	0.04 (-0.09, 0.17)	.6	0.04 (-0.08, 0.17)	.5
MetMet	0.04 (-0.12, 0.20)	.6	0.06 (-0.10, 0.21)	.5
Social Fearfulness x COMT	0.01 (-0.11, 0.12)	0.99	0.00 (-0.10, 0.11)	.9
OXTR	0.001 (-0.09, 0.09)	0.97	-0.01 (-0.10, 0.09)	.8
OXTR (general model)				
GG	0.00 (ref)	-	0.00 (ref)	-
GA	0.07 (-0.06, 0.19)	.3	0.06 (-0.06, 0.18)	.3
AA	-0.08 (-0.28, 0.13)	.5	-0.10 (-0.30, 0.10)	.3
Social Fearfulness x OXTR*				
Social Fearfulness x GA	0.07 (-0.08, 0.21)	.4	0.07 (-0.08, 0.22)	.4
Social Fearfulness x AA	0.01 (-0.20, 0.21)	.9	0.01 (-0.20, 0.21)	.9

Note. The adjusted model was adjusted for family stress, maternal educational level, and parity. Furthermore, all models included the main effects of social fearfulness and 5-HTTLPR.
Unless otherwise specified, additive m odels were used.
*For the interaction between social fearfulness and OXTR a general genetic model was used as the association between OXTR and sensitivity was not linear.