Dopamine system genes associated with parenting in the context of daily hassles

M. H. van IJzendoorn*[,1], M. J. Bakermans-Kranenburg[1] and J. Mesman

Centre for Child and Family Studies, Leiden University, The Netherlands

**Corresponding author: M. H. van IJzendoorn, Centre for Child and Family Studies, Leiden University, PO Box 9555, NL-2300 RB Leiden, The Netherlands. E-mail: vanijzen@fsw.leidenuniv.nl*

Received 2 July 2007, revised 25 September 2007, accepted for publication 26 September 2007

The current study examined the molecular genetic foundations of sensitive parenting in humans and is the first to test the interaction between genes and environment in modulating parental sensitive responses to children. In a community sample of 176 Caucasian, middle class mothers with their 23-month-old toddlers at risk for externalizing behavior problems, the association between daily hassles and sensitive parenting was investigated. We tested whether two dopamine-related genes, dopamine D4 receptor (*DRD4*) and catechol-O-methyltransferase (*COMT*) gene polymorphisms, modulate parents' vulnerability to the negative influence of daily hassles on sensitive parenting behavior to their offspring. Sensitive parenting was observed in structured settings, and parents reported on their daily hassles through a standard questionnaire. In parents with the combination of genes leading to the least efficient dopaminergic system functioning (*COMT* val/val or val/met, *DRD4*-7Repeat), more daily hassles were associated with less sensitive parenting, and lower levels of daily hassles were associated with more sensitive parenting $d = 1.12$. The other combinations of *COMT* and *DRD4* polymorphisms did not show significant associations between daily hassles and maternal sensitivity, suggesting differential susceptibility to hassles depending on parents' dopaminergic system genes. It is concluded that the study of (multiple) gene–environment interactions (in the current case: gene by gene by environment interaction, G × G × E) may explain why some parents are more and others less impacted by daily stresses in responding sensitively to their offspring's signals.

Keywords: Attachment, catechol-O-methyltransferase val[158]met, compliance, *COMT*, daily hassles, differential susceptibility, dopamine, dopamine D4 receptor gene, dopaminergic system, *DRD4*, mothers, parenting, sensitivity, stress, toddlers

Although parenting is crucial for child development, almost no studies have been conducted on the genetics of parental behavior in humans (Swain *et al.* 2007). In a series of programmatic studies on parenting in rodents, Meaney and colleagues provided evidence for genomic as well as non-genomic transmission of sensitive 'licking/grooming and arched-back nursing' in rats and showed that sensitive care enhanced the development of the dopaminergic system and stress regulation in the offspring (Meaney 2001, 2007).

In humans, sensitive parenting has frequently been investigated, in particular its role in the development of secure attachments that shape children's responses to stress and their willingness to be compliant to parental requests (Ainsworth *et al.* 1978; Belsky 2005; De Wolff & Van IJzendoorn 1997). Parental sensitivity refers to the ability to accurately perceive children's attachment signals and to respond to these signals in an adequate and prompt way (Ainsworth *et al.* 1978). Parental sensitivity has been shown to be affected by the strains and stresses implied in low socioeconomic status (Bakermans-Kranenburg *et al.* 2004), postnatal depression (Hoffman *et al.* 2006) and the experience of (parenting) daily hassles (Belsky *et al.* 1995, 1996a,b; Crnic & Low 2002; Phelps *et al.* 1998; Repetti & Wood 1997). However, much less is known about the genetic underpinnings of parental sensitivity and about the interaction between genes and environment in modulating sensitive responses to children. Previous studies focused on children's genetic differences in relation to the parenting they retrospectively perceived (Lucht *et al.* 2006) or actually experienced (Bakermans-Kranenburg & Van IJzendoorn 2006, 2007; Gervai *et al.* 2007), but as yet no molecular genetic basis of *observed* human parenting has been found, and studies on genetic differences interacting with the environment related to parenting are lacking. In the current study, we examine two crucial dopamine-related genes and their interaction with parents' daily hassles in addressing the question whether some parents are more and others are less vulnerable to the influence of stress in responding sensitively to their offspring.

The catechol-O-methyltransferase (*COMT*) gene is one of the candidate genes to be studied in the context of parenting as it influences the activity of dopamine in the prefrontal cortex through its catabolic effects on the neurotransmitter. The *COMT* gene, in humans localized on chromosome 22, band q11.2, plays a pivotal role in the regulation of dopaminergic activity, and the functional variation in the val[158]met polymorphism of this gene (Lotta *et al.* 1995) may contribute

[1] The first two authors contributed equally to this work.

doi: 10.1111/j.1601-183X.2007.00362.x

to differences in sensitive parenting under stress originating from daily hassles. The met allele codes for an enzyme with about one-fourth of the activity in degrading dopamine compared with the enzyme that is coded by the val allele (Chen *et al.* 2004). Smolka *et al.* (2005) suggested that for subjects with one or two *COMT* met alleles, increased limbic and prefrontal activation elicited by unpleasant stimuli might contribute to their lower emotional resilience against negative mood states, whereas in a comprehensive review, Bilder *et al.* (2004) hypothesized that subjects with one or more met158 alleles may better perform on tasks requiring a consistent behavioral program in the face of distraction. In a meta-analysis, Savitz *et al.* (2006) showed that the low-activity met allele is consistently associated with better performance on cognitive tasks, in particular those with a working memory component. The association with less distractibility may, however, also lead to decreased cognitive flexibility (Bilder *et al.* 2004; Drabant *et al.* 2006), thus both alleles seem to be associated with risks and benefits. As parental sensitivity requires continuous attention to the children's signals even in stressful circumstances, less distractibility may help parents to remain focused on their child.

The dopamine D4 receptor gene (*DRD4*) is another gene implicated in the dopaminergic system. The gene, located near the telomere of chromosome 11p, exhibits various polymorphisms, with variations in the number of 48-bp tandem repeats in exon 3. The three most common variants are 2R, 4R and 7R. The 7R gene variant (*DRD4*-7R) codes for a receptor that is less sensitive to endogenous dopamine compared with the receptors coded for by the shorter repeats. In previous studies, the *DRD4*-7R polymorphism showed associations with impulsive behavior in adults and attention-deficit hyperactivity disorder in children (Ebstein 2006; Swanson *et al.* 2007). Importantly, *DRD4*-7R (linked to attentional, motivational and reward mechanisms; Robbins & Everitt 1999) may make children more vulnerable to negative environmental influences in developing insecure, disorganized attachments (Lakatos *et al.* 2000, 2002, 2005; Gervai *et al.* 2005; but see Bakermans-Kranenburg & Van IJzendoorn 2007) and externalizing behavior problems (Bakermans-Kranenburg & Van IJzendoorn 2006, 2007), which are both related to stress dysregulation.

In the current gene by gene by environment interaction study, we investigate whether *COMT* val and *DRD4*-7R alleles (separately or in interaction) also make parents more vulnerable to the negative influence of daily hassles on sensitive parenting behavior to their offspring.

Materials and methods

Subjects

The current paper is based on data obtained in the Screening and Intervention of Problem behavior in Toddlerhood study (SCRIPT), which investigated the effectiveness of an early intervention program aimed at reducing externalizing problems in 1- to 3-year-old children by enhancing maternal sensitivity and adequate discipline strategies (VIPP-SD, see Van Zeijl *et al.* 2006). It consisted of a screening phase in a general population sample and a pretest–posttest randomized case–control intervention in a subsample of children with scores above the 75th percentile on the Child Behavior Checklist (CBCL) Externalizing Problems scale. Data for the current paper were derived from the screening and pretest phase. Permission for the study was obtained from the Committee for Medical Ethics of Leiden University Medical Centre and the Ethics Committee of the Faculty of Social and Behavioral Sciences of Leiden University.

Participants were recruited from community records of several cities and towns in the western region of the Netherlands. Several exclusion criteria (e.g. twins, serious medical condition in child or mother, single parenthood and non-Dutch cultural background) resulted in a target selection sample of 1954 children. Children with scores above the 75th percentile on the CBCL Externalizing Problems scale (age 1 year: scores $\geq$13; age 2 years: scores $\geq$19; age 3 years: scores $\geq$20) were selected for the intervention study. Of the 438 selected families, parents of 237 children (54%) agreed to participate in the entire intervention study and were invited for a pretest. During a 1.5-h laboratory session, mother and child completed several tasks coded afterwards from videotapes with observational measures, by independent coders, unaware of other data concerning the participants. The average time between the screening and the laboratory session was 4 months. There were no significant differences between selected families who agreed to participate in the intervention phase and those who did not regarding maternal age, child age or gender, presence of siblings and level of child externalizing problems. About 2 years after the intervention, the sample was contacted to take part in the collection of DNA material. Cheek cells were collected from 176 mothers. Mean age of the mothers was 33 years (SD = 4.0), and mean age of the children was 23 months (SD = 10.1) at pretest. Fifty-six per cent of the children were boys, and 60% of the children had siblings. The majority of the parents were well educated.

As data concerned the screening and pretest phases, the intervention and control groups were combined in the analyses. Internal consistency of questionnaire data was assessed in the general population screening sample (n = 2408).

Instruments

Daily hassles

In the screening phase, the mothers were asked to rate 25 indices of potentially stressful events (Kanner *et al.* 1981). They rated the intensity of hassles they experienced on a 5-point scale for each event (0 = no hassle to 4 = big hassle). Items concerned daily hassles related to life in general (e.g. money problems or trouble at work). Reliability and validity of this scale were shown by Kanner *et al.* (1981); in our sample alpha reliability was 0.87. The average level of hassles reported in the current sample was similar to that in a non-select community sample of families with 4-year-old children (Pannebakker 2007).

Maternal sensitivity

Mothers' sensitivity was observed in the laboratory session during a series of problem-solving tasks. Dyads were given three tasks during a total time of 15 min; they were asked to solve puzzles that were too difficult, considering the age of the child (different puzzles were used in each age group), and mothers were instructed to help their child in the way they usually did. Mothers' supportive presence, intrusiveness and clarity of instruction were rated on 7-point scales described by B. Egeland, M. F. Erickson, J. C. Clemenhagen-Moon, M. K. Hiester, J. Korfmacher, unpublished data, University of Minnesota, Minneapolis. These scales include but also extend the original scales for 'sensitive responsiveness' of Ainsworth *et al.* (1978), developed for parent–infant interaction in the first year after birth. The scales take the wider age range of the current sample into account and measure an age-appropriate concept of sensitivity that also pertains to the developmental domain of coping with cognitive challenges. The average intraclass correlation (single rater, absolute agreement) for intercoder reliability (for all separate pairs of seven coders) was 0.75 (n = 30). For overall sensitivity, ratings of the separate tasks were averaged, the intrusiveness scores were reversed and the standardized subscale scores were added.

Genes, Brain and Behavior (2007) doi: 10.1111/j.1601-183X.2007.00362.x

DNA isolation

Buccal swabs from the mothers were collected in lysis buffer (100 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 10 mM Tris, pH 8, 0.1 mg/ml proteinase K and 0.5% w/v sodium dodecyl sulfate) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). DNA concentrations were measured using the Quant-iT DNA Assay kit (Invitrogen, Breda, the Netherlands). The average yield was approximately 4 μg of genomic DNA per buccal swab sample.

Polymerase chain reaction amplification and genotyping

Catechol-O-methyltransferase

The region flanking amino acid position 158 from the *COMT* gene (Genbank accession number Z26491) was amplified from genomic DNA using primers *COMT*-F1 (5′-TGGACGCCGTGATTCAGGAG-3′) and *COMT*-R1 (5′-GGTGGGGAGGACAAAGTGCG-3′). Typical polymerase chain reaction (PCR) reactions were set up using 1–10 ng genomic template DNA, 10 pmol per primer, 5% dimethyl sulfoxide and BioThermAB polymerase (GeneCraft, Munster, Germany). Cycling conditions were as follows: initial denaturation step of 5 min at 94°C, followed by 36 cycles of 30 seconds at 94°C, 60 seconds at 62°C, 60 seconds at 72°C and a final extension step of 4 min at 72°C. Polymerase chain reaction fragments were sequenced using primer *COMT*-F (5′-ACTGTGGCTACTCAGCTGTG-3′; Eisenberg *et al.* 1999) and dye terminator chemistry. Sequence reactions were run on an ABI-3730 automated sequencer, and homozygous or heterozygous met/val variants were determined either by manual inspection of chromatograms or by automated sequence analysis using SeqScape software (v.2.1, Applied Biosystems, Foster City, CA, USA). Genotypes ($n = 48$ met/met, $n = 94$ met/val, $n = 34$ val/val) were in Hardy–Weinberg equilibrium, χ^2 (2, $n = 176$) = 1.01, $P = 0.61$.

Dopamine D4 receptor

For amplification, primers 5′-GCGACTACGTGGTCTACTCG-3′ and 5′-AGGACCCTCATGGCCTTG-3′ were used (Lichter *et al.* 1993). For genotyping of the *DRD4* gene (Genbank accession number AC021663), exon 3 fragments were amplified by an initial denaturation step of 5 min at 95°C, followed by 38 cycles of 45 seconds at 95°C, 30 seconds at 60°C, 1 min at 72°C and a final extension step of 5 min at 72°C. The number of repeats for each sample was determined by size fractionating the exon 3 PCR products on a 2% agarose gel. The 100-bp DNA ladder (Fermentas UAB, Vilnius, Lithuania) was used for size determination of PCR products. For three mothers, genotyping of *DRD4* was not successful. The main genotypes in the sample (2/4, 4/4, 4/7 and 7/7) were in Hardy–Weinberg equilibrium, χ^2 (3, $n = 140$) = 3.07, $P = 0.38$. Sixty mothers carried at least one *DRD4* 7R allele. The presence of *DRD4* 7repeat alleles was independent of *COMT* genotype, χ^2 (2, $n = 173$) = 3.58, $P = 0.17$.

Results

Bivariate correlations

In Table 1, the means, SDs and bivariate correlations for the main variables are presented. Mothers with a higher educational level appeared significantly more sensitive to their children, but educational level was not associated with daily hassles. Higher scores on parental sensitivity were associated with fewer daily hassles. The presence of the *DRD4*-7R allele was not associated with any of the main variables (computed as point biserial correlation), whereas the number of met alleles of *COMT* showed a significant association with daily hassles: mothers with val/met or met/met reported more daily hassles (see Table 1).

Multivariate regressions predicting maternal sensitivity

In Table 2, the results of the regression analysis on maternal sensitivity are presented. In the first step, child age and maternal educational level were entered. In the second step, daily hassles, *DRD4* (*DRD4*-7R or no *DRD4*-7R) and *COMT* (val/val, val/met or met/met) were entered. The predictors of the second step added significantly to the explained variance of maternal sensitivity, $F_{\text{change}}(3, 167) = 2.79$, $P = 0.04$. In the third step, the two-way interactions between *DRD4*, *COMT* and hassles (centered) were entered (Aiken & West 1991). The three two-way interactions did not contribute significantly to the prediction, $F_{\text{change}}(3, 164) = 1.60$, $P = 0.19$. In the final step, the three-way interaction of hassles, *DRD4* and *COMT* was entered. The interaction term contributed significantly to the equation, $F_{\text{change}}(1, 163) = 4.27$, $P = 0.04$.

The interpretation of the three-way interaction was facilitated by predicting maternal sensitivity from daily hassles, controlling for child age and maternal educational level, for separate groups with the various combinations of *DRD4* and *COMT*. In the subgroup of parents with a combination of *COMT* val/val or val/met *and* the *DRD4*-7R allele ($n = 40$), a strong association between hassles and maternal sensitivity was found: more daily hassles were associated with less sensitivity of the mothers to their child, and lower levels of daily hassles were associated with more sensitivity, $\beta = -0.49$, $P < 0.01$. The effect size for this association was

Table 1: Means, SDs and correlations for the main variables

	Mean	SD	Education	Sensitivity	Hassles	DRD4-7R†	COMT‡
Child age (months)	23.41	10.08	−0.10	0.12	−0.06	−0.13	0.11
Maternal education	3.68	1.04		0.37**	0.00	0.13	−0.08
Maternal sensitivity	0.12	2.26			−0.16*	0.09	0.07
Hassles	20.72	12.65				0.05	0.17*
DRD4-7R							0.01

*$P < 0.01$.

**$P < 0.05$.

†$n = 173$, 0 = DRD4-7R absent, 1 = DRD4-7R present.

‡0 = val/val, 1 = val/met, 2 = met/met.

Table 2: Regression analysis predicting maternal sensitivity from DRD4, COMT and daily hassles, controlling for child age and maternal education

	B	SE	β	*t*	*P*	R^2	*F*
Step 1[†]						0.16	16.57**
Age child	0.03	0.02	0.13	1.86	0.07		
Education mother	0.82	0.15	0.38	5.47	<0.01		
Step 2						0.20	8.51**
Hassles	−0.03	0.01	−0.18	−2.49	0.01		
DRD4-7R[‡]	0.15	0.17	0.06	0.88	0.38		
COMT[§]	0.39	0.24	0.12	1.64	0.10		
Step 3						0.23	5.97**
DRD4-7R × COMT	−0.11	0.24	−0.03	−0.47	0.64		
DRD4-7R × hassles	−0.02	0.01	−0.10	−1.27	0.17		
COMT × hassles	0.03	0.02	0.12	1.76	0.08		
Step 4						0.25	5.89**
DRD4-7R × COMT × hassles	0.04	0.02	0.15	2.07	0.04		

$n = 173$.

$^{**}P < 0.01$.

[†]The statistics are derived from the final block of the regression model.

[‡]0 = DRD4-7R absent, 1 = DRD4-7R present.

[§]0 = val/val, 1 = val/met, 2 = met/met.

$d = 1.12$, which is large. The association was not significant for any of the other groups (see Fig. 1): in the subgroup with *COMT* met/met and the shorter variants of *DRD4* ($n = 28$), the association between hassles and sensitivity (controlling for child age and maternal educational level) was $\beta = -0.01$ ($P = 0.97$); in the subgroup with *COMT* met/met and the *DRD4*-7R allele ($n = 20$), $\beta = 0.16$ ($P = 0.41$) and in the subgroup with *COMT* val/val or val/met and the shorter variants of *DRD4* ($n = 85$), $\beta = -0.13$ ($P = 0.23$). The association between daily hassles and maternal sensitivity (controlling for child age and maternal educational level) in the group of parents with *COMT* val *and DRD4*-7R was significantly different from this association in each of the other groups, with values for z_{diff} ranging from 2.03 to 2.38 ($0.01 < P < 0.04$). Parents with a combination of *COMT* val and the *DRD4*-7R allele were more sensitive when experiencing fewer hassles and less sensitive when experiencing more hassles.

Discussion

Daily hassles lead to less sensitive parenting, depending on the genetic makeup of the parents involved. We found that parents display less sensitive behavior to their children's attachment needs when they have to deal with more daily hassles, but only in the group of parents who have a *DRD4*-7R as well as a *COMT* val allele. At the same time, in the case of fewer daily hassles, this group shows *higher* levels of sensitive parenting compared with other parents. Both the *DRD4*-7R allele and the *COMT* val allele are associated with a less effectively functioning dopaminergic system. In the other groups of parents, the association between sensitive parenting and hassles is absent. Thus, the combination of *COMT* val and *DRD4*-7R appears to imply increased susceptibility to daily stresses in parents, for the better and the worse (Belsky 1997; Belsky *et al.* in press), leading to more sensitive interaction with their toddlers when they experience relatively few hassles and lower sensitivity when they are faced with more hassles. The fact that sensitive parenting is a well–documented, crucial determinant of young children's socio-emotional development (Cassidy & Shaver 1999) adds to the relevance of the current findings.

Daily hassles have been conceptualized as the irritating and annoying demands that to some degree are involved in any everyday transaction with the environment (Kanner *et al.* 1981). Kanner *et al.* (1981) and Lazarus (1984) found that the accumulation of relatively minor daily hassles as appraised by individuals may have greater impact on their sense of competence, well being and somatic health than major life events. Daily hassles interfere with parents' sensitivity to their children (Crnic & Low 2002) – although not in all parents. Some parents are more vulnerable to daily hassles than others, but the causes of this differential susceptibility are unknown (Phelps *et al.* 1998). Here we present the first molecular genetic study addressing this issue. We found 9% of the variance in parental sensitivity explained by *COMT*, *DRD4* and daily hassles. Of this variance, 2% was explained by the interaction between *COMT*, *DRD4* and daily hassles (G × G × E). Evans (1985) states that even moderator effects explaining as little as 1% of the total variance should be considered important.

The greater vulnerability of the *DRD4*-7R/*COMT* val group for negative effects of daily hassles on parental sensitivity seems to fit into the so-called diathesis-stress model (Clark *et al.* 1992; Phelps *et al.* 1998). In this model, negative

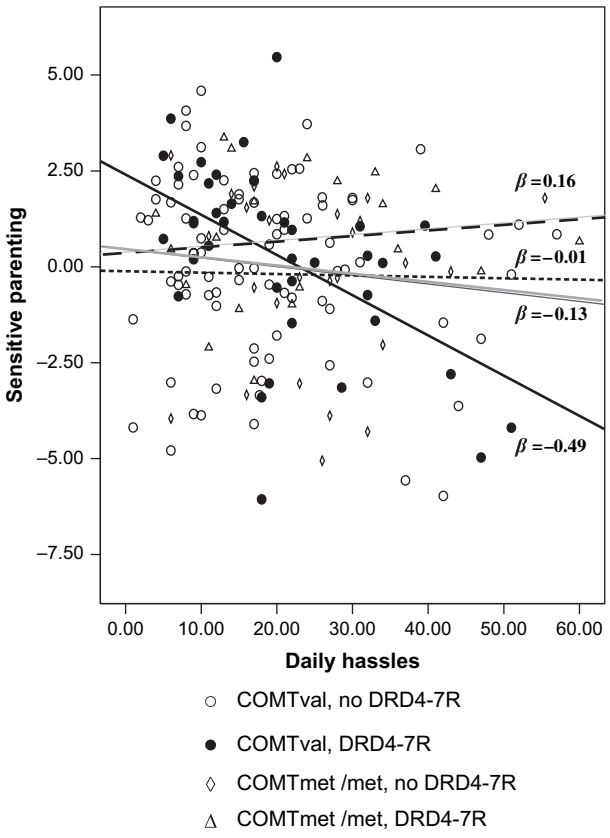

Figure 1: Association between daily hassles and sensitive parenting controlling for child age and maternal educational level. The graph shows a strong negative association between hassles and maternal sensitivity for parents with COMT val/val or val/met and the DRD4-7R allele ($\beta = -0.49$, $P < 0.01$) and no significant associations for parents with COMT val/val or val/met without DRD4-7R ($\beta = -0.13$, $P = 0.23$), for parents with COMT met/met and the DRD4-7R allele ($\beta = 0.16$, $P = 0.41$) and for parents with COMT met/met without DRD4-7R ($\beta = -0.01$, $P = 0.97$).

outcomes such as insensitive parenting emerge when an underlying vulnerability or diathesis is activated by environmental stressors; without stressful environment, the diathesis remains ineffective and the negative consequences absent. Originally, the diathesis was understood to be some traumatic event or experience with harsh or abusive parenting in childhood, but the model can easily be extended in the direction of biological or genetic vulnerabilities (Belsky 1997, 2005; Boyce & Ellis 2005a,b; Meehl 1962; Paris 2000) related to neurotransmitter systems such as the dopaminergic system.

From inspection of the scatterplot of the G × G × E interaction effect (see Fig. 1), however, it appears that the interaction term requires an explanation that is not covered by the stress-vulnerability model alone. The interaction term between *DRD4, COMT* and daily hassles appears to consist of two components. The first has already been discussed and relates to parents with the less efficient allele combination being more affected by the stress of daily hassles. The second component pertains to parents with the same genetic combination who are more sensitive to their children than comparison groups when daily hassles are relatively low. The interaction effect thus points to 'differential susceptibility' (Belsky 1997; Belsky *et al.* in press) instead of a stress-vulnerability model in that the genetic 'risk' combination may confer greater reactivity to the environment, a reactivity that can be either positive or negative, depending on environmental conditions. Conversely, the combination of 'non-risk' polymorphisms may be less responsive both to environmental stressors in the form of high hassles and to environmental support in the form of low hassles, so that the expected relation between hassles and parenting is absent in this group. (We thank one of the anonymous reviewers who suggested this interpretation.)

Animal research suggests that the *DRD4* receptor plays an important role in the dopaminergic modulation of the perception of stimuli (Falzone et al. 2002). Biochemical studies showed that the shorter exon III repeats code for a more efficient gene in terms of transcription, translation and second messenger generation compared with the *DRD4*-7R (Ebstein 2006). Seeger *et al.* (2004) suggest that the *DRD4*-7R allele is associated with a reward deficiency syndrome that may lead to sensation and novelty seeking as well as impulsivity. The short variants of *DRD4* are related to rigidity and inhibition (Ebstein 2006; Ebstein *et al.* 2002) or, to put it more moderately, to less distraction by irrelevant stimuli. Parenting in mildly stressful environments might be more effective if interference and distraction by child-independent stimuli from daily hassles is blocked or at least decreased.

Catechol-O-methyltransferase affects prefrontal functioning and working memory and has also been associated with emotional dysregulation. Greater connectivity of the amygdala and the hippocampus with the orbitofrontal cortex has been shown for met/met subjects (Drabant *et al.* 2006). This enhanced connectivity is associated with low levels of novelty seeking, with some inflexibility in processing affective information and with (affective) perseverance. Here we suggest that in normally functioning individuals with the *COMT* met/met genotype, the predisposition to focus their attention on relevant stimuli and to inhibit interference from other stimuli (Bilder *et al.* 2004; Drabant *et al.* 2006) may help to keep their attention focused on the child's attachment signals and needs in a mildly stressful context of daily hassles. At the same time, more *COMT* met alleles were also associated with the parent's report of more daily hassles. Thus, the finding that in the groups with *COMT* met the influence of hassles on parenting was absent cannot be ascribed to their experiencing fewer hassles. It also points to the possibility that *COMT* met may not always be functioning as a protective factor and *COMT* val as a risk or vulnerability factor (Drabant *et al.* 2006).

It should be noted that *DRD4* and *COMT* did not predict parental sensitivity separately but only in interaction. In his seminal review on the molecular genetic architecture of human personality, Ebstein (2006) proposed to go beyond single-gene association studies of personality and to consider various combinations of genetic polymorphisms (e.g. see Ebstein 2006). Here we focused on two genes implicated in

the dopaminergic system, rather than an exclusive focus on only one dopamine-related gene that in itself appeared to be unrelated to parenting (Gervai *et al.* 2007). Ebstein (2006) also argued that molecular genetic studies should go beyond the self-report of personality characteristics and that a focus on observed or experimentally manipulated features of a person's functioning is necessary to decrease error variance and to enhance the validity and precision of the phenotypic measures. In the current study, we addressed the question whether *observed* differences in sensitive parenting were related to genetic differences in situations of daily stress. Although we were not able to observe the daily hassles in the lives of the parents and had to rely on their own reports, we confirmed Ebstein's (2006) proposition that the environment interacting with genetic makeup should be taken into account when explaining human (parenting) behavior (e.g. Bakermans-Kranenburg & Van IJzendoorn 2007; Caspi & Moffitt 2006; Caspi *et al.* 2002; Rutter 2006). It should be noted that according to Lazarus (1984), the subjective appraisal of daily hassles is more important than the objective count of daily strains and stresses for the way in which hassles impact on the individual.

Increasingly, the study of gene–environment interaction, the interplay between 'nature' and 'nurture' deriving from genetic effects on susceptibility to environmental risks has been moving to the forefront (e.g. Bennett *et al.* 2002; Caspi *et al.* 2002, 2003; Fox *et al.* 2005; Kaufman *et al.* 2004). As shown by Suomi (1999) for primates and by Caspi *et al.* (2002, 2003) for humans, an individual's response to environmental influences may be moderated by his or her genetic makeup. In our own series of studies on children, a moderating role of the *DRD4* gene was found. Maternal unresolved loss or trauma was associated with infant attachment disorganization, but only for children with the *DRD4*-7R allele (Van IJzendoorn & Bakermans-Kranenburg 2006). In a related study on toddlers' externalizing behavior, maternal insensitivity was associated with externalizing (oppositional or aggressive) behaviors, but only in the presence of the *DRD4*-7R polymorphism in the child (Bakermans-Kranenburg & Van IJzendoorn 2006). In the current study, we focused on parents and found a similarly important role for the parental *DRD4* gene, in combination with *COMT*, also pointing at the greater susceptibility for environmental stresses of carriers of the *DRD4*-7R variant.

Our findings on the role of *DRD4* and *COMT* should be independently replicated. Multiple gene effects are based on an increasingly large number of statistical tests and are thus liable to false positives (Ebstein *et al.* 2002). Moreover, the number of participants with particular genotype combinations and the predicted phenotype becomes increasingly small (Van Gestel & Van Broeckhoven 2003). In our study, the risk of capitalization on chance was limited because we included only two genes, and the smallest subgroup for the combination of polymorphisms of the two genes was $n = 20$. Furthermore, we enhanced the power to find replicable outcomes by careful observational assessment of parenting, and we included assessment of the (risk) environment (Caspi *et al.* 2003; Rutter 2006; Rutter *et al.* 2006) with a validated standard questionnaire. Better measurement of the environment is, however, crucial (Luan *et al.* 2001; Rutter 2006; Wong *et al.* 2003), and in future studies our self-report assessment of daily hassles may be replaced by observational or even psychophysiological measures. Finally, the families in the current study had an externalizing toddler (75th percentile or above on the CBCL). Therefore, our findings may only apply to parents who perceive their children as difficult and non-compliant and who already at an early stage have difficulty managing their children. It is important to note, however, that the families in our study were two-parent families from predominantly middle-class background and without psychiatric disorders. The level of their daily hassles was similar to a non-select community sample of parents with 4 year olds. Nevertheless, replication in unselected samples is needed.

To our knowledge, the current study is the first to find a molecular genetic basis of observed human parenting, although parenting has been the subject of numerous studies in the past decades, resulting in the multivolume Handbook of Parenting (Bornstein 2002) and several specialized journals (e.g. Parenting). Because of the intriguing findings on the molecular genetic basis of parenting in studies on rodents (e.g. Meaney 2007; Meaney & Szyf 2005) and on non-human primates (e.g. Suomi 1999), it is time to address similar issues in the parenting of human offspring. Sensitive parenting is a complex activity in which affective information must be perceived and processed and a course of action chosen from a variety of competing activities. In the somewhat demanding and mildly stressful conditions of daily life, the ability to remain focused on the child's signals and needs seems crucial for sensitive parenting. We showed that dopamine-related genetic differences may play a role in sensitive parenting. Molecular genetic studies promise to elucidate differential susceptibility of parents to environmental stressors in taking sensitive care of their children.

References

Aiken, L.S. & West, S.G. (1991) *Multiple Regression: Testing and Interpreting Interactions*. Sage, Newbury Park, CA.

Ainsworth, M.D.S., Blehar, M.C., Waters, E. & Wall, S. (1978) *Patterns of Attachment. A Psychological Study of the Strange Situation*. Lawrence Erlbaum, Hillsdale, NJ.

Bakermans-Kranenburg, M.J. & Van IJzendoorn, M.H. (2006) Gene-environment interaction of the dopamine D4 receptor (*DRD4*) and observed maternal insensitivity predicting externalizing behavior in preschoolers. *Dev Psychobiol* **48**, 406–409.

Bakermans-Kranenburg, M.J. & Van IJzendoorn, M.H. (2007) Genetic vulnerability or differential susceptibility in child development: the case of attachment. *J Child Psychol Psychiatry*, doi: 10.1111/j.1469-7610.2007.01801.x

Bakermans-Kranenburg, M.J., Van IJzendoorn, M.H. & Kroonenberg, P.M. (2004) Differences in attachment security between African-American and white children: ethnicity or socio-economic status? *Infant Behav Dev* **27**, 417–433.

Belsky, J. (1997) Theory testing, effect-size evaluation, and differential susceptibility to rearing influence: the case of mothering and attachment. *Child Dev* **64**, 598–600.

Belsky, J. (2005) Differential susceptibility to rearing influence: an evolutionary hypothesis and some evidence. In Ellis, B. & Bjorklund, D. (eds), *Origins of the Social Mind: Evolutionary Psychology and Child Development*. Guildford, New York, pp. 139–163.

Belsky, J., Crnic, K. & Woodworth, S. (1995) Personality and parenting: exploring the mediating role of transient mood and daily hassles. *J Pers* **63**, 905–929.

Belsky, J., Woodworth, S. & Crnic, K. (1996a) Trouble in the second year: three questions about family interaction. *Child Dev* **67**, 556–578.

Belsky, J., Woodworth, S. & Crnic, K. (1996b) Troubled family interaction during childhood. *Dev Psychopathol* **8**, 477–495.

Belsky, J., Bakermans-Kranenburg, M. J. & Van IJzendoorn, M.H. (in press) for better and for worse: Differential susceptibility to environmental influences. *Current Directions in Psychological Science*.

Bennett, A.J., Lesch, K.P., Heils, A., Long, J.G., Lorenz, J.G., Shoaf, S.E., Champoux, M., Suomi, S.J., Linnoila, M.V. & Higley, J.D. (2002) Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Mol Psychiatry* **7**, 118–122.

Bilder, R.M., Volavka, J., Lachman, H.M. & Grace, A.A. (2004) The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology* **29**, 1943–1961.

Bornstein, M. (ed) (2002) *Handbook of Parenting*, 2nd edn. Lawrence Erlbaum, Mahwah, NJ.

Boyce, W.T. & Ellis, B.J. (2005a) Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev Psychopathol* **17**, 271–301.

Boyce, W.T. & Ellis, B.J. (2005b) Biological sensitivity to context: II. Empirical explorations of an evolutionary-developmental theory. *Dev Psychopathol* **17**, 303–328.

Caspi, A. & Moffitt, T.E. (2006) Opinion – gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* **7**, 583–590.

Caspi, A., McClay, J., Moffitt, T., Mill, J., Martin, J., Craig, I., Taylor, A. & Poulton, R. (2002) Role of genotype in the cycle of violence in maltreated children. *Science* **297**, 851–854.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A. & Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389.

Cassidy, J. & Shaver, P.R. (eds) (1999) *Handbook of Attachment: Theory, Research, and Clinical Applications*. The Guilford Press, New York.

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., Egan, M.F., Kleinman, J.E. & Weinberger, D.R. (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (*COMT*): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* **75**, 807–821.

Clark, D.A., Beck, A.T. & Brown, G.K. (1992) Sociotropy, autonomy, and life event perceptions in dysphoric and nondysphoric individuals. *Cogn Ther Res* **16**, 635–652.

Crnic, K. & Low, C. (2002) Everyday stresses and parenting. In Bornstein, M. (ed), *Handbook of Parenting: Practical Issues in Parenting*, 2nd edn, **Vol. 5**, Lawrence Erlbaum, Mahwah, pp. 243–267.

De Wolff, M.S. & Van IJzendoorn, M.H. (1997) Sensitivity and attachment: a meta-analysis on parental antecedents of infant attachment. *Child Dev* **68**, 571–591.

Drabant, E.M., Hariri, A.R., Meyer-Lindenberg, A., Munoz, K.E., Mattay, V.S., Kolachana, B.S., Egan, M.F. & Weinberger, D.R. (2006) Catechol O-methyltransferase val(158)met genotype and neural mechanisms related to affective arousal and regulation. *Arch Gen Psychiatry* **63**, 1396–1406.

Ebstein, R.P. (2006) The molecular genetic architecture of human personality: beyond self-report questionnaires. *Mol Psychiatry* **11**, 427–445.

Ebstein, R.P., Benjamin, J. & Belmaker, R.H. (2002) Behavioral genetics, genomics, and personality. In Plomin, R., DeFries, J.C., Craig, I.W. & McGuffin, P. (eds), *Behavioral Genetics in the Postgenomic Era*. American Psychological Association, Washington, pp. 365–388.

Eisenberg, J., Mei-Tal, G., Steinberg, A., Tartakovsky, E., Zohar, A., Gritsenko, I., Nemanov, L. & Ebstein, R.P. (1999) Haplotype relative risk study of catechol-O-methyltransferase (*COMT*) and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity val allele with ADHD impulsive-hyperactive phenotype. *Am J Med Genet* **88**, 497–502.

Evans, M.G. (1985) A Monte Carlo study of the effects of correlated method variance in moderated multiple regression analysis. *Organ Behav Hum Decis Process* **36**, 305–323.

Falzone, T.L., Gelman, D.M., Young, J.I., Grandy, D.K., Low, M.J. & Rubinstein, M. (2002) Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. *Eur J Neurosci* **15**, 158–164.

Fox, N.A., Nichols, K.E., Henderson, H.A., Rubin, K., Schmidt, L., Hamer, D., Ernst, M. & Pine, D. S. (2005) Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychol Sci* **16**, 921–926.

Gervai, J., Nemoda, Z., Lakatos, K., Ronai, Z., Toth, I., Ney, K. & Sasvari-Szekely, M. (2005) Transmission disequilibrium tests confirm the link between DRD4 gene polymorphism and infant attachment. *Am J Med Genet B Neuropsychiatr Genet* **132B**, 126–130.

Gervai, J., Novak, A., Lakatos, K., Toth, I., Donis, I., Ronai, Z., Nemoda, Z., Sasvari-Szekely, M, Bureau, J.-F., Bronfman, E. & Lyons-Ruth, K. (2007) Infant genotype may moderate sensitivity to maternal affective communications: attachment disorganization, quality of care, and the *DRD4* polymorphism. *Soc Neurosci* **2**, 307–319.

Hoffman, C., Crnic, K.A. & Baker, J.K. (2006) Maternal depression and parenting: implications for children's emergent emotion regulation and behavioral functioning. *Parent Sci Pract* **6**, 271–295.

Kanner, A.D., Coyne, J.C., Schaffer, C. & Lazarus, R.S. (1981) Comparison of two modes of stress measurement: daily hassles and uplifts versus major life events. *J Behav Med* **4**, 1–39.

Kaufman, J., Yang, B.Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J.H. & Gelernter, J. (2004) Social supports and serotonine transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci U S A* **101**, 17316–17321.

Lakatos, K., Toth, I., Nemoda, Z., Ney, K., Sasvari-Szekely, M. & Gervai, J. (2000) Dopamine D4 receptor (*DRD4*) gene polymorphism is associated with attachment disorganization in infants. *Mol Psychiatry* **5**, 633–637.

Lakatos, K., Nemoda, Z., Toth, I., Ronai, Z., Ney, K., Sasvari-Szekely, M. & Gervai, J. (2002) Further evidence for the role of the dopamine D4 receptor (*DRD4*) gene in attachment disorganization: interaction of the exon III 48-bp repeat and the 521 C/T promoter polymorphisms. *Mol Psychiatry* **7**, 27–31.

Lakatos, K., Toth, I., Danis, I., Nemoda, Z. & Gervai, J. (2005) Infant attachment disorganization: genetic and environmental contributions. *J Psychophysiol* **19**, 129.

Lazarus R.S. (1984) Puzzles in the study of daily hassles. *J Behav Med* **7**, 375–389.

Lichter, J.B., Barr, C.L., Kennedy, J.L., Vantol, H.H.M., Kidd, K.K. & Livak, K.J. (1993) A hypervariable segment in the human dopamine receptor D(4) (Drd4) gene. *Hum Mol Genet* **2**, 767–773.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I. & Taskinen, J. (1995) Kinetics of human soluble and membrane-bound catechol O-methyltransferase – a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* **34**, 4202–4210.

Luan, J.A., Wong, M.Y., Day, N.E. & Wareham, N.J. (2001) Sample size determination for studies of gene-environment interaction. *Int J Epidemiol* **30**, 1035–1040.

Lucht, M., Barnow, S., Schroeder, W., Grabe, H.J., Finckh, U., John, U., Freyberger, H.J. & Henrmam, F.H. (2006) Negative perceived paternal parenting is associated with dopamine D-2 receptor exon 8 and GABA(A) alpha 6 receptor variants: an explorative study. *Am J Med Genet B Neuropsychiatr Genet* **141B**, 167–172.

Meaney, M.J. (2001) Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci* **24**, 1161–1192.

Meaney, M.J. (2007) *Maternal regulation of individual differences in reproductive behavior in the female offspring*. Paper presented at the biennial meeting of the Society for Research in Child Development, March 29-April 1, Boston (MA), USA.

Meaney, M.J. & Szyf, M. (2005) Maternal care as a model for experience-dependent chromatin plasticity? *Trends Neurosci* **28**, 456–463.

Meehl, P.E. (1962) Schizotaxia, schizotypy, schizophrenia. *Am Psychol* **17**, 827–838.

Pannebakker, F. D. (2007) *Morality from infancy to middle childhood*. Unpublished PhD dissertation, Leiden University, Leiden, The Netherlands.
Paris, J. (2000) Predispositions, personality traits, and posttraumatic stress disorder. *Harv Rev Psychiatry* **8**, 175–183.
Phelps, J.L., Belsky, J. & Crnic, K. (1998) Earned security, daily stress, and parenting: a comparison of five alternative models. *Dev Psychopathol* **10**, 21–38.
Repetti, R. & Wood, J. (1997) Effects of daily stress at work on mothers' interactions with preschoolers. *J Fam Psychol* **11**, 90–108.
Robbins, T.W. & Everitt, B.J. (1999) Motivation and reward. In Zigmond, M.J. (ed.), *Fundamental Neuroscience*. Academic Press, San Diego, CA, pp. 1246–1260.
Rutter, M. (2006) *Genes and Behavior. Nature-Nurture Interplay Explained*. Blackwell, Oxford.
Rutter, M., Moffitt, T.E. & Caspi, A. (2006) Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry* **47**, 226–261.
Savitz, J., Solms, M. & Ramesar R. (2006) The molecular genetics of cognition: dopamine, *COMT* and BDNF. *Genes Brain Behav* **5**, 311–328.
Seeger, G., Schloss, P., Schmidt, M.H., Rüter-Jungfleisch, A. & Henn, F.A. (2004) Gene-environment interaction in hyperkinetic conduct disorder (HD + CD) as indicated by season of birth variations in dopamine receptor (*DRD4*) gene polymorphism. *Neurosci Lett* **366**, 282–286.
Smolka, M.N., Schumann, G., Wrase, J., Grusser, S.M., Flor, H., Mann, K., Braus, D.F., Goldman, D., Buchel, C. & Heinz, A. (2005) Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *J Neurosci* **25**, 836–842.
Suomi, S.J. (1999) Attachment in rhesus monkeys. In Cassidy, J. & Shaver, P.R. (eds), *Handbook of Attachment: Theory, Research, and Clinical Applications*. The Guilford Press, New York, pp. 181–197.
Swain, J.E., Lorberbaum, J.P., Kose, S. & Strathearn, L. (2007) Brain basis of early parent-infant interactions: psychology, physiology, and in vivo functional neuroimaging studies. *J Child Psychol Psychiatry* **48**, 262–287.
Swanson, J.M., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G.A., Volkow, N., Taylor, E., Casey, B.J., Castellanos, F.X. & Wadwa, P.D. (2007) Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev* **17**, 39–59.
Van Gestel, S. & Van Broeckhoven, C. (2003) Genetics of personality: are we making progress? *Mol Psychiatry* **8**, 840–852.
Van IJzendoorn, M.H. & Bakermans-Kranenburg, M.J. (2006) *DRD4* 7-repeat polymorphism moderates the association between maternal unresolved loss or trauma and infant disorganization. *Attach Hum Dev* **8**, 291–307.
Van Zeijl, J., Mesman, J., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Juffer, F., Stolk, M.N., Koot, H.M. & Alink, L.R.A. (2006) Attachment-based intervention for enhancing sensitive discipline in mothers of 1- to 3-year-old children at risk for externalizing behavior problems: a randomized controlled trial. *J Consult Clin Psychol* **74**, 994–1005.
Wong, M.Y., Day, N.E., Luan, J.A., Chan, K.P. & Wareham, N.J. (2003) The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *Int J Epidemiol* **32**, 51–57.

Acknowledgments

This study is part of the research project SCRIPT, conducted at the Centre for Child and Family Studies, Leiden University, the Netherlands. The study is supported by grant 2200.0097 from ZonMw (Netherlands Organization for Health Research and Development) to M.H. van IJ. and Femmie Juffer. Support from the Netherlands Organization for Scientific Research NWO (VENI, VIDI and SPINOZA Prize) to J.M., M.J.B.-K. and M.H. van IJ. respectively, is also gratefully acknowledged. We thank Drs Jantien van Zeijl, Mirjam Stolk, Lenneke Alink, Femke Pijlman, Femmie Juffer and Hans Koot for their contributions to the various parts of the study and Base-Clear for the genotyping. Last but not least, we thank the parents and children who participated in our study as well as the students who assisted in various phases of the SCRIPT project.