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Dopaminergic, Serotonergic, and Oxytonergic Candidate Genes Associated with Infant Attachment Security and Disorganization? In Search of Main and Interaction Effects

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Abstract

Background and methods—In two birth cohort studies with genetic, sensitive parenting, and attachment data of more than 1,000 infants in total, we tested main and interaction effects of candidate genes involved in the dopamine, serotonin, and oxytocin systems (DRD4, DRD2, COMT, 5-HTT, OXTR) on attachment security and disorganization. Parenting was assessed using observational rating scales for parental sensitivity (Ainsworth, Bell, & Stayton, 1974), and infant attachment was assessed with the Strange Situation Procedure.

Results—We found no consistent additive genetic associations for attachment security and attachment disorganization. However, specific tests revealed evidence for a co-dominant risk model for COMT Val158Met, consistent across both samples. Children with the Val/Met

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genotype showed higher disorganization scores (combined effect size $d = 0.22$, $CI = 0.10; 0.34$, $p < .001$). Gene-by-environment interaction effects were not replicable across the two samples.

Conclusions—This unexpected finding might be explained by a broader range of plasticity in heterozygotes, which may increase susceptibility to environmental influences or to dysregulation of emotional arousal. This study is unique in combining the two largest attachment cohorts with molecular genetic and observed rearing environment data to date.

Keywords

attachment; Strange Situation Procedure; candidate genes; parenting; sensitivity; GxE

Attachment is defined as the child's need to seek proximity to a favorite, protective caregiver in times of stress (e.g., illness, danger) and to derive comfort from the attachment figure in stressful settings (Cassidy, 2008). Insecure and especially disorganized attachments elevate risk for psychopathology in adolescence and adulthood (Sroufe, Egeland, Carlson, & Collins, 2005). Formation of an attachment relationship, considered essential for offspring survival (Bowlby, 1969/1982; Suomi, 2008), has been found to be influenced by the interactive history of an infant and its caregiver, in particular sensitive parenting, and, to a lesser extent, socio-demographic factors and psychosocial characteristics of the parents (Belsky & Fearon, 2008). An emphasis on environmental origins of attachment-related individual differences is consistent with behavior-genetic studies of twins that estimated the contribution of genetic factors to attachment security and disorganization to be negligible (Bokhorst et al., 2003; O'Connor & Croft, 2001; Roisman & Fraley, 2008).

Although behavioral genetic studies have found main effects on attachment security to be elusive, there are at least two reasons to believe that genetic differences might play a modest role in the formation of attachments. First, parental sensitivity only explains a small part of the total variation in infant attachment security (Bakermans-Kranenburg, Van IJzendoorn, & Juffer, 2003; De Wolff & Van IJzendoorn, 1997). Because parents' representations of their own childhood attachment experiences were found to be rather strongly associated with infant attachment without an equally strong mediating mechanism of parental behavior, an intergenerational transmission gap has been proposed for attachment security as well as for attachment disorganization (Belsky, 2005; Madigan et al., 2006; Van IJzendoorn, 1995). One way of bridging the transmission gap would be through genetic mechanisms (Belsky, 2009; Bokhorst et al., 2003; Main, 1999). Second, frequently cited work by Lakatos and colleagues (Lakatos et al., 2000) a decade ago presented evidence of a genetic main effect on disorganized attachment involving a 48 base pair variable number tandem repeat (VNTR) in the promoter region of the Dopamine D4 receptor gene (DRD4). In a homogeneous sample of 90 low-risk Caucasian children, the 7-repeat allele was associated with higher risk for disorganized attachment. These results stimulated several replication efforts in rather small samples (Bakermans-Kranenburg & Van IJzendoorn, 2004; Spangler, Johann, Ronai, & Zimmermann, 2009), and overall the evidence of a direct association between DRD4 and disorganized attachment did not seem convincing (Bakermans-Kranenburg & Van IJzendoorn, 2007). Larger samples are required to settle the issue of genetic influences on attachment security and disorganization.

In two large cohorts of infants, we assessed the 'usual genetic suspects' in the domain of social-emotional development (Ebstein, 2006), most of which have already been examined in previous attachment studies. Polymorphisms in the dopaminergic, serotonergic, and oxytonergic systems were selected to explore whether these are associated with the quality of infants' attachment behavior. The dopaminergic system is involved in attentional, motivational, and reward mechanisms (Robbins & Everitt, 1999). Common variations in dopaminergic genes DRD4 48 bp VNTR, DRD2/ANKK1 and COMT Val158Met are

associated with regulation of dopamine levels (D'Souza & Craig, 2006). Behaviorally, carrying the minor allele of these polymorphisms (respectively, DRD4 48 bp 7-repeat; DRD2/ANKK1 T[A1]; COMT rs4680 G [val]) has been related to variations in infant temperament (Ebstein, 2006) and ADHD (Faraone & Khan, 2006). Although temperament has not been found to be related to attachment security per se it might be implicated in children's behavior in the Strange Situation procedure to assess attachment security (Vaughn, Bost, & Van IJzendoorn, 2008). A protective effect has been reported for COMT heterozygotes (Val/Met) showing dopamine levels associated with optimal neurobehavioral outcomes, compared with both homozygous groups (Wahlstrom, White, & Luciana, 2010). Neonatal neurobehavioral organization as assessed with Brazelton's Neonatal Behavioral Assessment Scale (NBAS) was found related to more secure attachment (Grossmann et al., 1985) and less attachment disorganization (Spangler, Fremmer-Bombik & Grossmann, 1996). The associations between the dopaminergic system and attachment-related phenotypes render the genes involved in the dopaminergic system potential candidates.

The serotonin system is involved in affect and emotion. A 44 bp insertion/deletion segment of the serotonin transporter gene 5-HTT (5-HTTLPR) is associated with less efficient transcription and serotonin uptake in the synapse (Greenberg et al., 1999; Heils et al., 1996), and the short allele is related to psychiatric disorders (Ebstein, 2006; Rutter, 2006). The oxytocinergic system is related to social and parenting behaviors, and both oxytocin levels and polymorphisms in the oxytocin receptor gene (OXTR rs53576 and rs2254298; in particular for the minor A-allele) are associated with the formation of social bonds in both human and animal studies (Bakermans-Kranenburg & Van IJzendoorn, 2008; Carter, Boone, Pournajafi-Nazarloo, & Bales, 2009; Feldman, Gordon, Schneiderman, Weisman, & Zagooory-Sharon, 2010; Insel, 2010). Both 5-HTT and OXTR have been associated with sensitive responsiveness towards infants (Bakermans-Kranenburg & Van IJzendoorn, 2008), which might indicate a role of these genes in attachment-related behavior. Our hypotheses concerning the main effects of the candidate genes involved in the dopamine, serotonin, and oxytocin systems suggest that the minor alleles of the pertinent genetic polymorphisms will elevate the chance for infants to be insecurely attached or to show disorganization of attachment.

However, the most important genetic effects on attachment might be hidden in interaction with environmental factors (Bakermans-Kranenburg & Van IJzendoorn, 2006). A promising avenue for the study of genetic influences on attachment may therefore be the careful assessment of the interplay between genetic differences and child-rearing influences. The most relevant 'candidate environment' in the case of attachment formation is parental sensitivity, which has been documented to be consistently, albeit moderately, associated with attachment security (for correlational and experimental meta-analytic evidence see Bakermans-Kranenburg, et al., 2003; De Wolff & Van IJzendoorn, 1997). Several studies (Barry et al., 2008; Gervai et al., 2007; Spangler, et al., 2009; Van IJzendoorn & Bakermans-Kranenburg, 2006) have presented evidence for interactions between candidate genes (DRD4, 5-HTT) and parental sensitivity on the quality of attachment but samples have been rather small for the purpose of discovering robust gene-environment interactions. Spangler and colleagues (Spangler et al., 2009) reported a combined effect of the short allele of the serotonin transporter gene SLC6A4 (5-HTT) and low maternal sensitivity on attachment disorganization in 96 low-risk Caucasian infants, and Barry, Kochanska, and Philibert (2008) found in their study of 88 typically developing infants that the typical association between maternal responsiveness and security was obtained for carriers of the short allele of the 5-HTT genotype (*ss/sl*), but not for those at low genetic risk for insecurity (i.e., *ll*). These findings call for replication in larger samples.

Replicating genetic analyses across the two largest attachment cohorts to date provides a unique opportunity to test effects of candidate genes involved in the dopamine, serotonin, and oxytocin systems on attachment security and disorganization, as well as the effects of these genes in interaction with parenting quality. As main and interaction effects of genes on developmental outcomes have been found to be rather elusive in many behavioral and medical domains, and findings remain equivocal until replicated in different samples (Rutter, 2006), we here compare the genetic findings derived from two independent studies on attachment and decide a priori to take only those results into account that could be replicated across these two samples. According to the STREGA statement (Little et al., 2009, p. 99), 'In the fast-moving field of genetic association studies, the risk of new methodological pitfalls is high. (...) Generally, the credibility of gene-disease associations is low if the evidence comes from single studies of small scale and cannot be replicated.'. The use of standardized observational assessments of attachment and environment in two independent, well-powered cohorts of Caucasian infants, and the application of state-of-the-art genotyping of specific candidate genes may thus lead to robust findings.

Materials and Methods

Setting

This report is based on two investigations, the Generation R Study, a prospective cohort study investigating development from fetal life into young adulthood in Rotterdam, the Netherlands (see Jaddoe et al., 2007; Jaddoe et al., 2008), and the NICHD Study of Early Child Care and Youth Development (SECCYD), a prospective study carried out in 10 sites in the USA following children from birth to age 17.5 years (NICHD, 2005).

Detailed studies were performed in an ethnically homogeneous sub-sample of children of Dutch national origin from the Generation R Study. These children, their parents and their grandparents were born in the Netherlands, which was a selection criterion in order to reduce the risk of confounding (population stratification) by ethnicity. Detailed measurements of child development were obtained in both studies. The SECCYD followed an ethnically diverse sample, though the focus of the present inquiry was on the sub-set of Caucasian participants. Written informed consent was obtained from parents of all participants in both studies, which were approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam and the Internal Review Boards of the SECCYD participating universities, respectively.

Study Population

In the Generation R study, DNA was collected from cord blood samples at birth. SECCYD DNA was obtained from buccal cheek cells when children were 15 years old. In both studies infants and their parent participated in the Strange Situation Procedure (SSP) at age 15 months. In Generation R, quality of attachment and maternal sensitive parenting was available for 663 parent-child dyads; availability of genotype information ranged from $n = 506$ to $n = 547$ for specific SNPs and VNTRs. In SECCYD, information on attachment and sensitivity was available for 1191 dyads; in the ethnically homogeneous group that was the focus of the current study DNA was available for $n = 478$ to $n = 522$ infants, depending on the specific SNPs and VNTRs. Non-response analysis indicated significant differences between the groups with and without genotypic data in Generation R mainly on perinatal variables. Children without genotypic data had lower gestational age, birth weight and Apgar scores ($ps < .01$). These births may have been more problematic, raising logistical difficulties to sample cord blood for DNA. SECCYD non-response analysis indicated that Caucasians with genotypic and infant attachment data differed from Caucasians lost to follow-up before age 15 years or who did not provide genetic data; those in the current

analysis were more likely to be female ($p < .05$) and have mothers who were somewhat older ($p < .01$) and more educated ($p < .01$) at study onset.

Characteristics of the children and mothers of the current samples are displayed in Table 1. In Generation R, gender was distributed almost evenly: Forty-eight percent of the children were girls. A majority of the children (60%) were firstborn. Birth parameters were normal with a mean gestational age at birth of 40 weeks, an average birth weight of 3547 grams, and 4% of 1 minute APGAR scores below 7. Socio-economic status was high in that 65% of the women were higher educated, i.e. had completed at least 3 years of higher vocational or academic education. During pregnancy, mothers worked for an average of 28 hours per week. Almost 11% continued smoking when the pregnancy was known, and 56% continued drinking (small amounts of) alcohol. Almost all mothers were married or living with a partner (5% were single parents). In the SECCYD, gender was also distributed evenly: Fifty-two percent of the children were females. Forty-eight percent of the children were firstborn. Birth parameters were normal with a mean gestational age at birth of 39 weeks and an average birth weight of 3537 grams. Additionally, 71% of the women were higher educated, operationalized as having at least a high school education at the study onset (participant age 1 month). When participants were age 15 months, mothers worked for an average of 23 hours per week and 7% of the mothers were single parents.

Procedures and Measures

Maternal sensitive responsiveness—In Generation R maternal sensitive responsiveness was observed during two episodes in the 14 months lab visit; a psychophysiological assessment of the child, and a break, using Ainsworth's rating scales for sensitivity (Ainsworth, et al., 1974). We used the sensitivity and cooperation scales, which were aggregated by standardizing the scores on both scales for the separate episodes (psychophysiological assessment and break), and calculating a mean score based on the number of available observations. Cronbach's alpha for the reliability (across scales and episodes) was $\alpha = .75$. The intercoder reliability was $r = .70$ ($n = 82$; intraclass correlation, absolute agreement). Mean duration of the psychophysiological assessment was 12.4 minutes ($SD = 2.9$), mean duration of the break was 4.9 minutes ($SD = 2.2$).

In the NICHD SECCYD mother-child interactions were videotaped during 15-min semi-structured tasks at 6 and 15 months. At both 6 and 15 months, an *a priori* maternal sensitivity composite was constructed by summing ratings for sensitivity to non-distress, positive regard, and intrusiveness (reversed). Internal consistencies of these *a priori* composites were .75 for the 6 months composite, and .70 for the 15 month composite, intercoder reliabilities on scales were $> .80$ (NICHD ECCRN, 1998). Observations of maternal sensitivity from the two time points ($r = .39$, $p < .01$) were standardized and averaged to form a composite for the current analysis. We chose to make optimal use of the diverging sensitivity assessments in both samples in view of the fact that the subjects from both studies were not integrated into one overall sample but were used as independent replications with similar hypotheses and statistical approaches but somewhat varying assessments. If replication can be established with these varying approaches the results might be considered robust.

Strange Situation Procedure—In both studies, mother-infant dyads were observed in the Strange Situation Procedure (SSP, Ainsworth, Blehar, Waters, & Wall, 1978) when the infant was about 15 months old. The SSP is a well-validated, widely used procedure to measure the attachment quality. It consists of seven 3-minute episodes designed to evoke mild stress to trigger attachment behavior (Ainsworth, et al., 1978). Mild stress is evoked by introducing the infant to an unfamiliar lab environment, a female stranger engaging with the

infant, and the parent leaving the room twice for maximal 3 minutes. The infant's behavior upon reunion with the parent is critical for coding attachment behaviors such as proximity and contact seeking, avoidance and resistance. A slightly shortened version of the SSP was used in Generation R. Pre-separation and separation episodes were shortened by one minute each, keeping the critical reunion episodes intact (Luijk et al., 2010).

Attachment behaviors may be categorized as secure (B) or insecure (A, C, D; Main & Solomon, 1990). When stressed, secure (B) infants seek comfort from their mothers, which proves effective, enabling the infant to return to play. Avoidant (A) infants show little overt distress, while turning away from or ignoring mother on reunion. Resistant (C) infants are distressed and angry, but ambivalent about contact, which does not effectively comfort and allow the child to return to play. Examples of disorganized/disoriented (D) behaviors are prolonged stilling, rapid approach-avoidance vacillation, sudden unexplained affect changes, severe distress followed by avoidance, and expressions of fear or disorientation upon return of mother.

Attachment behavior was coded according to established coding systems (Ainsworth, et al., 1978) by two or three highly-trained, reliable coders. Inter-coder agreement was calculated on 70 SSPs in Generation R and 1191 double-coded SSPs in the SECCYD. For ABCD classification, inter-coder agreement was 77% and 83% ($\kappa = .63$ and $.69$); agreement on disorganized versus non-disorganized attachment classification was 87% and 90% ($\kappa = .64$ and $.64$), respectively.

Richters and associates (Richters, Waters, & Vaughn, 1988) developed a method to score attachment in a continuous way. The continuous Attachment Security Scale has been widely used (e.g., Kochanska, Aksan, Knaack, & Rhines, 2004). Van IJzendoorn and Kroonenberg (Van IJzendoorn & Kroonenberg, 1990) adapted and validated the algorithm for use with Strange Situation interactive scales without scores for crying. The resulting algorithm yields a continuous score for attachment that is strongly associated with the insecure vs. secure attachment classifications. Higher security scores indicate a more secure attachment relationship. Continuous scores for disorganization were derived directly from coding the conventional 9-point scale for disorganization (Main & Solomon, 1990), with higher scores indicating more disorganized behavior. Inter-coder reliability (intraclass correlation coefficients [ICC]) for the continuous attachment security and disorganization scales were .88 and .88, respectively, in Generation R ($n = 70$) and were .92 and .84, respectively, in SECCYD ($n = 1191$). It should be noted that the inter-coder reliabilities for attachment classifications were lower (kappas from .63 to .69). We chose to conduct our analyses on the more reliable continuous attachment scores in order to enhance statistical power, and to be less dependent on subtle borderline classification cases which might have lowered somewhat the inter-coder reliabilities of the well-trained coders in our studies. Empirical evidence is emerging that the validity of the continuous scores might at least equal the (predictive) power of the traditional classifications (Fralely & Spieker, 2003).

Genotyping—Genotyping was performed for genes in the dopaminergic system; DRD4 48 bp VNTR, DRD2 (rs1800497), COMT Val158Met (rs4680), the serotonergic system; 5-HTTLPR, and the oxytonergic system; OXTR (rs53576 and rs2254298). See Table 2 for the risk alleles, and Table 3 for a display of minor allele frequencies (MAF). Frequency distributions conformed to the Hardy-Weinberg equilibrium (HWE), except for OXTR rs53576 ($\chi^2 = 4.96$; $p = .03$) in Generation R and DRD4 48 bp VNTR ($\chi^2 = 14.17$; $p < .001$) in SECCYD. An electronic appendix provides detailed information about extraction and genotyping procedures.

Statistical analyses—Preliminary ANOVA and correlational analyses evaluated whether demographic variables were related to genotype and attachment security. Associations between the pertinent gene polymorphisms and attachment security and disorganization were tested using regression analyses applying additive genetic models. In these models, genes are analyzed additively, meaning that participants are viewed as carrying 0, 1 or 2 copies of the minor (often ‘risk’-) allele. For DRD4 48 bp VNTR, DRD2, COMT, 5-HTT VNTR, and OXTR previous studies have suggested increased risk for carriers of the DRD4 48 bp 7-repeat (Ebstein, 2006), the A1 allele of DRD2 (Berman, Ozkaragoz, Young, & Noble, 2002), the short allele of 5-HTT (Lesch et al., 1996; Philibert et al., 2007), the A allele of OXTR (Bakermans-Kranenburg & Van IJzendoorn, 2008), and a beneficial effect for COMT heterozygotes (Wahlstrom, et al., 2010). These models were tested in regression analyses using dichotomous gene risk models. In these risk models, genes are analyzed dichotomously, i.e. carrying vs. not carrying the proposed risk allele. Results for additive and risk models may be different. Interactions between candidate genes and maternal sensitivity were tested in the regression analyses. Maternal sensitivity was centered prior to analyses. There was no reason to assume that SNPs which are not in linkage disequilibrium (LD) can confound each other or affect the $G \times E$ interactions. Furthermore, different number of observations were missing for different genotypes. Thus, we decided to conduct separate regressions for each of the candidate genes instead of including all genes and interactions into one regression equation. Moreover, in an overall regression individual $G \times E$ interactions become difficult to interpret if they would show co-variation with other predictors or interactions. Attachment security and disorganization, as orthogonal constructs (Van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999), were analyzed separately. Assuming a power of .80 and significance level of .05 (2-sided) (using Quanto 1.2.4 software, <http://hydra.usc.edu/GxE>), we were able to detect genetic effects of approximately 1.5% of explained variance in both samples.

Results

Distribution of attachment

Distribution of attachment classifications was as follows in Generation R and SECCYD: 58.2% and 69.8% secure ($n = 323$ and $n = 370$), 17.7% and 15.7% insecure-avoidant ($n = 98$ and $n = 83$), 23.4% and 14.5% insecure-resistant ($n = 130$ and $n = 77$). In Generation R, no classification could be assigned for $n = 4$ (0.7%) children (All SECCYD participants were assigned to their best fitting category). Of all children, 21.8% and 13.4% were classified as disorganized ($n = 121$ and $n = 71$), 78.2% and 83.2% were non-disorganized ($n = 434$ and $n = 441$). SECCYD excluded 18 (3.4%) difficult to classify cases from the ABCD groupings. Mean Attachment Security Scale scores in Generation R and SECCYD were 0.18 ($SD = 2.60$) and 1.21 ($SD = 3.17$); mean disorganization scores were 3.44 ($SD = 1.90$) and 2.39 ($SD = 2.01$). Table 2 presents means and standard deviations of security and disorganization scores for the separate genotypes.

Background variables

Of all background characteristics (see Table 1), in the Generation R sample only breastfeeding at six months was associated with attachment security ($p < .01$), genotype ($p < .05$), and maternal sensitivity ($p < .01$). Children breastfed at six months were more secure, less often carried the minor Val allele of COMT, and had more sensitive mothers. Taking breastfeeding into account as a covariate did not change the Generation R results. None of the demographic variables in Table 1 was simultaneously associated with attachment quality, genotype and maternal sensitivity in the SECCYD. To maximize power we minimized the number of covariates in the analyses and only included covariates correlating with the three main variables.

Additive genetic models

Using an additive genetic model, in both samples none of the genetic associations for attachment security and attachment disorganization reached significance. Carriers of the 5-HTT short allele were more often securely attached, but only in the Generation R sample (Table 3).

Genetic risk models

Table 4 and 5 present the results of regression analyses for dichotomous risk models for DRD2, DRD4 VNTR, COMT, 5-HTT VNTR, and OXTR. DRD4 associations were non-significant. For 5-HTT, short-allele carriers were more often securely attached, but only in Generation R. For COMT, no associations with attachment security emerged. However, COMT heterozygotes were more disorganized in both samples, see Table 5 (combined effect size $d = 0.22$, 95% CI = 0.10; 0.34, $p < .001$). This finding was the only significant result that was replicable across both samples.

Gene x Environment effects

In each of the samples only few significant GxE interactions were found, and they were not consistent across the two samples. Using dichotomous risk models to minimize the number of tests we found a significant interaction between DRD4 and parental sensitivity on attachment security in the SECCYD ($p = .004$) (see Table 4). The interaction implied that the association between sensitivity and security was not significant for carriers of the DRD4 7-repeats whereas those infants without the 7-repeats developed higher levels of security if their mother was more sensitive. In the Generation R sample however the trend was in the opposite direction (see Table 4). The interaction between COMT and parental sensitivity on attachment disorganization in Generation R ($p = .04$) was far from significant in the SECCYD sample ($p = .70$) (see Table 5).

Discussion

In these two large cohort studies, no consistent evidence emerged for additive effects of candidate genes putatively involved in attachment security and disorganization. Thus, the 'usual suspects' (Ebstein, Israel, Chew, Zhong, & Knafo, 2010) in the dopamine, serotonin, and oxytocin systems were not related to attachment quality. Furthermore, proposed risk models for DRD2, DRD4, 5-HTT, and OXTR failed to provide unequivocal results. No effects were found in either study for insecure or disorganized attachment in carriers of the DRD2 minor-T(A1)-allele, DRD4 7-repeat, and A-allele of OXTR. 5-HTT short-allele carriers proved more securely attached in Generation R, but this finding was not replicated in the SECCYD. Previous studies by Gervai and her team (e.g., Lakatos et al., 2000), Spangler and his colleagues (e.g., Spangler et al., 2009), and by Barry, Kochanska, and Philibert (2008) reported genetic main effects and/or interactive effects of genotype and parental sensitive-responsiveness on attachment, but their samples were about four times smaller than each of the current samples. The lack of replication in the two largest attachment samples to date leads us to the conclusion that these earlier studies presented intriguing but insufficiently supported hypotheses.

That said, a co-dominant effect of the COMT Val/Met proved replicable across the studies (a small combined effect of $d = 0.22$). In carriers of the Val/Met genotype, disorganization scores were higher compared to both Val/Val and Met/Met carriers, a disadvantage also referred to as negative heterosis (Comings & MacMurray, 2000). Co-dominant effects for COMT Val/Met have been reported for neurobehavioral functioning (Gosso et al., 2008; Wahlstrom, et al., 2010) and schizophrenia (for a meta-analysis, see Costas et al., 2010). However, these studies showed evidence of *positive* heterosis. Molecular heterosis is

thought to be biologically plausible. Several studies (e.g. Tunbridge, Harrison, & Weinberger, 2006) suggest that there is an inverted U-shape with opposing gene expression occurring in heterozygotes compared to the homozygotes. Furthermore, the range of expression of gene products could be greater in heterozygotes, providing a broader window for plasticity or response to stress (Comings & MacMurray, 2000).

Evidence from this inquiry might suggest the latter. COMT val/met carriers may be more susceptible to environmental influences, which in turn may increase risk for attachment disorganization provided the small effect identified is not a product of Type 1 error. Of course, the increased susceptibility to the environment might also result in effective gene \times environment interactions which we did not find for this genotype. For attachment disorganization we did not assess the most promising candidate environment, i.e. frightening or frightened parenting (Madigan et al., 2006). An additional explanation might be the involvement of COMT Val158Met in regulation of emotional arousal (Drabant et al., 2006), which is considered central to disorganized attachment. Disorganized infants inability to regulate stress and emotions in arousing situations is striking, and their dysregulation is an early predictor of later psychopathology (Fearon, Bakermans-Kranenburg, Van IJzendoorn, Lapsley, & Roisman, 2010; Sroufe, et al., 2005). As this is the first study that reveals a replicated co-dominant effect of COMT on attachment, further studies are needed that investigate the effects of the COMT val/met genotype in combination with challenging environments, and assess outcomes related to the child's plasticity in emotion regulation.

Genetic pathways are frequently indirect and subject to numerous biological and environmental influences (Ebstein, et al., 2010; Kendler, 2005). Several previous attachment G \times E studies have suggested that genetic effects may be contingent upon gene-environment co-action (Gervai, et al., 2007; Spangler, et al., 2009; Van IJzendoorn & Bakermans-Kranenburg, 2006; see also Rutter, 2006). Nevertheless, we did not find G \times E interactions that were replicable across the two samples. Previously reported associations for genes involved in attachment (DRD4, 5-HTT) could not be replicated in the two cohorts. The contrast with previous findings might indicate the importance of large samples to test for reliable G \times E effects, particularly in case of a phenotype that cannot be assessed without some error.

Population stratification, sufficient power and accurate assessment of the phenotype are crucial methodological aspects (Ebstein, 2006; Ioannidis, 2007; Little et al., 2009). High-quality G \times E studies with careful measurement of the environment and the outcome variables are essential, as well as explicit hypotheses about how a *specific* gene and a *specific* environmental condition interact to predict a specific outcome (Bakermans-Kranenburg & Van IJzendoorn, 2010). Here the study populations were selected for Caucasian ethnicity, securing an ethnically homogenous sample that might restrict the generalizability of the results but also make them more robust. Although only small single-gene effects were anticipated (Plomin & Davis, 2009), power was sufficient to detect rather small effects. Furthermore, the phenotype was assessed carefully, as the SSP is the gold standard for assessing attachment quality. Finally, direct replications were possible by using the two largest attachment cohorts with molecular genetic data to date.

Nevertheless, the absence of a replicable G \times E effect in explaining variation in attachment security and disorganization may be related to the assessment of the outcome or the candidate environments in the current studies. The assessments of attachment and sensitivity in the SECCYD sample were based on gold standard procedures in this field of inquiry, and they showed the expected co-variation, with an effect size equal to the combined effect size of a series of earlier, smaller studies (NICHD, 2005; De Wolff & Van IJzendoorn, 1997). The unexpected association between sensitivity and attachment disorganization found in one

of the analyses of the SECCYD data should be taken as a spurious and non-replicated outcome.

In the Generation R study a slightly modified Strange Situation Procedure was used, with pre-separation and separation episodes shortened by one minute each. This modified procedure however was stressful enough to yield the expected distribution of secure and insecure attachments. Moreover, in a previous report on the Generation R study we showed that infant attachment quality was related to cortisol stress reactivity as assessed before and after the SSP, with resistant infants showing the largest increase in cortisol excretion after the SSP and disorganized infants displaying a more flattened diurnal slope than non-disorganized infants (Luijk et al., 2010), indicating the validity of the procedure. However, in the Generation R sample no significant association between maternal sensitivity and attachment security was found. The lack of association runs counter to meta-analytic evidence on the relation between parental sensitivity and infant attachment security, not only in correlational studies (see De Wolff and Van IJzendoorn, 1997, though it should be noted that effect sizes were found to be significantly smaller in larger samples) but also in experimental intervention studies (Bakermans-Kranenburg et al., 2003). We note that the assessment of sensitivity in Generation R was less than optimal as it took place during a rather brief session with simultaneous psychophysiological assessments, and this may have decreased the association between observed sensitivity and infant attachment security.

In terms of predicting attachment, sensitivity to positive signals of the infant in settings in which the parents can fully concentrate on their child might not be the optimal way of measuring this complex construct. Parent-infant interactions in situations with competing demands (Pederson et al., 1990) might entail more ecological validity, and parental responses to infants' negative or distress signals may be more powerful in shaping attachment (Cassidy, 2008; Goldberg et al., 1999; Thompson, 1997). In both studies the sensitivity assessments did not include these more challenging components of parenting. For attachment disorganization the most important determinant has been found to be frightening or atypical parenting behaviors (Lyons-Ruth & Jacobvitz, 2008; Madigan et al., 2006). In the current studies this type of parenting has not been assessed. Furthermore, other risk factors in the infants' environment that may lead to attachment disorganization have not been assessed either, such as parental psychopathology (e.g., bipolar depression) or family violence (Cyr et al., 2010). In samples with more variety in clinical symptoms or in risk environments and with parenting assessments in more challenging settings replicable gene \times environment effects might be revealed.

Genetic contributions to attachment may operate in ways not tested in this study. For example, epistatic effects could play a role (e.g., Pezawas et al., 2008). Before evaluating these gene-gene interactions, more knowledge is needed about functionality and specific pathways of targeted genes. Genome-wide analyses (GWAS) and pathway analyses might uncover genetic associations beyond the usual suspects. Also, effects of deletions or multiplications of larger DNA segments—copy number variations (CNVs)—are known to affect protein expression and gene function. These CNVs might act as vulnerability factors for neurodevelopmental phenotypes (Merikangas, Corvin, & Gallagher, 2009). Furthermore, epigenetic processes merit consideration, as these can modify gene expression and neural function without changing nucleotide sequence (Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010; Zhang & Meaney, 2010).

Conclusion

Attachment is a developmental milestone and attachment disorganization a major risk factor for later-life psychopathology. Here we found evidence for negative heterosis, with carriers

of the COMTval/met genotype showing more attachment disorganization than both Val/Val and Met/Met carriers. This finding was replicated in both samples and we suggest that this heterosis might reflect greater vulnerability to a negative environment or to dysregulation of emotional arousal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SSP	Strange Situation Procedure
NICHD SECCYD	National Institute of Child Health and Human Development Study of Early Child Care and Youth Development
HWE	Hardy-Weinberg Equilibrium
MAF	Minor Allele Frequency
GWAS	Genome Wide Association Study
SNP	Single Nucleotide Polymorphism
VNTR	Variable Number Tandem Repeat
LD	Linkage Disequilibrium

Key points

- Studies have reported diverging molecular genetic findings for attachment security and disorganization and the interaction with maternal sensitivity, often with modest sample size.
- In the two largest attachment cohorts to date, genetic main and interaction effects on attachment were explored.
- No consistent evidence emerged for effects of candidate genes, neither for interaction with maternal sensitivity.
- A co-dominant effect of the COMT-gene was found in both samples; COMT Val/Met carriers showed higher disorganization scores ($d = 0.22$).
- The usual genetic suspects did not explain attachment differences in a replicable way.

Table 1
Sample characteristics for Generation R and NICHD SECCYD

Child characteristics	Generation R	NICHD SECCYD
Child gender, % female	48.3	51.5
Birth weight in grams	3547 (579)	3537 (496)
Gestational age in weeks	40.2 (1.4)	39.3 (1.4)
Apgar score, % < 7	4.2	--
Parental characteristics		
Age at intake mother	31.9 (3.9)	29.4 (5.3)
Maternal educational level, % low/medium	34.6	22.6
Hours working per week, mother	28.2 (12.6)	22.5 (19.6)
Marital status, % single	5.0	6.8
Smoking during pregnancy, %	10.6	--
Alcohol during pregnancy, %	56.0	--
Breastfeeding at 6 months, %	31.0	51.8
Parity, % nulliparous	60.4	47.7

Note. Unless indicated otherwise, values are Mean (SD). -- = Not assessed or not measured prospectively.

Table 2
Means and Standard Deviations for Attachment Security and Disorganization Scores as related to DRD2, DRD4 VNTR, COMT 5-HTT VNTR, and OXTR in both Samples

Gene	Risk model	Generation R - Genotype (M, SD)			SECXYD - Genotype (M, SD)		
		aa	Aa	AA	aa	Aa	AA
		M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)
Security		N	N	N	N	N	N
DRD2 rs1800497	T (A1)	0.29 (2.62)	0.04 (2.56)	0.35 (2.73)	1.19 (3.05)	1.29 (3.38)	0.58 (3.83)
		346	143	17	333	167	12
DRD4 VNTR	7+	0.08 (2.61)	0.39 (2.39)	-0.11 (3.32)	1.11 (3.22)	1.66 (2.88)	0.67 (3.74)
		336	161	15	377	85	16
COMT rs4680	homozygous	0.22 (2.50)	0.26 (2.65)	0.15 (2.63)	0.77 (3.32)	1.31 (3.00)	1.39 (3.29)
		135	250	121	140	245	137
5-HTT VNTR	short	-0.24 (2.50)	0.43 (2.61)	0.31 (2.68)	1.03 (3.31)	1.34 (3.09)	1.17 (3.15)
		171	257	113	135	147	230
OXTR rs53576	A	0.19 (2.45)	0.14 (2.65)	0.18 (3.02)	1.29 (3.25)	1.02 (3.09)	1.70 (3.07)
		224	269	52	216	234	62
OXTR rs2254298	A	0.19 (2.61)	0.08 (2.62)	1.62 (2.39)	1.16 (3.20)	1.31 (3.05)	1.05 (6.06)
		430	111	6	396	104	3
Disorganization		M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)
DRD2 rs1800497	T(A1)	3.38 (1.89)	3.69 (1.88)	3.38 (2.26)	2.31 (1.96)	2.56 (2.11)	2.33 (2.02)
		347	143	17	333	167	12
DRD4 VNTR	7+	3.52 (1.95)	3.41 (1.87)	2.57 (1.43)	2.33 (1.97)	2.75 (2.27)	2.56 (2.45)
		337	161	15	377	85	16
COMT rs4680	homozygous	3.07 (1.95)	3.73 (1.81)	3.39 (1.96)	2.12 (1.77)	2.58 (2.13)	2.31 (1.97)
		136	250	121	140	245	137
5-HTT VNTR	short	3.45 (1.90)	3.41 (1.85)	3.41 (2.04)	2.47 (2.10)	2.27 (2.00)	2.35 (1.95)
		171	257	113	135	147	230
OXTR rs53576	A	3.60 (1.92)	3.38 (1.91)	3.09 (1.84)	2.50 (1.93)	2.28 (2.01)	2.39 (2.24)
		225	269	52	216	234	62

Gene	Risk model	Generation R - Genotype (M, SD)			SECCYD - Genotype (M, SD)		
		aa	Aa	AA	aa	Aa	AA
OXTR rs2254298	A	M (SD)	3.62 (1.95)	2.00 (1.55)	2.35 (2.06)	M (SD)	2.51 (1.90)
			431	111	6	396	104

Note. aa: homozygous for the typical ('wildtype') allele, Aa: heterozygous, AA: homozygous for minor allele. The risk model indicates the specific risk allele for the gene, which is the minor allele (except for COMT). Higher security scores indicate more security; higher disorganization scores indicate more disorganization. Methods for coding converged across samples.

Table 3
Main Effects of Candidate Genes on Attachment Security and Disorganization Scores for both Samples, Additive Models

Gene	Marker	Minor allele	Generation R					NICHD SECCYD						
			MAF (%)	N	B	95% CI	r	p	MAF (%)	N	B	95% CI	r	p
Security														
Dopaminergic system														
DRD2	rs1800497	T	17	513	-0.15	-0.57; 0.28	-0.03	.50	19	512	-0.02	-0.54; 0.50	.00	.95
DRD4	48bp VNTR	7+	19	543	0.16	-0.25; 0.57	.04	.45	12	478	0.19	-0.38; 0.76	.03	.52
COMT	rs4680	G (val)	49	507	-0.02	-0.34; 0.30	-0.01	.92	50	522	0.31	-0.06; 0.68	.07	.10
Serotonergic system														
5-HTT	44bp VNTR	short	45	541	0.32	0.021; 0.63	.09	.04	59	512	0.05	-0.29; 0.38	.01	.77
Oxytonergic system														
OXTR	rs53576	A	34	546	-0.02	-0.36; 0.33	-0.01	.93	35	512	0.06	-0.35; 0.47	.01	.77
OXTR	rs2254298	A	11	548	0.05	-0.45; 0.55	.01	.84	11	503	0.13	-0.52; 0.78	.02	.70
Disorganization														
Dopaminergic system														
DRD2	rs1800497	T	17	513	0.18	-0.12; 0.49	.06	.24	19	512	0.19	-0.14; 0.52	.05	.26
DRD4	48bp VNTR	7+	19	543	-0.23	-0.53; 0.08	-0.07	.15	12	478	0.28	-0.09; 0.64	.07	.14
COMT	rs4680	G (val)	49	507	0.16	-0.07; 0.40	.07	.17	50	522	0.10	-0.14; 0.34	.04	.42
Serotonergic system														
5-HTT	44bp VNTR	short	45	541	-0.03	-0.25; 0.20	-0.01	.82	59	512	-0.05	-0.26; 0.17	-.02	.67
Oxytonergic system														
OXTR	rs53576	A	34	546	-0.25	-0.50; 0.01	-.08	.06	35	512	-0.11	-0.37; 0.15	-.04	.42
OXTR	rs2254298	A	11	548	0.04	-0.32; 0.40	.01	.82	11	503	0.10	-0.31; 0.52	.02	.63

Note. Additive models are presented. MAF refers to minor allele frequencies. *B* denotes change in security and disorganization scores per unit change in the predictor.

Table 4
Main and Interaction Effects for Dichotomous Genetic Risk Models for Security Scores in Generation R and SECCYD

Gene	Risk model	Generation R			NICHD SECCYD				
		B	SE	p	B	SE	p		
Security									
DRD2	T (A1)								
DRD2		-.21	.25	-.04	.39	.04	.29	.01	.88
Sensitivity		.14	.17	.04	.43	.22	.14	.09	.11
Sens*DRD2		.00	.29	.00	.99	.08	.22	.02	.73
DRD4	7 +								
DRD4		.28	.24	.05	.25	.45	.36	.06	.21
Sensitivity		.04	.17	.01	.82	.40	.13	.17	<.01
Sens*DRD4		.42	.30	.08	.16	-.78	.27	-.15	<.01
COMT	homozygous								
COMT		-.07	.23	-.01	.77	-.22	.28	-.04	.42
Sensitivity		.04	.19	.01	.84	.08	.17	.03	.65
Sens*COMT		.21	.28	.05	.45	.20	.22	.06	.35
5-HTT	short								
5-HTT		.64	.24	.12	<.01	.19	.32	.03	.55
Sensitivity		.08	.25	.03	.74	.37	.19	.15	.05
Sens*5-HTT		.08	.30	.02	.78	-.18	.23	-.06	.45
OXTR (rs53576)	A								
OXTR		-.04	.23	-.01	.86	-.15	.28	-.02	.59
Sensitivity		.13	.23	.04	.58	.36	.17	.15	.04
Sens*OXTR		-.03	.29	-.01	.93	-.21	.22	-.07	.36
OXTR (rs2254298)	A								
OXTR		-.03	.27	.00	.92	.21	.35	.03	.54
Sensitivity		.13	.16	.04	.40	.21	.13	.09	.10
Sens*OXTR		.09	.33	.01	.78	.17	.25	.03	.51

Note. Dichotomous risk models. B denotes change in security scores per unit change in the predictor.

Table 5
Main and Interaction Effects for Dichotomous Genetic Risk Models for Disorganization Scores in Generation R and SECCYD

Gene	Risk model	Generation R			NICHD SECCYD				
		B	SE	p	B	SE	p		
Disorganization									
	T (A1)								
DRD2		.28	.18	.07	.13	.24	.19	.06	.19
DRD2		-.15	.13	-.07	.23	-.03	.09	-.02	.71
Sensitivity		.08	.21	.02	.69	.24	.14	.09	.09
Sens*DRD2									
DRD4	7 +								
DRD4		-.19	.18	-.05	.30	.36	.23	.07	.12
Sensitivity		-.09	.13	-.04	.46	.00	.08	.00	.99
Sens*DRD4		-.09	.22	-.02	.68	.17	.17	.05	.34
COMT	homozygous								
COMT		-.52	.17	-.14	<.01	-.35	.18	-.09	.04
Sensitivity		.06	.14	.03	.66	.09	.11	.06	.42
Sens*COMT		-.41	.20	-.12	.04	-.05	.14	-.03	.70
5-HTT	short								
5-HTT		-.05	.18	-.01	.77	-.15	.20	-.03	.45
Sensitivity		-.14	.18	-.06	.45	.24	.12	.15	.05
Sens*5-HTT		-.01	.22	.00	.97	-.26	.15	-.14	.08
OXTR (rs53576)	A								
OXTR		-.27	.17	-.07	.11	-.21	.18	-.05	.25
Sensitivity		-.17	.17	-.07	.30	.07	.11	.04	.54
Sens*OXTR		.05	.21	.02	.81	.02	.14	.01	.90
OXTR (rs2254298)	A								
OXTR		.14	.20	.03	.50	.16	.22	.03	.48
Sensitivity		-.11	.11	-.05	.33	.03	.08	.02	.67
Sens*OXTR		-.18	.24	-.04	.47	.09	.16	.03	.59

Note. Dichotomous risk models. B denotes change in security scores per unit change in the predictor.