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Genetic and environmental influence on attachment disorganization

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Abstract

Background: Empirical studies demonstrate that maternal sensitivity is associated with attachment security in infancy, while maternal frightening/frightened behavior is related to attachment disorganization. However, attachment disorganization is also predicted by individual dispositions in infancy. Indeed, recent studies indicate a link between attachment disorganization and DRD4 gene polymorphisms, thus suggesting a genetic vulnerability for attachment disorganization. The aims of our study were twofold, to test a) a possible direct link between molecular genetic variations and attachment disorganization, and b) a possible gene-environment interaction with a moderating effect of early maternal caregiving. **Methods:** Attachment security and disorganization, as well as quality of maternal behavior were assessed in the infants of the Regensburg Longitudinal study IV (N=106) at the age of 12 months. DNA samples were collected in order to assess the exon III repeat polymorphism in the coding region, the –521 C/T SNP in the regulatory region of the DRD4 gene, and a repeat polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene. **Results:** Significant associations were found between attachment disorganization and the short polymorphism of the serotonin transporter gene. Also, a gene-environment interaction indicated that this genetic association was only valid for infants of mothers exhibiting low responsiveness. No other significant genetic associations with attachment disorganization were apparent. **Conclusions:** The study suggests a gene-environment interaction whereby biological determinants of attachment disorganization are moderated by social experiences. Different pathways of the development of attachment disorganization are discussed based on a bio-behavioral model of development.

Keywords: attachment, gene-environment interaction, DRD4, serotonin-transporter.

Genetic and environmental influence on attachment disorganization

Attachment theory provides a widely used framework for explaining influences of early social experiences on later normal and psychopathological development (Sroufe, 1996). The attachment system may be conceptualized as a biologically based behavioral system, regulatinges the proximity to the caregiver depending on the child's emotional state and environmental challenges (Bowlby, 1989; Ainsworth, Blehar, Waters & Wall, 1978). Differences in the organization of this behavioral system are known indicatiors of the quality of attachment. These may be assessed at the end of the first year of life by Ainsworth's strange situation (Ainsworth et al., 1978). The resulting attachment classifications in infancy incorporate two conceptually different behavioral dimensions. First, the dimension of security of the attachment pattern, and second, the dimension of organization of the specific attachment pattern (Carlson, & Sroufe, 1999; Spangler & Grossmann, 1999)

Determinants of Attachment Security

The security dimension of the attachment system can be observed amongst the established and consistent patterns of emotional regulation in caregiver-child dyads (Zimmermann, 1999). In the case of a secure attachment, the infant regulates negative emotions effectively by seeking proximity to the parent. Insecure-avoidantly attached infants avoid proximity and ineffectively try to regulate their emotions individually without their caregivers's help, while insecure-ambivalently attached infants keep close contact, but are not able to use the caregiver's help for effective regulation.

Maternal sensitivity has repeatedly been identified as an important developmental precursor of attachment security (e.g. Ainsworth et al., 1978; Grossmann, Grossmann,

Spangler, Suess & Unzner, 1985; van den Boom, 1994). In addition, several studies have reported longitudinal associations between newborn's behaviors, like low social orientation or low regulation of internal states as assessed by the NBAS, and later insecure attachment classifications (e.g. Waters, Vaughn, & Egeland, 1980; Crockenberg, 1981; Grossmann et al., 1985). This evidence suggests that individual dispositions may increase the probability of developing an insecure attachment pattern. However, intervention studies have also shown that an improvement in maternal sensitivity increases the rate of secure attachment patterns in early childhood, even with in highly irritable infants (van den Boom, 1994).

Determinants of Disorganized Attachment

In addition to the security dimension, Main and Solomon (1990) described attachment disorganization as an additional attachment pattern, in which infants do not show a consistent behavioral strategy in coping with emotional challenges instigated from the strange situation. Indeed, the observable behaviors are marked by functional contradictions, incompleteness or interruptions of movements, breaks, stereotypes, confusion, and apprehension. Attachment disorganization can be classified in addition to the traditional attachment security patterns. The regulatory difficulties associated with disorganized attachment pattern have also been demonstrated in bio-behavioral studies. Infants classified as disorganized exhibited an increased cardiac activity, and an elevation of salivary cortisol level during the strange situation (Spangler & Grossmann, 1993; Heertsgard, Gunnar, Erickson, & Nachmias, 1995; Spangler, & Grossmann, 1999), thus indicating more physiological stress.

About 15% of one-year-old infants in low-risk and up to 86% in high-risk populations showed disorganized attachment behavior in the strange situation (Main &

Solomon, 1990; Van IJzendoorn, Schuengel, & Bakermans-Kranenburg, 1999; Barnett, Garniban, & Cicchetti, 1999). In high-risk samples, high rates of disorganization have been found in maltreated infants (Lyons-Ruth, & Jacobvitz, 1999; Barnett et al., 1999). These findings have supported the assumption that specific frightening or frightened maternal behaviors might be developmental precursors to attachment disorganization (Main, & Hesse, 1990). Empirical studies of high risk samples have shown that mothers of infants with attachment disorganization show higher rates of atypical maternal behavior, such as hostile-intrusive behaviors and communication errors (Lyons-Ruth, Repacholi, McLeod, & Silva, 1991; Lyons-Ruth, Bronfman, & Parsons, 1999; Madigan, Pederson, & Moran, 2006). In addition, maternal frightened or frightening behavior has also been observed more often, but not exclusively, in mothers of infants with attachment disorganization (e.g. Schuengel, Bakermans-Kranenburg, & van IJzendoorn, 1999, Hesse, & Main, 2006; Goldberg, Benoit, Blokland & Madigan, 2003). There is a significant association between low maternal sensitivity and attachment disorganization in low-SES samples or samples of adolescent mothers (van IJzendoorn et al., 1999; Moran, Forbes, Evans, Tarabulsy, & Madigan, 2008). Interventions in high-risk populations aiming at improving parenting can be relatively effective in changing the infants' attachment disorganization status (e.g. Lyons-Ruth et al., 1999). Thus, in high-risk samples, high rates of attachment disorganization might be explained by inappropriate maternal behaviors.

In contrast, in a low risk sample, Spangler, Fremmer-Bombik and Grossmann (1996) found that disorganized infants have been characterized by low levels of behavioral organization (i.e. self-regulation), already as newborns. However, there was no significant difference in maternal sensitivity between mothers of disorganized and

non-disorganized infants. Goldberg and colleagues (2003) found, in a low-risk sample, that a substantial proportion of the mothers of infants with attachment disorganization (38 %) were not coded as disruptive in their parenting. There were also no significant differences in maternal fearful or intrusive behaviors between the disorganized and the non-disorganized groups. As maternal behaviors did not mediate the link between maternal unresolved state of mind and infant attachment disorganization, the authors concluded that both infant factors and contextual factors might contribute to the development of attachment disorganization. Other studies have also identified child-related factors, such as neurological impairment (Pipp-Siegel, Siegel &, Dean, 1999) or autism, that seem to contribute to the same behavior charateristics as attachment disorganization.

Based on these findings, the development of attachment disorganization in low-risk families may be influenced by the infants' individual dispositions. In these cases, attachment disorganization might be conceived as an individual construct, in contrast to infant attachment security, which is commonly conceived as a relationship characteristic.

This notion is supported by studies indicating the contribution of genetic factors to attachment disorganization. Again, in a low-risk sample, Lakatos and colleagues (2000) found that the 7-repeat allele of the D4 dopamine receptor (DRD4) gene was significantly associated with attachment disorganization in infants. Additional analyses, including the -521 C/T single nucleotide polymorphism in the regulatory region of the DRD4 gene showed that the previous association with the 7-repeat allele was significant solely in the presence of the -521 T-allele (Lakatos et al., 2002; Gervai et al., 2005). However, an attempt to replicate these findings in a Dutch twin study failed to confirm

the association (Bakermans-Kranenburg, & van IJzendoorn, 2004). Similarly, behavioral genetic analyses within the same sample did not yield major genetic effects on the explained variance of attachment disorganization (Bokhorst et al., 2003). Thus, to this day, a final conclusion about the contribution of specific genetic differences on the development of attachment disorganization can not yet be drawn.

Molecular-Genetic Effects on Behavior Organization

In regard to phenotypic behaviors, infants classified as disorganized are specifically alarmed, have a restricted ability to resolve the resulting anxiety in the strange situation (Hesse, & Main, 2006), and show clear deficits in effective self-regulation. Several studies have found an association between the long variant (7 repeat) of the DRD4 polymorphism and ADHD or aggressiveness in children (Savitz, & Ramesar, 2004; Holmes, Payton, Barrett, Hever, Fitzpatrick, Trumper et al., 2000; Nobile et al., 2007).

In addition, several studies have shown the effects of gene-gene-interaction on temperament, more specifically including polymorphisms of the serotonin transporter (5-HTTLPR). Lakatos and colleagues (2004) found that infants with the 7-repeat DRD4 allele and the homozygous short form of the 5-HTTLPR (7+, s/s) showed more anxiety and resistance to the stranger's initiation of interaction. In a longitudinal study, newborns with the homozygous short form (s/s) of the 5-HTTLPR and the short version of the DRD4-polymorphism had the lowest scores on orientation (Ebstein et al., 1998). Also within the same sample, at two months of age, the infants carrying the s/s form of the 5-HTTLPR polymorphism displayed more negative emotionality in daily caregiving interactions than infants carrying the other forms of this polymorphism (Auerbach et al., 1999). In adults, other molecular genetic studies have revealed significant associations

between the short version of the serotonin transporter and individual characteristcs, such as anxiety, impulsiveness, aggressivity, emotional dysregulation, stress reactivity, as well as higher sensitivity to risk factors for the development of depression (Lesch, Bengel, Heils, Sabol, Greenberg, Petri et al., 1996; Greenberg Li, Q., Lucas, F., Hu, S., Sirota, L.A., Benjamin, J., et al., 2000; Carver, & Miller, 2006; Caspi, Sugden, Moffitt, Taylor, Craig, Harrington, et al., 2003; Hariri, & Holmes, 2006).

Beside direct associations between the polymorphisms of these candidate genes and observable behavior or personality traits, gene-environment effects explain the reasons how genetic or environmental risk factors are moderated and why direct effects will not always be found (Moffitt, Caspi, & Rutter, 2006). Regarding attachment, an interplay between attachment security, as well as disorganization, and regulatory biological systems has repeatedly been demonstrated (e.g. Spangler, & Grossmann, 1993; Nachmias, Gunnar, Mangelsdorf, Parritz, & Buss, 1996; Spangler, & Schieche, 1998). Recent findings also indicate a genetic moderation of the relation between disruptive maternal behavior and attachment disorganization (Gervai, Novak, Lakatos, Toth, Danis, Ronai et al., 2007). In fact, only in the absence of the DRD4 7-repeat polymorphism the authors have found a significant effect of disruptive maternal behavior on the development of attachment disorganization. In addition, van IJzendoorn and Bakermans-Kranenburg (2006) have reported a signifcant association between maternal unresolved trauma and infant attachment disorganization, only in the presence of the DRD4 7-repeat allele, which indicates a higher susceptibility in this group. However, the authors did not report a significant interaction of genetic polymorphims and atypical maternal behavior on the development of attachment disorganization.

Thus, there is some evidence that early regulatory deficits during infancy increase the risk for developing attachment disorganization (Spangler et al., 1996). Also, there is some, but not unequivocal, evidence that attachment disorganization in infancy is associated with molecular genetic polymorphisms of the DRD4 gene. Moreover, behaviors that are typically interpreted as signs of attachment disorganization (e.g. difficulties in organizing attention and behavior in stressful situations) may not be phenotypically distinguishable from behaviors that are commonly prevalent in individuals with regulatory deficitsbased on the genetically influenced organization of the dopamine or serotonine system (Savitz, & Ramesar, 2004; Carver, & Miller, 2006).

Based on the concept of multi-causality (Cicchetti, & Rogosch, 1996), we suggest different developmental pathways to the same phenotypic patterns of disorganized attachment. While some infants may exhibit disorganized attachment behaviors because of their dispositional restrictions of behavioral organization, others may develop attachment disorganization as a result of disruptive experiences with the attachment figure, e.g. by frightened or frightening parental behavior. While the dispositional pathway may influence regulatory capacities in general, the impact of inappropriate caregiving might primarily explain the high rate of disorganized infants in high-risk samples. In contrast, the effects of disruptive parenting on attachment disorganization may be smaller in low-risk-samples, as maltreatment or frightening behaviors' occurrence and intensity might be limited. Beside these two direct pathways, the possibility of a third pathway characterized by a gene-environment-interaction may reveal moderating effects as well.

The main objective of this study was to investigate the role of genetic differences in the development of attachment disorganization. It is essential to try a second attempt

at replicating the initial findings, which emphasized a genetic impact on attachment disorganization (Lakatos et al., 2000, 2002), as Bakermans-Kranenburg and van IJzendoorn (2004) failed to do so. Thus, the first aim of this study was an attempt to replicate the link between attachment disorganization and the 7-repeat variant of the DRD4 polymorphism. Second, we wanted to explore possible additional associations between polymorphisms of the dopamine transporter (-521 C/T) and the serotonin transporter (5-HTTLPR) and attachment disorganization. Finally, we wanted to investigate gene-environment interactions by examining the moderating role of maternal sensitivity on these molecular genetic polymorphisms in regards to the development of attachment disorganization.

In terms of attachment security, genetic associations have not been reported hitherto. As attachment security is commonly conceived as a relationship construct, such genetic associations theoretically are also not expected. Nevertheless, for comparison, analyses were conducted in parallel for attachment security.

Method

Subjects and Procedure

The sample consisted of 106 healthy German, Caucasian, low-risk infants (53 girls/53 boys). The children's families represent a fairly wide range of socioeconomic status, including 42% upper middle class, 24% middle class and 27% lower class, as assessed by the father's education and occupation, as well as the total family income. The mother was the infant's primary caretaker in all but one case, in which the father took part in the study. Sixteen mothers (15 %) were working outside the family in part-time jobs at the end of the first year mark. During mothers' working time, these infants

were cared for by the father or the grandmother. None of the infants was in daycare during the first year. Informed consent was obtained from the parents at each assessment period.

At the age of 12 months, the infants were observed during the strange situation, a 20-minute situation involving a sequence of episodes which progressively activates the attachment system by an unfamiliar environment, the arrival of a stranger and two brief separations from the mother (Ainsworth et al., 1978). Following this procedure, they were observed during a 30-minute free play session, in which the mother was advised to fill in a questionnaire but to freely respond to the infant as she usually would. In a follow-up assessment at the age of twelve, cheek-cells were collected from 96 of the original 106 subjects, for genetic analyses.

Behavioral analyses

The quality of infant-mother attachment was analyzed by trained observers from the strange situation video-tapes using the procedures described by Ainsworth et al (1978) for traditional classification of attachment security (secure, insecure-avoidant, insecure-resistant), and by Main and Solomon (1990) for classification of disorganization (D). Reliability for security of attachment was 90% (kappa=.87). For D-classification, there were two different observers trained and examined with tapes analyzed by an expert judge (Erik Hesse). Reliability for the two observers for the D-status (5 or above) was 80% and 82% (kappa = .63). In case of disagreement in attachment classification, conference scores were used.

Quality of maternal behavior was analyzed from the videotaped play session. First, global ratings of maternal sensitivity according to Ainsworth et al. (1978) were done by trained observers. Sensitivity was defined as the mother's ability to appropriately

perceive and interpret infant signals and needs, as well as to promptly and adequately respond to the infant. Ratings were done by using Ainsworth's 9-point sensitivity scale. The inter-rater reliability, based on 30 play situations, was kappa=.80. Second, a micro-analytic procedure, based on event-sampling, was developed to separately assess the different components of maternal sensitivity, the perception of infant signals, as well as the promptness and appropriateness of the responses to the signals.

Infant signals were defined as any instance of vocalization, negative facial expression and behavior directed to the mother (approaching, looking for at least 3 sec, giving an object, grasping for her questionnaire). These were only used to calculate the maternal behavior measures. Maternal behavior was coded according to six different measures in the two dimensions maternal responsiveness and regulation quality. The specific maternal responsiveness measures were (1) *the proportion of perceived signals* (% of signals followed by any maternal responses, which ranged from short glances to behavioral breaks indicating attention to obvious infant-directed behavior), (2) *the proportion of responded signals* (% of signals followed by an infant-directed active behaviors, going beyond short glances or looks), and (3) *the proportion of prompt responses* (% of signals responded to within 3 seconds). Measures for regulation quality were (4) *the proportion of appropriate responses* (% of signals with responses agreeing with the child's wish or need, e.g. mother providing he infant with a desired object or mother comforting the infant when distressed), (5) *the proportion of emotionally positive responses* (% of signals with responses characterized by affectionate, respectful, and sensitive behavior), and (6*) the proportion of episodes (% of signals) with sustained regulation* (e.g. mother finally comforting the infant; mother allowing the infant to play with pencil as long as he/she wants).

Reliability analysis was based on 12 play situations. Agreement for detection of infant signals was 81%. For the maternal behavior scales, kappa ranged from .76 to 1.0. As there were high correlations within the responsiveness measures (ranging from .82 to .92) and within the regulation quality scales (ranging from .81 to .92) and as the correlations between the single measures of the two dimensions were only moderate (ranging from .36 to .62), composite measures were computed for the two dimensions based on z-transformed scores. Maternal responsiveness was calculated as the mean of the proportion of perceived, responded and promptly responded signals, while maternal regulation quality was calculated as the mean of appropriate, emotionally positive warm and sustained responses. The correlation between maternal responsiveness and regulation quality was $r = .57$ ($p < .001$). The correlation of the two measures with the global sensitivity scale was $r = .29$ ($p < .01$) and $r = .53$ ($p < .001$) for maternal responsiveness and regulation quality, respectively.

Molecular genetic analyses

Genotyping was performed at the Institute of Psychiatry, University of Regensburg (Germany) for DRD4 exon III repeat polymorphism and at the Institute of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University (Budapest, Hungary) for the 5-HTTLPR polymorphism and the -521 C/T SNP, each time blind to the psychological data. Genomic DNA was isolated from buccal swabs using published procedures (Freeman et al., 1997).

For exon III 48-bp VNTR polymorphism in DRD4 primers were 5´ GCG ACT ACG TGG TCT ACT CG 3´ and 5´ AGG ACC CTC ATG GCC TTG 3´. PCR cycling conditions were 15 min at 95° C, followed by 35 cycles of 45 s at 95° C (denaturation), 30 s at 50° C (annealing), and 30 s at 72° C (elongation), with a final extension for 7

min at 72° C using a Multicycler PTC 200 gradient machine (Biozym Diagnostik, Germany). PCR products were separated by 2.0% agarose gel electrophoresis and stained with ethidium bromide for UV visualisation (Schoots & Van Tol, 2003).

Investigation of the –521C/T SNP was carried out by two independent methods to increase the reliability of the genotyping procedure. The first approach consists of a single-tube bidirectional allele-specific amplification, whereby the reaction mixture contains two outer (sense: 5' GGA ATG GAG GAG GGA GCG GG 3'; antisense: 5' CGC TCC ACC GTG AGC CCA GTA T 3') and two allele-specific (C-specific: 5' GGA GCG GGC GTG GAG GGC 3', T-specific: 5' GCC TCG ACC TCG TGC GCA 3') primers. The resulting "T"- and "C"-specific PCR-fragments differ in size, thus the genotype can be unambiguously determined based on the obtained electrophoretic pattern. The second technique employs a restriction digestion after the amplification step (restriction fragment length polymorphism). The same outer primers are used to amplify the region of interest. This is followed by the application of the *Fsp* I endonuclease (its recognition site is TGCGCA, the "T" is the –521CT polymorphic site). The outer primers are designed to incorporate a non-polymorphic digestion site in the PCR-fragment depending on the presence of a "C" or "T" allele. For more details of the two procedures see Szantai et al. (2005).

The 5-HTTLPR variable number of tandem repeats (VNTR) polymorphism was investigated by employing two flanking primers for the polymerase chain reaction (sense primer: 5' GGC GTT GCC GCT CTG AAT GC 3', antisense primer: 5' GAG GGA CTG AGC TGG ACA ACC AC 3'; thermocycling was initiated at 95° C for 10 min to activate HotStar DNA polymerase (Qiagen) followed by 35 cycles of 1 min denaturation at 95° C, 1 min of annealing at 65° C and 1 min extension at 72° C,

completed by 10 min of extension at 72° C). In both VNTR polymorphisms dGTP was replaced with dITP in 50% in order to avoid allelic drop-out in heterozygotes. The length of the generated PCR-amplicons directly shows the repeat number (i.e. short or long allele) (Nemoda et al., 2001).

Results

Regarding attachment security, 61 out of 96 (64%) infants were classified as securely attached, while 18 (19%) and 14 (15%) infants were categorized as insecure-avoidant and insecure-resistant, respectively. Three infants were unclassifiable. Regarding attachment disorganization, 23 (24%) infants were classified as disorganized. Among the D-infants, the number of children having an underlying secure, insecure-avoidant and insecure-resistant attachment relationship, was 15, 1 and 4, respectively. Attachment classification was not associated with the infants' sex.

The allele-wise distribution of the DRD4 was comparable to European and Middle East populations (see Chang, Kidd, Livak, Pakstis & Kidd, 1996).While the most frequent variant was the 4-repeat (67.9%), the frequency of the 7-repeat was 13.2%, and of the 2-repeat was 9.5%. The remaining rare alleles summed up to a frequency of 9.6%. The genotype frequencies were 71 (75 %) and 24 (25 %) for the 7- and the 7+ polymorphism, respectively. Frequencies of the -521 C and T alleles were 49.5% and 50.5%, respectively, which are comparable to Lakatos et al. (2002). The genotype frequencies were 24 (25 %), 47 (49 %) and 25 (26 %) for the CC, CT and TT genotype, respectively. Frequencies of short and long alleles of 5-HTTLPR were 41.7% and 58.3%, respectively, which are comparable to European population (see Gelernter, Kranzler, & Cubells, 1997). The genotype frequencies were 35 (37 %), 42 (44 %) and 19 (20 %) for the ll, ls and ss genotypes, respectively.

The 5-HTTLPR and the -521 C/T genotypes were in the Hardy-Weinberg equilibrium, χ^2 (2, N=96) =.96, and χ^2 (2, N=96) = .06, N.S. Due to small cell counts for specific DRD4 genotypes, Hardy-Weinberg equilibrium was tested only for combinations of presence and absence of the 7-repeat polymorphisms, which also were in the equilibrium, χ^2 (2, N=96) =.32, N.S. There was no significant effect of infant sex on the distribution of genotypes.

To assess associations between attachment and gene markers, chi-square analyses were conducted. There were no significant associations between attachment security (secure vs. insecure) and any of the three gene markers. Thus, securely and insecurely attached infants did not show genetic differences (see Table 1). In contrast, the analyses for attachment disorganization revealed a significant relation between attachment disorganization and the 5-HTTLPR genotype, linear χ^2 (2, N=96) = 6.57, p = .02). Only a very low proportion of disorganized infants was found amongst infants with two long alleles (11.4%), while the proportion was more than twice as high (26.2%) amongst those with one long allele, and almost four times as high (42.1%) amongst those with two short alleles (see Table 1). Attachment disorganization was not related to the DRD4 and the -521 C/T polymorphism and any combination of their alleles.

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Before examining gene-environment interactions in the development of attachment disorganization, it was tested whether maternal behavior ratings varied according to the infants genotypes. None of the ANOVAs resulted in significant effects, indicating independence of infant genotype and maternal behavior. In order to

investigate whether the genetic impacts on disorganization were moderated by the quality of maternal behavior, the infants were grouped depending each of the maternal behavior scales by median-split. A maternal responsiveness (low vs. high) x 5-HTTLPR (ll, sl, ss) two-way ANOVA for attachment disorganization revealed a main effect of 5-HTTLPR, $F(2,89) = 4.24$, $p=.02$, $\eta^2 = .10$), which was qualified by an interaction between maternal responsiveness and 5-HTTLPR, $F(2,89) = 3.58$, $p = .03$, $\eta^2 = .07$). While the proportion of D infants was low and unrelated to the 5-HTTLPR genotype for the high responsiveness group (see Figure 1), the proportion of D infants increased with the number of short alleles on the low responsiveness group; Duncan-post-hoc tests revealed that the proportion of D infants was significantly higher in the s/s group ($p<.05$) and tended to be higher in the s/l group ($p<.10$) as compared to the l/l group. No interaction effects were found for the maternal regulation quality and the maternal sensitivity scale. After finding an interaction between genes and maternal behavior on attachment disorganization, this effect was also tested regarding attachment security, but this did not yield significant results.

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Discussion

The aims of this study were to investigate molecular genetic correlates of attachment disorganization and their possible moderation by maternal behavior in low-risk families. The hypothesis of possible genetic effects was based on earlier findings showing an association between attachment disorganization and individual dispositions in newborns (Spangler et al., 1996), on findings indicating relations between genetic

polymorphisms and attachment disorganization (Lakatos et al., 2000) and other molecular genetic studies showing that behaviors typical of infants with attachment disorganization are associated with differences in molecular genetic polymorphisms of candidate genes affecting the serotonin or dopamine system (Ebstein et al., 1998).

The results of this study did not replicate the findings by Lakatos and colleagues (2000), who found a significant association between attachment disorganization in infancy and polymorphisms of the DRD4, and the -521 C/T gene. On the other hand, attachment disorganization in infancy was significantly increased in carriers of the short allele polymorphism of the serotonin transporter gene (5-HTTLPR), thus attesting for a genetic predisposition for the development of attachment disorganization.

The association of attachment disorganization with the serotonin transporter supports the hypothesis that individual dispositions in regulatory capacities contribute to the development of attachment disorganization (Spangler et al., 1996). A possible developmental pathway could lead from individual difficulties in self-regulation of attention and behavior already detectable in newborn age to later attachment disorganization in infancy observable in a stressful situation like the strange situation. The poor orientation and high negative emotionality found in infants carrying two short 5-HTTLPR alleles (Ebstein et al., 1998; Auerbach et al., 1999) would support this perspective.

However, these results may only be interpreted as a probabilistic association and not as a deterministic link. The significant gene-environment interaction shows that the genetic effect was moderated by early maternal responsiveness. That is, the association between the short allele variation of the 5-HTTLPR and attachment disorganization is only significant when maternal responsiveness is low, but not in those cases where

mothers were highly responsive to their infants. From this perspective, maternal behavior constitutes a protective factor against genetic risk.

Taking an alternative perspective, low parental responsiveness increases the probability for infants to develop attachment disorganization, but only for individuals carrying the short version of the 5-HTTLPR allele. Thus, the presence of two long alleles of the serotonin gene may be interpreted as a protective factor, at least when exposed to low responsive mothers. The protective effect associated with two long 5-HTTLPR alleles has also been reported by Caspi and colleagues (2003) with respect to the influence of stressful life events on the development of depression. Additionally, in a comparative study, Barr and colleagues (2003) found that, in peer-reared rhesus macaques carrying the short serotonin transporter allele, the behavioral and emotional regulation was restricted compared to mother-reared primates.

A gene-environment interaction with regard to attachment disorganization recently has also been reported (Gervai et al., 2007). Combining a low and a high risk sample, maternal disruptive behavior was found to impact infant attachment disorganization, but only for those infants without a DRD4 7-repeat allele. When experiencing disruptive parenting, carriers of the 7-repeat allele did not show an increased rate of attachment disorganization. In contrast, van IJzendoorn and Bakermans-Kranenburg (2006) found, in a small sample, that the 7-repeat genotype heightened the risk of disorganization in infants of mothers with unresolved trauma, as assessed by the Adult Attachment Interview. These findings indicate a common contribution of genetic characteristics and parental attachment characteristics, as assessed at the behavioral or the representational level, to the development of infant attachment disorganization, but are far from showing an unequivocal picture.

Therefore, a careful interpretation of the association between genetic characteristics and attachment disorganization as probabilistic and not as deterministic seems to be necessary. While the presence of two long 5-HTTLPR alleles seems to be protective against the development of attachment disorganization, infants with at least one short allele had a higher risk to develop attachment disorganization. The present findings do not exclude disorganization as developing from the consequence of inappropriate caregiver behavior (e.g. Schuengel et al., 1999; Lyons-Ruth et al., 1991). From a developmental perspective, there may be at least two differential pathways to disorganized attachment. Some infants may develop disorganized attachment due to inappropriate caregiving (Moran et al., 2008), while for others, their dispositional self-regulation restrictions may contribute to their display of disorganized attachment behaviors, and more so in low-risk samples. In addition, the results showed that a heightened genetic risk for attachment disorganization only becomes manifest when mothers show low responsiveness. From a different perspective, infants carrying two long alleles of the 5-HTTLPR may be more resilient to experiences of parental behaviors commonly inductive of anxiety and stress.

These results are in line with the threshold model for the development of attachment disorganization proposed by Bernier and Meins (2008). According to this approach, both infants' individual characteristics and specific contextual factors may influence the threshold when caregiving behavior has an effect on the development of attachment disorganization.

Regarding attachment security, associations with genetic characteristics were not found in this study. Conceiving attachment security – in contrast to attachment disorganization - as a relational construct (e.g. Spangler & Grossmann, 1999) we did not

expect to find links with genetic characteristics. However, from this relational perspective, significant associations between attachment security and quality of maternal behavior would have been expected, but this was not to case in this study. This might be explained by the age at the assessment of maternal sensitivity, which was twelve months in this study. There are some indications that maternal sensitivity earlier in life may be more predictive of attachment security than later assessments. Isabella (1993) found that mothers of securely and insecurely attached infants differed in sensitive responsiveness assessed at the age of 1, but not at the ages of 4 and 9 months. Similarly, Grossmann and colleagues (1985) reported significant associations between maternal sensitivity and later attachment quality, for maternal sensitivity ratings at two and six months, but not at ten months.

Clearly, several limitations of this study must be taken into consideration, due to methodological issues. With respect to standards of molecular-genetic studies (Lesch at al., 1996), the sample size of this study was relatively small, which decreases the power of statistical analyses, and reduces the possibility to test other possible gene-gene or gene-environment interactions. In addition, the effect sizes in the analyses of this study were quite small, which indicates that the variance explained by genetic influences was limited. Finally, as the error probabilities were not corrected for multiple testing, the number of statistical tests reported increased the risk of possible spurious results. Although Savitz and Ramesar (2004) conclude in their review that most significant associations between DRD4 and serotonin transporter polymorphism and personality have been found in small samples, a generalization of the results of this study may be too premature and not yet justifiable until replications have been published.

In conclusion, the findings of this study suggest a genetic predisposition for the

development of attachment disorganization, indicating a higher risk for infants carrying the short version of the serotonin transporter polymorphism. Most important, the direct genetic risk was moderated by the quality of maternal behavior. In other words, specific genetic characteristics may lower or heighten the infants' reactivity towards non-responsive parenting in the development of attachment disorganization.

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- Differences in attachment security in infancy are influenced by the caregiver's sensitivity. Thus, attachment security primarily can be conceived as a relationship construct.
- Attachment disorganization, a risk factor for socio-emotional development, has been associated with the caregiver's disruptive or frightening/frightened behaviors. However, individual dispositions, expressed in newborn behavior and genetic characteristics, also predict attachment disorganization.
- The findings suggest a gene-environment interaction in the development of attachment disorganization: High maternal responsiveness seems to provide a social buffer against a genetic risk.
- Clinical implications: Findings indicate a (genetically based) vulnerability in self-regulation as a precursor of maladaptive development. The gene-environment interaction suggests that early attachment-related prevention in terms of parent education or training programs may alter the consequences of such dispositions.

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Figure Captions

Fig. 1) Relative frequency of disorganization (D) depending on maternal responsiveness and the 5-HTTLPR genotype. The number of infants carrying ll, sl and ss alleles was 14, 24, and 8 for the low responsiveness group and 20, 18 and 10 infants of the high responsiveness group, respectively.

Table 1. Associations between attachment security and disorganization and dopamine receptor gene polymorphisms (DRD4 and -521 C/T) and the serotonin transporter gene polymorphism (5-HTTLPR) [a]

Genotype	Attachment security			Attachment disorganization		
	Secure	insecure	χ^2 (D.F.)	D	Non-D	χ^2 (D.F.)
DRD4 7-	44	25	χ^2 (1) = .26	19	52	χ^2 (1) = 1.00
DRD4 7+	16	7	N.S	4	20	N. S.
-521 CC	18	6	χ^2 (2) = 2.07	8	16	χ^2 (2) = 1.56,
-521 CT	27	19	N.S.	10	37	N.S.
-521 TT	16	7		5	20	
5-HTTLPR l/l	23	11	χ^2 (2). = .16	4	31	χ^2 (2) = 6.57,
5-HTTLPR l/s	26	15	N.S.	11	31	p = .02
5-HTTLPR s/s	12	6		8	11	

[a] number of subjects

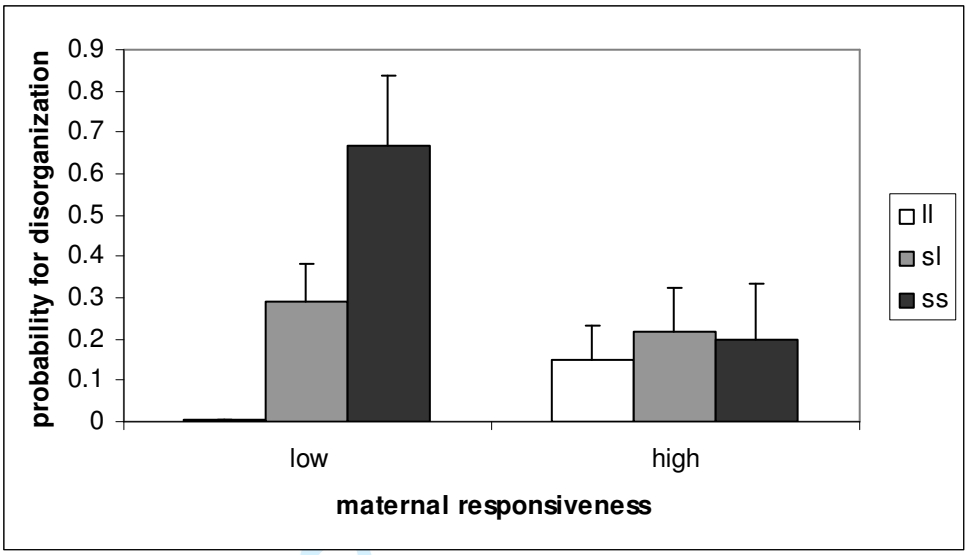

Fig. 1