

BRIEF REPORT

Genetic Moderation of Stability in Attachment Security From Early Childhood to Age 18 Years: A Replication Study

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A longstanding question for attachment theory and research is whether genetically based characteristics of the child influence the development of attachment security and its stability over time. This study attempted to replicate and extend recent findings indicating that the developmental stability of attachment security is moderated by oxytocin receptor (*OXTR*) genetic variants. Using longitudinal data from over 550 individuals, there was no evidence that *OXTR* rs53576 moderated the association between attachment security during early childhood and overall coherence of mind (“security”) during the Adult Attachment Interview at age 18 years. Additional analyses involving a second commonly investigated *OXTR* variant (rs2254298) and indices of individuals’ dismissing and preoccupied attachment states of mind also failed to provide robust evidence for oxytonergic moderation of the stability in attachment security across development. The discussion focuses on research strategies for investigating genetic contributions to attachment security across the life span.

Keywords: attachment, stability and change, oxytocin, candidate genes, replication

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One of attachment theory’s central assumptions is that the mental representations of close relationships that individuals construct within their early attachment relationships are relatively stable across development (Bowlby, 1988; Sroufe, Egeland, Carlson, & Collins, 2005). More precisely, a secure attachment relationship during early childhood is expected to provide a foundation for a more secure attachment state of mind by young adulthood. Although individual longitudinal studies in this area have produced widely varying results, recent findings from the two largest sample investigations indicate that the test-retest stability of security from early childhood to young adulthood is quite modest. Specifically, in both the elevated-risk Minnesota Longitudinal Study of Risk and Adaptation (MLSRA) cohort ($N \approx 140$; Raby, Cicchetti, Carlson, Egeland, & Collins, 2013) and the normative risk NICHD Study of Early Child Care and Youth Development

(SECCYD) cohort ($N = 857$; Groh et al., 2014) the test-retest stability of security from early childhood to young adulthood was equivalent to a small effect size (i.e., a correlation of $\sim .15$; see also Piquart, Feussner, & Ahnert, 2013).

The evidence of rather modest stability in security over the first two decades of life has motivated research into the sources of change in attachment security across development. To date, much of the research on this topic has focused on the role of experiences within the family during childhood and adolescence that might account for shifts in attachment security from infancy to young adulthood. However, attachment scholars have proposed that all children may not be equally affected by these later experiences (e.g., Bowlby, 1988; Lewis, Feiring, & Rosenthal, 2000; Thompson, 2006; Waters, Weinfield, & Hamilton, 2000). Specifically, genetically based characteristics of the child have been hypothesized to shape or constrain the stability of attachment security by influencing individuals’ perceptions of and responses to subsequent interpersonal experiences in ways that render attachment-related representations more or less resistant to change. This hypothesis integrates ideas from both temperament research and attachment theory, perspectives that have historically offered competing explanations of the origins of attachment security (Vaughn, Bost, & van IJzendoorn, 2008).

In addition, theory and research on the neurobiology of social development have highlighted the importance of the oxytocin system for interpersonal functioning across the life span. For

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example, intranasal administration of oxytocin and endogenous peripheral levels of oxytocin and have been associated with individual differences in social cognition and behavior among infants, children, and adults (Feldman, 2012; Galbally, Lewis, van IJzendoorn, & Permezel, 2011; van IJzendoorn & Bakermans-Kranenburg, 2012). Experimental research with animal models and correlational studies with humans also suggest that variations in the oxytocin receptor gene (*OXTR*) contribute to interpersonal outcomes (e.g., Carter, 2014; Donaldson & Young, 2008; Ebstein, Israel, Chew, Zhong, & Knafo, 2010). In particular, in some studies the G variant of the rs53576 single nucleotide polymorphism (SNP) has been associated with enhanced processing of social-emotional information, including—most importantly for the present investigation—improved memory of social stimuli and experiences.

Despite its theoretical significance, researchers have only recently begun to test the idea that genetic factors may moderate the effects of early relationship experiences on attachment security during adulthood (e.g., Bakermans-Kranenburg, van IJzendoorn, Caspers, & Philibert, 2011; Reiner & Spangler, 2010). Because initial studies were limited by their reliance on retrospective measures of the early caregiving environment, Raby et al. (2013) investigated the potential genetic contributions to attachment stability using prospective data from the higher-risk MLSRA. They reported evidence that *OXTR* moderated the stability of attachment security, such that infant attachment security predicted the security of adults' attachment representations, as measured by the Adult Attachment Interview (AAI), only for individuals carrying the G/G variant of the *OXTR* rs53576 SNP. This finding was relatively robust within the MLSRA, as *OXTR* rs53576 moderated the association between infant attachment and AAI security measured at two time-points. Moreover, this finding was specific to variation in *OXTR*, as similar effects were not observed for the serotonin transporter or dopamine D4 receptor variable number tandem repeat polymorphisms. Based on these findings, Raby et al. (2013) proposed that *OXTR* may facilitate the consolidation of early attachment representations and their preservation across development. Specifically, the rs53576 G/G genotype was hypothesized to render early attachment representations more resistant to change across development compared to A allele carriers.

The findings from Raby et al. (2013) are potentially important for our understanding of stability and change in attachment for several reasons. First, Raby et al. (2013) provided initial longitudinal evidence for a widely proposed but rarely tested idea; namely, that the degree to which attachment representations established during early childhood are carried into adulthood may partially depend on individuals' genetic characteristics. Second, the identification of genetically distinct subsets of individuals who are more or less likely to exhibit stability in attachment security may help account for the modest overall stability of attachment security. Third, the Raby et al. (2013) findings advance our understanding of the neurobiology of attachment by providing evidence that oxytonergic variants contribute to the stability of attachment security across development. As previously discussed, these findings are consistent with other evidence from humans and nonhuman animals that highlight the importance of the oxytocin system and its underlying genetic components for close relationship functioning (e.g., Carter, 2014; Donaldson & Young, 2008; Ebstein et al., 2010).

As Raby et al. (2013) noted, it is nonetheless critical for their initial findings to be replicated with additional longitudinal data since candidate gene studies have proven difficult to replicate. For example, using data from the SECCYD, Roisman and Booth-LaForce (2014) reported that commonly investigated molecular genetic variants, including the two *OXTR* SNPs noted above, did not consistently moderate the associations between caregiving experiences during childhood and adolescence and AAI attachment states of mind at age 18 years. Importantly, however, Roisman and Booth-LaForce (2014) did not directly address the question of genetic moderation of *stability* in attachment security, as caregiving quality is conceptually and empirically distinct from early attachment security. In addition, a recent meta-analysis indicated that the two most intensively studied *OXTR* SNPs (rs53576 and rs2254298) are not reliably associated with biological, psychological, or behavioral outcomes in humans (Bakermans-Kranenburg & van IJzendoorn, 2014). For these reasons, we attempted to replicate the findings of Raby et al. (2013) using AAI data collected during an age 18 year follow-up of the SECCYD cohort. The SECCYD dataset is well suited for this purpose because it contains measures of attachment that closely parallel those collected by the MLSRA as well as molecular genetic data for a subsample of over 550 participants.

We also sought to extend research on this topic in two ways. First, we included information about rs53576, which was the focus of Raby et al. (2013), as well as rs2254298, a second *OXTR* SNP available within the SECCYD dataset. Both SNPs are common in investigations of the oxytonergic correlates of socioemotional behavior (Bakermans-Kranenburg & van IJzendoorn, 2014). Second, we included dimensional indices of adults' dismissing and preoccupied states of mind, which have been shown to underlie AAI attachment security (e.g., Haltigan, Roisman, & Haydon, 2014). This allowed us to examine whether the interaction between *OXTR* polymorphisms and early childhood attachment security is unique to a specific form of attachment (in)security.

Method

Participants

Analyses for this report are based on the subsample of participants from the SECCYD who completed at least two assessments of attachment security during early childhood, provided DNA information at the age 15 year assessment, and completed the AAI at the age 18 follow-up assessment ($N = 579$). Sample sizes further varied as a function of the availability of genetic data for each polymorphism ($n = 564$ for *OXTR* rs54576; $n = 576$ for *OXTR* rs2254298). Informed consent was acquired from parents during childhood and when the age 18 assessment occurred prior to participant age 18. Assent was acquired from participants during early childhood, and informed consent was acquired from the participants when they were age 18 years or older.

Measures

Early childhood attachment security. Three measures of attachment security were collected during early childhood: the Strange Situation Procedure (SSP; Ainsworth, Blehar, Waters, & Wall, 1978) was completed at 15 months, the Attachment Q-Set

(AQS; Waters & Deane, 1985) was completed at 24 months, and the Modified Strange Situation Procedure (MSSP; Cassidy, Marvin, & the MacArthur Working Group on Attachment, 1992) was completed at 36 months. Interrater agreement was 83% ($\kappa = .69$) for the SSP assessment and 76% ($\kappa = .58$) for the MSSP assessment. The intraclass correlation for the AQS was .96. Attachment security information at these three ages was aggregated by following the decision rules described by Groh et al. (2014). First, dichotomous measures of attachment security were created at each age. For the SSP and MSSP, children were classified as secure or insecure (avoidant, resistant, disorganized/unclassifiable). For the AQS, children whose Q-sorts were correlated at .30 or above with the security criterion sort were classified as secure (vs. insecure). Next, a composite measure of early childhood attachment security was created for participants with two or more early attachment assessments by dividing number of times the child was classified as securely attached by the total number of attachment assessments available for that child ($M = .61$, $SD = .29$).

Adult attachment security. At approximately age 18 years, participants completed the AAI, an hour-long, semistructured interview wherein participants describe their childhood relationships with their parents and recall specific incidents of separation, rejection, loss and trauma. AAI transcripts were rated by six trained and certified coders. The current analyses focused on the 9-point coherence of mind scale drawn from the Main and Goldwyn (1984-1998) coding system and the dismissing and preoccupied state of mind prototype scores from Kobak's (1993) Adult Attachment Interview Q set. The 9-point coherence of mind rating captures the extent to which the individual discusses and freely evaluates their childhood attachment experiences in an organized and emotionally contained manner and was used by Raby et al. (2013) as a dimensional measure of the overall security of individuals' attachment states of mind ($M = 5.03$, $SD = 1.42$). The AAI Q set dismissing state of mind dimension reflects the degree to which the individual freely evaluates (or defensively dismisses) early childhood experiences with caregivers ($M = -0.02$, $SD = 0.40$), whereas the preoccupied state of mind dimension reflects the degree to which the individual becomes emotionally dysregulated while discussing early experiences with caregivers and experiences of loss or trauma ($M = -0.25$, $SD = 0.21$).

Interrater reliability was calculated based on a subsample of 178 AAIs (21% of the total AAI sample). The intraclass correlation for the coherence of mind rating was .85. For the AAI Q set data, 90% of the reliability cases had reliability estimates of .6 or higher (after Spearman-Brown correction). For those cases in which the two coders were unreliable, a third (and rarely a fourth) coder rated the transcript and data from the two coders with the highest reliability above .60 were averaged and used in analyses (final $M = .77$, $SD = .08$).

Oxytocin SNPs. Participants provided buccal cheek cells for DNA analysis when they were age 15 years. DNA extraction and genotyping were performed at the Genome Core Facility in the Huck Institutes for Life Sciences at Penn State University under the direction of Deborah S. Grove, Director for Genetic Analysis. Taqman SNP Genotyping Assays were performed using an Allelic Discrimination Assay protocol (Applied Biosystems, Foster City, CA). Forty nanograms of DNA were combined in a volume of 5 μ l with 2X Universal PCR Mix (Applied Biosystems) and 1/20 the volume of the Taqman SNP assay in a 384 well plate. A Pre-Read

was performed and then PCR as follows: a 10 min hold at 95 °C, followed by 40–45 cycles of 15 sec at 92 °C, and then 1 min at 60 °C in a 7900HT PCR System. After amplification, a Post-Read was performed to analyze. Automatic and manual calls were made.

In this subsample, 11% ($n = 64$) of the samples were genotyped twice in order to calculate reliability, with discrepancies resolved via a third genotyping. For *OXTR* rs53576, there was 92% agreement ($\kappa = .86$, $p < .001$), and 4% of available samples could not be genotyped. For *OXTR* rs2254298, there was 100% agreement, and 2% of available samples could not be genotyped. Both the *OXTR* rs53576 (A/A, $n = 62$; A/G, $n = 266$; G/G, $n = 236$; $\chi^2[1] = 1.02$, $p = .31$) and *OXTR* rs2254298 (A/A, $n = 7$; A/G, $n = 129$; G/G, $n = 440$; $\chi^2[1] = 0.52$, $p = .47$) genotype distributions were in Hardy–Weinberg equilibrium. Because the functional significance of these SNPs and whether they function in a dominant or additive manner is presently unknown, analyses were conducted with both additive and dominant codings of the genetic data. Since results were essentially identical, only the results of the genetic dominance models ($G/G = 0$; A/A or $A/G = 1$) are presented below in order to parallel the findings reported by Raby et al. (2013). The two *OXTR* SNPs were not correlated with one another when coded using an additive or a dominance model.

Analytic Approach

Separate linear regressions were conducted for the three adult attachment outcomes and for the two *OXTR* SNPs. Terms representing the main effects of early childhood attachment security and *OXTR* variation were entered in an initial step, followed by terms representing the interactions between early childhood attachment security and the *OXTR* SNPs. In order to maximize statistical power and parallel the analyses of Raby et al. (2013), child ethnicity (1 = White/non-Hispanic vs. 0 = other) was included as a control for potential confounding due to population stratification. Follow-up analyses based on only the 80% of the sample that that was White/non-Hispanic yielded results that were identical to those reported below.

Power Analysis

The current sample of approximately 550 SECCYD participants is nearly four times the size of the sample used in Raby et al. (2013; $N = 143$) and sufficiently powered to detect interaction effects that are comparable in magnitude to those reported in the original study ($R^2 \approx .02$). More specifically, the current study had 80% power to detect interaction effects that explain approximately 1% of the variance in the outcome variables (Duncan & Keller, 2011).

Results

Preliminary Analyses

Preliminary analyses were conducted to ensure that the results with the current subsample are consistent with previous reports based on larger samples of SECCYD participants. Consistent with Groh et al. (2014), attachment security during early childhood modestly predicted more coherence ($r = .16$, $p < .001$), lower dismissing scores ($r = -.14$, $p = .001$), and lower preoccupied

scores during the AAI at age 18 ($r = -.13, p = .001$). Consistent with Roisman, Booth-LaForce, Belsky, Burt, and Groh (2013), *OXTR* rs53576 and *OXTR* rs2254298 were not associated with early childhood attachment security (all $r_{\text{partial}} < .03$). Similarly, *OXTR* variants were not associated with AAI coherence or preoccupied states of mind (all $r_{\text{partial}} < .06$). However, as reported by Roisman and Booth-LaForce (2014), dismissing attachment states of mind were modestly associated with *OXTR* rs2254298 ($r_{\text{partial}} = .12, p = .003$) but not *OXTR* rs53576 ($r_{\text{partial}} = .02, p = .66$).

Predicting AAI States of Mind From Early Childhood Attachment Security \times *OXTR* Interactions

Focal analyses examined whether the modest stability in attachment security was moderated by polymorphisms in the oxytocin receptor gene. We initially focused on predicting coherence of mind during the AAI (see Tables A1 and A2 in the online supplemental materials). Results provided no evidence that *OXTR* rs53576 or *OXTR* rs2254298 conditioned the associations between early childhood attachment security and AAI coherence.

Next, we evaluated whether the association between early childhood attachment security and dismissing states of mind was moderated by *OXTR* rs53576 or *OXTR* rs2254298 (see Tables A3 and A4 in the online supplemental materials). Once again, two-way interactions involving early childhood attachment security and the *OXTR* variants were not significant.

Finally, we tested whether the association between early childhood attachment security and preoccupied states of mind was moderated by the *OXTR* SNPs. All two-way interactions involving early childhood attachment security and *OXTR* rs53576 or *OXTR* rs2254298 variants were not significant (see Tables A5 and A6 in the online supplemental materials).

Robustness Checks

Because the MLSRA sample featured in Raby et al. (2013) is composed exclusively of participants born into poverty and the SECCYD includes participants from a wide range of family incomes, we tested whether *OXTR* moderation of attachment stability was further conditional on family income. These analyses were conducted in order to approximate, to the extent possible, the demographic characteristics of the participants featured in Raby et al. (2013), and we had no hypotheses regarding potential biological or social processes that may explain why genetic moderation might vary as a function of families' incomes. Although preoccupied attachment states of mind were predicted by the interaction between early childhood attachment security, *OXTR* rs53576, and poverty status at the time of the child's birth, this three-way interaction did not survive corrections for multiple testing and was not robust to an alternate operational definition of family financial resources (see the online supplemental materials for more information).

At the request of reviewers, we also conducted a set of additional tests using each of the three early attachment security measures and indices of infant attachment avoidance and attachment resistance. These analyses did not yield robust evidence that *OXTR* moderation of the stability of attachment security varied as a function of when early attachment security was assessed or the specific form of attachment (in)security.

Discussion

The current study used longitudinal data from over 550 individuals who participated in the age 18 year follow-up of the SECCYD cohort in order to attempt to replicate and extend recent evidence indicating that variants in the oxytocin receptor gene moderate the stability of attachment security from early childhood to young adulthood. Consistent with prior findings, the overall stability of attachment security was modest in this subsample (see Groh et al., 2014, for findings with the full sample). Moreover, despite having a sample nearly four times larger the original study by Raby et al. (2013), the current study failed to replicate the evidence that individuals homozygous for the rs53576 G allele exhibit more stability in attachment security from early childhood to young adulthood compared to A allele carriers. Additional analyses using a second commonly investigated *OXTR* SNP (rs2254298) and indices of the more specific forms of attachment (in)security during young adulthood produced no evidence for oxytonergic moderation of the associations between attachment security during early childhood and individuals' attachment states of mind at age 18 years.

Altogether, the results from this large-sample, longitudinal study failed to provide robust evidence that the two most commonly investigated oxytonergic polymorphisms moderate the stability of attachment security from early childhood to early adulthood. Nonetheless, it would be premature to conclude from these findings that genetic factors have no role in the development of adult attachment states of mind or the stability of attachment security over time. Subtle differences between the present study and the investigation by Raby et al. (2013)—such as the genetic ancestries of the samples, the ages the AAIs were administered, or base rates of the more specific forms of attachment (in)security—could potentially account for the discrepant findings. Oxytonergic contributions to attachment stability might also be substantially smaller in magnitude than originally estimated and therefore could not be detected as statistically significant in this study despite its relatively large sample size. Lastly, it is possible that molecular genetic variants not included in our analyses are involved in moderating the stability of attachment security across development.

Given the many failures replicating candidate gene findings in the attachment domain (e.g., Roisman & Booth-LaForce, 2014; Roisman et al., 2013), we recommend two ways of moving forward. First, molecular-genetic investigations will require the use of very large sample sizes to have sufficient statistical power to reliably detect the individual contributions of specific genetic variants. Relatedly, attachment researchers should consider utilizing data-driven approaches, such as genome wide association studies, along with the continuing effort to attempt to replicate candidate gene findings with large, independent samples. Second, the implementation of well-powered quantitative behavioral genetic investigations would provide much needed estimates of the heritability of adult attachment states of mind and their stability over time. Although a recent large-sample twin study indicated there are substantial heritable contributions to attachment security during mid-adolescence (Fearon, Shmueli-Goetz, Viding, Fonagy, & Plomin, 2014), relevant research with adults has been based on relatively small samples and has not been conclusive (e.g., Caspers, Yucuis, Troutman, Arndt, & Lang-

behn, 2007; Torgersen, Grova, & Sommerstad, 2007). Future research of this type would be valuable in advancing our understanding of the developmental origins of attachment outcomes over the life course.

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