Research paper

Maternal *DRD2, SLC6A3,* and *OXTR* genotypes as potential moderators of the relation between maternal history of care and maternal cortisol secretion in the context of mother-infant separation

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ABSTRACT

A mother's cortisol secretion is importantly associated with her own mental health and her infant's cortisol secretion. This study investigated the influences of maternal history of care and maternal *DRD2, SLC6A3,* and *OXTR* genotypes on maternal cortisol in the context of infant stress. A community sample of 296 mother-infant dyads completed a maternal separation at infant age 17 months. Maternal salivary cortisol, buccal cells, and self-reported history of care were collected. Multilevel models revealed that history of care had a greater influence on maternal baseline cortisol (but not cortisol trajectory) for mothers with more plasticity alleles of *SLC6A3* (10R) and *OXTR* (G), relative to mothers with fewer or no plasticity alleles. Findings indicate that a mother's history of care is related to her cortisol secretion in anticipation of infant stress, but that this relation depends on her genetic characteristics. Findings are discussed in relation to the maternal protective system and anticipatory cortisol secretion.

1. Introduction

Patterns of cortisol secretion are set early in life (Gunnar & Donzella, 2002), are transmitted across generations (Yehuda et al., 2000) from mother to infant (Atkinson et al., 2013), and have important health implications, playing a role in nearly all physical and mental health conditions (Jessop & Turner-Cobb, 2008). As such, a mother's cortisol secretion is particularly important to examine, given that it has implications for her own health as well as her infant's cortisol secretion and health (Atkinson, Jamieson, Khoury, Ludmer, & Gonzalez, 2016; Debiec & Sullivan, 2014; Gunnar & Hostinar, 2015). For example, lower levels of maternal cortisol secretion in the context of infant stress are associated with lower levels of postpartum depressive symptoms, and may function to regulate and protect the infant's physiological response, i.e., buffer against infant cortisol hyperreactivity (Khoury et al., 2016). Currently, there is a dearth of research on maternal cortisol secretion in the context of infant stress (Atkinson et al., 2016). Thus, the

aim of this study was to examine the early environmental and genetic predictors of maternal cortisol secretion in the context of infant stress.

In terms of early environmental predictors, a mother's history of care that she received in early childhood is a well-established determinant of her cortisol secretion. For example, Tyrka, Price, Marsit, Walters, and Carpenter (2012) found that, in healthy adults, low levels of childhood parental care (as assessed with the Parental Bonding Instrument, Parker, Tupling, & Brown, 1979) are associated with increased methylation of the glucocorticoid receptor gene, which in turn predicts atypically attenuated cortisol responses to the dexamethasone/corticotropin-releasing hormone test (i.e., when dexamethasone and corticotropin-releasing hormone are administered). Tyrka et al.'s (2012) findings occurred over and above the effects of current depressive symptoms and perceived stress, suggesting the importance of early parenting on human epigenetics and cortisol. Perhaps as a result of such epigenetic influences (e.g., Jawahar, Murgatroyd, Harrison, & Baune, 2015; Perroud et al., 2011; Perroud et al., 2014), the

effects of a mother's history of care on her cortisol patterns are enduring, and can last until older adulthood (Engert et al., 2010).

The intergenerational transmission of cortisol patterns also suggests that genetic factors may play important roles in impacting cortisol secretion, and may do so in interaction with the early environment (e.g., Bakermans-Kranenburg, van IJzendoorn, Mesman, Alink, & Juffer, 2008; Ludmer et al., 2015). Pertinent to this discussion are the definitions of various theories of gene x environment (GxE) interactions. *Diathesis-stress* theory posits that individuals with specific genetic characteristics are genetically vulnerable to the adverse effects of negative rearing environments. In contrast, *differential susceptibility* theory suggests that genetically "susceptible" individuals experience *both* the worst outcomes if reared in impoverished environments *and* the best outcomes if reared in enriched environments (Belsky & Pluess, 2009). *Vantage sensitivity* suggests that individuals with specific genetic characteristics are exclusively susceptible to the positive effects of enriched environments. Research pertinent to how differential context associates with each of the three GxE theories is extremely rare, such that it is impossible to make specific hypotheses in this regard (Del Giudice, 2016). For example, Dalton, Hammen, Najman, and Brennan (2014) found that youth genotype interacted with family environment quality to predict youth depression at age 15 in a differential susceptibility manner, but that this same GxE interaction predicted depression after age 15 in a diathesis-stress manner. Other studies have found the GxE model to differ for the same interaction depending on the informant (i.e., parent versus teacher, Roisman et al., 2012), type of psychosocial challenge (Ludmer et al., 2015), and adult attachment style (Cassidy, Woodhouse, Sherman, Stupica, & Lejuez, 2011).

The ambiguity regarding the contexts in which the different GxE theories emerge is compounded by lack of statistical symmetry across studies in this area. To address this issue, Roisman et al. (2012) proposed stringent statistical criteria for establishing the GxE models, which have been adopted in several recent studies (e.g., Beach et al., 2014; Dalton et al., 2014; Ludmer et al., 2015). These include: i) *Regions of Significance on environmental factors* (*RoS on X*): demonstration that the outcome variable and the plasticity genotype are correlated at high and/or low ends of the environmental variable (Roisman and colleagues recommend bounding by $+/-2SD$ from the mean of the environmental variable); ii) *Proportion of interaction index (PoI):* ratio of improved outcomes for the plasticity genotype over the sum of improved outcomes and harmful outcomes; and iii) *Linearity:* apparent differential susceptibility effects can be artefacts of imposing a linear predictor model on a nonlinear diathesis stress or vantage sensitivity phenomenon, and thus analyses should be repeated when introducing quadratic effects: (environmental variable)^2 and genotype x (environmental variable)^2. While these statistics are an important step toward clarifying the contexts in which each GxE model occurs, the RoS on X test is biased by sample size, power, and environmental ranges, and the PoI index lacks clear statistical guidelines regarding which values indicate which GxE interaction type (Del Giudice, 2016; Roisman et al., 2012). Given our limited understanding of GxE models and the statistics necessary to differentiate between them (Del Giudice, 2016), it appears that, at this time, the crucial piece of information is not the type of interaction, but the fact that there is an interaction.

Additional statistical refinements to GxE research that have subsequently been proposed include presenting results without "binning" alleles (e.g., Bradley et al., 2011; Ludmer et al., 2015; Villani et al., in press). Binning alleles involves creating dichotomous groups of "plasticity genotype" and "non-plasticity genotype" individuals, and it is problematic because in many cases it unjustifiably assumes allele dominance in heterozygous individuals (Ludmer et al., 2015). To avoid such ambiguities, it is important to code all genes without "binning", i.e., by tallying the number of candidate alleles (an individual homozygous for plasticity alleles would be scored 2, an individual heterozygous for plasticity and non-plasticity alleles would be scored 1, and an individual homozygous for non-plasticity alleles would be scored 0).

With further regard to methodological caution, the current study focuses specifically on three candidate genes chosen a priori that may interact with maternal history of care to influence maternal cortisol secretion: dopamine receptor (*DRD2*), dopamine transporter (*SLC6A3*), and oxytocin receptor (*OXTR*). The *DRD2* gene is localized to chromosome 11q23 and the single nucleotide polymorphism (SNP) rs1800497 (Taq1A) involves a C to T substitution and resides in the overlapping *ANKK1* (ankyrin repeat and kinase domain containing 1) gene (Neville, Johnstone, & Walton, 2004). The A1 and A2 alleles correspond to the A and G alleles, respectively. The A1 allele has been associated with dysregulated hypothalamic-pituitary-adrenal (HPA) function (Belda & Armario, 2009) as well as with heightened susceptibility to environmental influences (Belsky & Pluess, 2009). With respect to *SLC6A3,* a 40-base pair VNTR downstream of this gene has been found to alter the density of the dopamine transporter protein *in vitro* differentially based on the presence or absence of 9- or 10-repeat alleles (VanNess, Owens, & Kilts, 2005). The 10-repeat allele (10R) is associated with dysregulated HPA function (Alexander et al., 2011) as well as with heightened susceptibility to environmental influences (Belsky & Pluess, 2009).

DRD2 and *SLC6A3* were selected for the current study given i) the influence of dopamine on parent-child interactions (Van IJzendoorn, Bakermans-Kranenburg, & Mesman, 2008), ii) the influence of dopamine on the medial prefrontal cortex and amygdala, which regulate HPA function (e.g., Pruessner, Champagne, Meaney, & Dagher, 2004; Zhang, Chretien, Meaney, & Gratton, 2005), and iii) our previous findings that infant *DRD2* and *SLC6A3* genotypes moderate the association between maternal depressive symptoms and infant cortisol reactivity (Ludmer et al., 2015). Conceptual replication of Ludmer et al.'s (2015) GxE findings is needed, given that only 27% of GxE replication attempts are statistically significant, compared to 96% of novel GxE studies (Duncan & Keller, 2011). As such, to conceptually replicate Ludmer et al.'s (2015) findings, the current study assesses whether *maternal DRD2* and *SLC6A3* genotypes interact with the mother's *own* early caregiving environment (i.e., history of care) to influence *maternal* cortisol secretion in the context of infant stress.

Further extending Ludmer et al.'s (2015) findings, genes related to oxytocin function may also play a role in maternal cortisol secretion, given that the two central functions of oxytocin are to enable social (e.g., parental) bonding and to reduce stress (e.g., Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Gordon et al., 2008). The *OXTR* gene, located on chromosome 3p25, containing four exons and three introns, has been found to impact social behavior and stress physiology (e.g., Rodrigues, Saslow, Garcia, John, & Keltner, 2009). A SNP in the third intron, rs53576 (G/A), is of interest given associations with cortisol reactivity (Chen et al., 2011; Norman et al., 2012), parenting (Bakermans-Kranenburg & van IJzendoorn, 2008; Bradley et al., 2011), and maternal differential susceptibility (e.g., Sturge-Apple, Cicchetti, Davies, & Suor, 2012). For example, in a low-risk community sample, mothers with GG genotypes (i.e., genotypes signifying more efficient oxytocin function) display more observable sensitivity to their toddlers (Bakermans-Kranenburg & van IJzendoorn, 2008). In a sample of healthy male students participating in the Trier Social Stress Test either alone or with social support (operationalized as having the help of a close female friend to prepare for the task), Chen et al. (2011) found that only G carriers showed lower cortisol responses to the test in the context of social support (reflecting vantage sensitivity). In a low-income sample, Bradley et al. (2011) found that individuals with the G/G genotype, relative to individuals with other genotypes, reported the most difficulty with emotion regulation (an important correlate of cortisol reactivity, e.g., Quirin, Kuhl, & Düsing, 2011) if they reported a high history of childhood maltreatment (reflecting diathesis stress). Thus, given the role of *OXTR* in cortisol secretion, maternal bonding, and susceptibility to the influences of childhood care, *OXTR* genotypes may moderate the degree to which maternal history of care impacts maternal cortisol secretion in the context of infant stress.