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Genetic sensitivity to the caregiving context: The influence of 5httlpr and BDNF val66met on indiscriminate social behavior☆,☆☆

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ABSTRACT

Evidence that gene × environment interactions can reflect differential sensitivity to the environmental context, rather than risk or resilience, is increasing. To test this model, we examined the genetic contribution to indiscriminate social behavior, in the setting of a randomized controlled trial of foster care compared to institutional rearing. Children enrolled in the Bucharest Early Intervention Project (BEIP) were assessed comprehensively before the age of 30 months and subsequently randomized to either care as usual (CAUG) or high quality foster care (FCG). Indiscriminate social behavior was assessed at four time points, baseline, 30 months, 42 months and 54 months of age, using caregiver report with the Disturbances of Attachment Interview (DAI). General linear mixed-effects models were used to examine the effect of the interaction between group status and functional polymorphisms in Brain Derived Neurotrophic Factor (BDNF) and the Serotonin Transporter (5htt) on levels of indiscriminate behavior over time. Differential susceptibility, relative to levels of indiscriminate behavior, was demonstrated in children with either the s/s 5httlpr genotype or met 66 BDNF allele carriers. Specifically children with either the s/s 5httlpr genotype or met66 carriers in BDNF demonstrated the lowest levels of indiscriminate behavior in the FCG and the highest levels in the CAUG. Children with either the long allele of the 5httlpr or val/val genotype of BDNF demonstrated little difference in levels of indiscriminate behaviors over time and no group × genotype interaction. Children with both plasticity genotypes had the most signs of indiscriminate behavior at 54 months if they were randomized to the CAUG in the institution, while those with both plasticity genotypes randomized to the FCG intervention had the fewest signs at 54 months. Strikingly children with no plasticity alleles demonstrated no intervention effect on levels of indiscriminate behavior at 54 months. These findings represent the first genetic associations reported with indiscriminate social behavior, replicate previous gene × gene × environment findings with these polymorphisms, and add to the growing body of literature supporting a differential susceptibility model of gene × environment interactions in developmental psychopathology.

1. Introduction

Gene × environment studies have been a source of both tremendous enthusiasm as well as frustration. Although the initial demonstration of gene × environment interactions were promising [1], failed replications of both candidate gene and genome wide association studies (GWAS) have dampened the enthusiasm for genetic studies in psychiatry [2,3]. The lack of consistent findings appears to challenge the utility of genetic studies for complex phenotypes, as well as the standard genetic risk and resilience model. An alternative conceptualization, differential susceptibility, may offer significant insight into these discrepant findings and enhance the understanding of the influence genetic variation has on complex psychological and behavioral phenotypes. In this model, specific polymorphisms confer a differential responsiveness to the environment, instead of risk or resilience *per se* [4]. These "plasticity alleles" are predicted to enhance outcomes in positive environments yet elevate vulnerability in adverse environments [5,6]. Individuals with the non-plastic, "fixed" alleles, are expected to demonstrate few differences in behavior or

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outcomes between positive or negative environments. Randomized controlled trials provide a unique opportunity to directly test this model as there is a specific manipulation of the environmental context and established outcome measures by which responsiveness to the environmental change can be measured [7]. Randomized controlled trials of alterations of the environment, particularly the caregiving environment, though common in animal studies are rare in human research and thus represent an important source for hypothesis testing related to differential susceptibility.

Extremes of early caregiving adversity, including severe social deprivation as a result of institutional rearing, are associated with a range of clinical and behavioral problems that in normative social conditions often result in significant impairment. One behavioral construct which has been demonstrated across studies of children reared in institutions and proposed to be part of a deprivation specific pattern (as reviewed in [8]) is indiscriminate social behavior. Indiscriminate social behavior is also elevated in children exposed to early maltreatment [9]. The core features of indiscriminate behavior include lack of reticence with unfamiliar adults, inappropriate social boundaries and affection with strangers, willingness to accompany strangers, and failure to check back with a familiar caregiver when in an unfamiliar setting. While these behaviors may have an unidentified adaptive purpose in inconsistent or inadequate caregiving environments, these same behaviors in normative environments are impairing across multiple domains in part consistent with a mismatch theory of early behavior. Recently, construct and criterion validity as well as stability of indiscriminate behaviors have been reported in a longitudinal study of institutionalized children [10–12]. Despite its etiologic association with adverse caregiving [13] indiscriminate behavior persists in a significant proportion of children years after restoration of adequate caregiving environment [11,13–15] indicating that other factors contribute to both the development and the persistence of indiscriminate social behavior.

A model of genetically driven differential susceptibility in this context would predict that indiscriminate behavior in some children, "sensitive" individuals, would be quite responsive to changes in the caregiving environment whereas others, "fixed" individuals, would demonstrate little change when moved to an improved environment. This model predicts that children who carry differentially susceptible genetic alleles would demonstrate both the greatest amount of symptoms in the adverse environment and *the least amount* of symptoms in the positive environment. To test this theory directly we explored the contribution of genotype to change in levels of indiscriminate behavior in the setting of a randomized controlled trial of foster care compared to institutional rearing, the Bucharest Early Intervention Project [16–19].

The biological substrate of indiscriminate behavior is unknown. However, given the association of indiscriminate behavior with a range of negative behavioral and psychological outcomes we selected functional polymorphisms in two genes, the 5httplr in the serotonin transporter gene and the met66val polymorphism in BDNF. These genes were selected *a priori* because they have been associated with differential susceptibility in both preclinical animal studies and human research, have established roles in social behavior, and have been associated with a range of psychopathology [3,20–31]. There exists significant evidence, particularly for the 5httlpr, that individuals with the short ("s") allele, particularly those with the s/s genotye, are not only at increased risk for psychopathology with exposure to high levels of stress or adversity, but these same individuals also appear to benefit disproportionately from supportive environments [32–34]. BDNF is critically involved in neuroplasticity and neurodevelopment and BDNF levels have been found to moderate the association between early adversity and anxiety [35]. The functional val66met polymorphism has been studied across psychopathology. Although a number of studies have demonstrated that the val allele is the protective allele a recent meta-analysis revealed that the met allele was protective for neuroticism [36]. The differential impact of the met allele was also demonstrated in association with high levels of exercise and depression as well as protective relative to psychological disorders when associated with elevated fear processing [37,38]. Because variation in gene expression levels have been demonstrated during early development for both genes we further evaluated whether these genotypes exhibited any differential timing effects.

The differential susceptibility model further predicts a multiplicative genetic effect where the responsiveness to the environment may be greater in individuals with more than one plasticity allele. Given that both gene–gene and epistatic interactions have been demonstrated repeatedly with these specific polymorphisms [20,39–43] we examined both their independent and combined impact on indiscriminate social behavior. We hypothesized that cumulative genetic plasticity would be associated with the greatest sensitivity to the caregiving environment. We predicted that children with both plasticity genotypes would exhibit the greatest number of symptoms in the negative caregiving environment (institutional) but the lowest amount of symptoms in the positive caregiving environment (foster care). We further predicted that children with no plasticity alleles would exhibit little difference in indiscriminate behavior between the extreme caregiving environments [25,44,45].

2. Methods

2.1. Participants

Participants were enrolled in the Bucharest Early Intervention Project (BEIP) [46], a randomized controlled trial of foster care as an alternative to institutional care in Romania. The study sample, with inclusion and exclusion criteria, has been described elsewhere [47,48]. Briefly, participants included 136 abandoned children between 6 and 30 months of age who were living in one of six institutions in Bucharest, Romania. Following baseline assessments, 68 of the children (33 males and 35 females) were randomly assigned to care as usual (CAUG) and 68 (34 males and 34 females) were randomly assigned to foster care (FCG). Children were excluded for medical reasons including diagnosed genetic syndromes, significant evidence of fetal alcohol syndrome or microcephaly. The foster care network was created and supported by the project as an intentional alternative to institutional care [49].

Following randomization, all subsequent decisions regarding placement were made by the Romanian National Authority for Child Protection in accordance with Romanian law, with the expectation that no child removed from an institution and placed in project supported foster care would be returned to an institution. Over the four years of the project, there was considerable movement within the groups (Fig. 1). Nevertheless, all analyses that include FCG or CAUG reported herein follow intent to treat, so that children are analyzed within their originally assigned group.

At 54 months of age, 112 children from the initial randomization continued to participate in the study (53 CAUG and 59 FCG). Complete psychopathology data at all four time points, BDNF and 5httlpr genotyping were obtained on 98 (CAUG 45, FCG 53) children.

2.2. Measures

2.2.1. The Disturbances of Attachment Interview (DAI) [50]

The DAI is a semi-structured interview of the caregiver about signs of disturbed or disordered attachment behavior in the child, including signs of indiscriminate social behavior. Ratings of three items, checking back with a caregiver when in unfamiliar settings, reticence with unfamiliar adults, and willingness to "go off" with a stranger, were coded on a 3-point Likert scale, where "0" was "rarely or minimally" demonstrated a behavior, "1" was "sometimes or somewhat" demonstrates a behavior, and "2" was "clearly" demonstrates a behavior. A