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A neurogenetics approach to defining differential susceptibility to institutional care

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Abstract

An individual's neurodevelopmental and cognitive sequelae to negative early experiences may, in part, be explained by genetic susceptibility. We examined whether extreme differences in the early caregiving environment, defined as exposure to severe psychosocial deprivation associated with institutional care compared to normative rearing, interacted with a biologically informed genoset comprising *BDNF* (rs6265), *COMT* (rs4680), and *SIRT1* (rs3758391) to predict distinct outcomes of neurodevelopment at age 8 ($N = 193$, 97 males and 96 females). Ethnicity was categorized as Romanian (71%), Roma (21%), unknown (7%), or other (1%). We identified a significant interaction between early caregiving environment (i.e., institutionalized versus never institutionalized children) and the a priori defined genoset for full-scale IQ, two spatial working memory tasks, and prefrontal cortex gray matter volume. Model validation was performed using a bootstrap resampling procedure. Although we hypothesized that the effect of this genoset would operate in a manner consistent with differential susceptibility, our results demonstrate a complex interaction where vantage susceptibility, diathesis stress, and differential susceptibility are implicated.

Keywords

cognitive function, neurodevelopment, genoset, gene x environment

Introduction

Institutional rearing is characterized by multiple, rotating caregivers, unfavorable child-to-caregiver ratios, and limited social, cognitive, and language stimulation (McGoron et al., 2012; Sheridan, Drury, McLaughlin, & Almas, 2010; Smyke, Dumitrescu, & Zeanah, 2002; Smyke et al., 2007; Zeanah et al., 2003, 2009). Caregiving staff often have insufficient education and face challenging working conditions, as such attention to the individual needs of children in these environments is minimal or absent (Smyke et al., 2007). Previous studies have found both cognitive deficits and neuroanatomical differences in children exposed to early institutional rearing when compared to typically developing children (Fox, Almas, Degnan, Nelson, & Zeanah, 2011; Nelson et al., 2007; Sheridan et al., 2010; Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012). While institutional care is significantly predictive of negative neurocognitive outcomes, individual differences in both the impact of early adversity, as well as the degree of recovery following placement in improved caregiving environments, has been demonstrated (Fox et al., 2011).

Increasing evidence, including studies of children exposed to institutional care, suggests that genetic variation may confer a differential susceptibility to the environment, rather than specific vulnerability or resilience (Beaver, Wright, DeLisi, & Vaughn, 2012; Drury et al., 2012; Karlson et al., 2010; Simons et al., 2011; Zheng et al., 2008). Differential susceptibility predicts that specific genetic variants influence responsivity to the environment, in a "for better or worse manner," such that the same variant that leads to negative outcomes in a negative environment, such as institutional care, would be associated with the most positive outcome in positive caregiving settings (Belsky, Pluess, & Widaman, 2013; Roisman et al., 2012). Vantage sensitivity, a recent extension of differential susceptibility, extends this hypothesis further and posits that some genetic variants only confer positive benefits in positive environments, with no disadvantage seen in negative environments (Pluess & Belsky, 2013). The responsivity, in either model, is predicted to occur via the influence of these genetic variants on the underlying neurobiological circuits implicated in both neurodevelopment and response to caregiving (Bakermans-Kranenburg & van Ijzendoorn, 2007, 2011; Belsky & Beaver, 2011; Belsky & Pluess, 2009; Pluess & Belsky, 2013). Given the polygenic nature of neurodevelopment, analysis of the cumulative impact of well-defined genes known to interact (i.e., "genosets") is expected to be more predictive of complex neurocognitive phenotypes than single gene studies (Nikolova, Ferrell, Manuck, & Hariri, 2011). Careful consideration of several factors is required when defining the specific genes incorporated into a particular genoset.

Previous studies comparing neurodevelopmental outcomes in typically developing children compared to those with a history of institutional care have reported significant differences, particularly

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in the prefrontal cortex (PFC) (Behen et al., 2009; Chugani et al., 2001; Hostinar, Stellern, Schaefer, Carlson, & Gunnar, 2012; Sheridan et al., 2012). Specifically, post-institutionalized (PI) children have been found to have more diffuse connectivity and decreased glucose metabolism in the PFC (Behen et al., 2009; Chugani et al., 2001). Gee and colleagues (2013) extended these findings and demonstrated differential amygdala to PFC connectivity in PI youth compared to children from typical families. In rodent models maternal deprivation was associated with altered prefrontal dendritic growth (Pascual & Zamora-Leon, 2007). In addition, our previous results have demonstrated decreased gray matter volume in children with a history of institutionalization (Sheridan et al., 2012). The link between PFC function and measures of executive function (EF) is well-established (Kane & Engle, 2002; Miller, Erickson, & Desimone, 1996; Surmeier, 2007). Two established EF tasks from the Cambridge Automated Neurocognitive Testing Battery (CANTAB) associated with PFC function are the Spatial Working Memory (SWM) and Stockings of Cambridge (SOC) tasks (Luciana & Nelson, 2000). Functional neuroimaging studies have linked SWM performance to the dorsal and ventral lateral PFC (Nelson et al., 2000; Owen, Doyon, Petrides, & Evans, 1996). Abnormalities or damage to the PFC have been shown to result in impaired function on SWM tasks (Luna et al., 2002; Owen, Downes, Sahakian, Polkev, & Robbins, 1990). Similarly, performance on the SOC task, a test of spatial working memory and EF, has independently been associated with functioning of the dorsal and ventral lateral PFC (Baker et al., 1996; Egerhazi, Berecz, Bartok, & Degrell, 2007; Owen et al., 1996). Early institutional care and poorer performance on both SWM and SOC CANTAB tasks has been demonstrated (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009; Bos, Fox, Zeanah, & Nelson, 2009; Pollak et al., 2011). These findings suggest that studies examining the interaction between genetic factors and early institutional care ought to focus on neurocognitive outcomes specifically related to the PFC, including EF tasks and neuroanatomical measures.

The growing number of integrated gene and protein databases, such as String (string-db.org, Franceschini et al., 2013) and ConsensusPathDB (consensuspathdb.org, Kamburov, Stelzl, Lehrach, & Herwig, 2013), provide multi-level evidence of genetic regulatory, protein-protein, and signaling interactions. These databases provide a rich source of integrated information crucial to the construction of biologically informed multi-locus genosets for hypothesis testing in gene by environment (g x e) studies of complex neurocognitive traits (Bogdan, Hyde, & Hariri, 2012). Empirically constructed genosets that further incorporate a neurodevelopmental perspective, can enhance the likelihood of detecting effects, minimize the risk of false positive associations, and ideally provide initial insight into underlying mechanisms (Califano, Butte, Friend, Ideker, & Schadt, 2012; Carayol et al., 2010; Kohannim et al., 2012; Nikolova et al., 2011).

An integrated network approach was used to create a biologically informed multi-locus genoset containing three interacting genes each associated with PFC structure and function, neural plasticity, IQ, and experience-expectant neurodevelopment: Brain-derived neurotrophic factor (*BDNF*, rs6265), Catechol-O-methyltransferase (*COMT*, rs4680), and Sirtuin1 (*SIRT1*, rs3758391). The following criteria were applied to construct this genoset. Firstly, each gene was independently associated with differences in cognitive functioning (Bruder et al., 2005; Kuningas, Putters, Westendorp, Slagboom, & van Heemst, 2007; Malhotra et al., 2002; Savitz, Solms, & Ramesar, 2006; Sheldrick et al., 2008; Trotman, Cubells, Compton, & Walker, 2010), including working memory and spatial learning (Egan et al., 2003; Ho et al., 2006; Honea et al., 2009; Kuningas et al., 2007; Schulz-Heik et al., 2011; Sheldrick et al., 2008). Secondly, each gene has an established biological role in the development of the central nervous system (CNS; Bosse et al., 2012; Rahman & Islam, 2011; Winterer & Goldman, 2003). Thirdly, each gene has been associated with the PFC and development. Fourthly, as the cumulative effect was to be tested, evidence of either a direct molecular interaction or epistatic effects (i.e., where the effect of a genetic variant is altered or masked by effects of a variant in another gene (Cordell, 2002)) between the selected genes was needed. These three genes have complex, yet definable hierarchical relationships (Gao et al., 2010; Witte et al., 2012). *BDNF* is integral in neuronal survival, synaptic plasticity, and neurogenesis (Berton et al., 2006; Bosse et al., 2012; Burton et al., 2007; Chatterjee et al., 2007; Chen et al., 2004; Pencea, Bingaman, Wiegand, & Luskin, 2001). In cellular studies, the *BDNF* rs6265(*Met*) polymorphic variant has been found to have reduced activity-dependent secretion of *BDNF* in cortical neurons (Chen et al., 2004). In rodents, *BDNF* influences the proliferation of dopaminergic neurons (Burton et al., 2007; Pencea et al., 2001), regulates the release and uptake dynamics of pre-synaptic dopamine signaling (Bosse et al., 2012) and *BDNF* PFC expression has been found to be altered by maternal deprivation (Burton et al., 2007; Chatterjee et al., 2007). In children, *BDNF* rs6265 genotype was associated with subgenual anterior cingulate gray matter volume in individuals exposed to early life adversity (Gerritse et al., 2012).

The *COMT* gene encodes a post-synaptic enzyme that is the primary regulator of dopamine catabolism in the PFC (Gogos et al., 1998; Huotari et al., 2002; Mier, Kirsch, & Meyer-Lindenberg, 2010). At the cellular level studies have demonstrated that *COMT* rs4680(*Met*) results in reduced *COMT* enzyme activity (Lotta et al., 1995), indicating a potential effect of *COMT* genotype on dopamine metabolism (Mier et al., 2010; Savitz et al., 2006). Performance on SWM and SOC tasks is sensitive to dopamine levels and genetic variation in *COMT* has been found to influence working memory and decision making in both adults and children (Barnett, Heron, Goldman, Jones, & Xu, 2009; Hoare & Sevar, 2007; Roussos, Giakoumaki, Pavlakis, & Bitsios, 2008). Furthermore, in human adults, *COMT* rs4680 genotype has been shown to interact with *BDNF* rs6265 genotype to influence cortical plasticity (Savitz et al., 2006; Witte et al., 2012).

SIRT1 regulates neurodevelopmental plasticity (Gao et al., 2010; Michan et al., 2010), axonal degeneration (Araki, Sasaki, & Milbrandt, 2004), and dendritic branching and arborization (Michan et al., 2010). Molecular studies have shown *SIRT1* rs3758391(*C*) confers lower p53 binding affinity (Naqvi et al., 2010), which is expected to lead to decreased up-regulation of *SIRT1* expression via the miR-34a feedback loop (Yamakuchi, 2012). In cellular studies, *SIRT1* expression regulates dopaminergic cell apoptosis (Park, Jeong, & Kim, 2011) and dopaminergic axonal protection (Araki et al., 2004). Reduced *SIRT1* expression results in decreased micro RNA mediated up-regulation of *BDNF* expression (Gao et al., 2010). Similar to *BDNF* and *COMT*, *SIRT1* is essential for synaptic plasticity, myelination, and dendritic branching (Li, Xu, McBurney, & Longo, 2008; Michan et al., 2010). In adults, *SIRT1* has been linked to cognitive functioning (Kuningas et al., 2007).

Together these data indicate that *BDNF*, *COMT*, and *SIRT1* interact to influence neurodevelopment, via regulation of dopamine

Table 1. Demographics for ever institutionalized group and never institutionalized group groups for ethnicity, sex, and age (years).

		Group	
		Ever institutionalized group *n* (%)	Never institutionalized group *n* (%)
Ethnicity	Romanian	56 (29)	81 (42)
	Roma	36 (17)	5 (30
	Unknown	13 (7)	0 (0)
	Other	1 (1)	1 (1)
Sex	Male	56 (29)	41 (21)
	Female	50 (26)	46 (24)
Age (years)	*M* (*SD*)	8.6 (0.4)	8.4 (0.3)

system homeostasis, in an experience-dependent manner (Park et al., 2011). We therefore tested whether the interaction between this genoset and the presence or absence of early care in an institutional setting would predict PFC-related neurodevelopmental outcomes. Leveraging a longitudinal study of children exposed to institutional care and a group of children with no exposure to institutional care recruited from the same maternity hospitals in Romania, we tested the interaction between genoset and early experience on four different distinct outcomes: (1) full-scale IQ; (2) SWM; (3) the SOC; and (4) total gray matter volume of the PFC.

A priori, we assigned genotypes as "susceptible" based on existing molecular evidence, and subsequently explored the interaction in terms of differential susceptibility. High susceptibility was defined by *BDNF Met* allele carriers, *COMT Met/Met* genotype, and *SIRT1 C* carriers. We predicted that individuals with more "susceptible" genotypes would have the most positive outcomes in the positive environment (e.g., children without a history of institutionalization (never institutionalized group, NIG), but the most negative outcomes in children with exposure to the negative environment (e.g., children with a history of institutional care, the ever institutionalized group, EIG)).

Materials and methods

Participants

Participants were drawn from the Bucharest Early Intervention Project (BEIP) ($N = 193$, 97 males and 96 females) (Zeanah et al., 2003). A total of 187 children residing in any of six institution orphanages in Bucharest, Romania, were screened and 51 children excluded for medical reasons (i.e., genetic syndromes, fetal alcohol syndrome, or microcephaly). The 136 remaining children (age 6–22 months at baseline assessment), who had all spent at least half their life in institution care, comprised the EIG. Ethnicity for all groups was categorized as Romanian (71%), Roma (21%), unknown (7%), or other (1%). The study sample has previously been described in detail (Nelson et al., 2007; Zeanah et al., 2003). All subsequent decisions regarding placement after randomization were made by the Romanian National Authority for Child Protection in accordance with Romanian law. A group of comparison children comprised the NIG and were recruited from the same maternity hospitals as the EIG ($N = 87$). All children with valid genotype data and valid measures of neurodevelopment for each outcome were included ($N = 193$, EIG = 106, NIG = 87), resulting in different sample sizes for specific outcomes. Demographics for EIG and NIG groups, including ethnicity, age, and sex, are shown in Table 1.

Human subjects

The study was approved by Institutional Review Boards at Boston Children's Hospital, Harvard Medical School, Tulane University, University of Maryland, and the local commissions on child protection in each sector of Bucharest. Ethical issues have been discussed by the present authors (Zeanah, Fox, & Nelson, 2012; Zeanah et al., 2006), and others (Miller, 2009; Millum & Emanuel, 2007).

Cognitive function

Wechsler Intelligence Scale for Children. At 8 years of age, 106 EIG and 87 NIG children were assessed using the Wechsler Intelligence Scale for Children (WISC-IV; Wechsler, 2003), and had DNA available. The WISC-IV was translated into Romanian by study staff, and administered in the BEIP laboratory by trained and reliable Romanian research assistants. The standardized full-scale IQ composite score, calculated based on 10 subtest scores, was used in the present analyses. This data has been used in previous studies in this sample (Fox et al., 2011).

CANTAB. CANTAB data were available for 100 EIG and 47 NIG children. Two EF tests were used in this analysis: SWM and SOC. Validation of neurodevelopmental assessment of cognitive function using the CANTAB is described by Luciana and Nelson (2000) and is supported by a range of studies in both typically and atypically developing children and adults (Fried, Hirshfeld-Becker, Petty, Batchelder, & Biederman, 2012; Smith, Need, Cirulli, Chiba-Falek, & Attix, 2013; Torgersen, Flaatten, Engelsen, & Gramstad, 2012; Vinţan, Palade, Cristea, Benga, & Muresanu, 2012). The SWM test is a self-ordered search task that measures the subject's ability to retain spatial information and to manipulate remembered items (Chamberlain et al., 2010). The strategy score, reflecting the search strategy efficiency, was the dependent variable.

For the SOC test, the child is provided with puzzles of increasing difficulty and instructed to solve problems quickly in the least number of moves. The number of problems solved in the minimum number of moves was the dependent variable. An increased number of problems solved in the minimum number of moves is reflective of better performance.

PFC gray matter volume

Structural magnetic resonance images and DNA was available on a subset of individuals: 44 EIG and 17 NIG children. Structural magnetic resonance images were acquired at Regina Maria Health Center (Bucharest, Romania) on a Siemens Magnetom Avanto 1.5 T syngo system. Images were obtained using a transverse magnetization-prepared rapid gradient echo three-dimensional sequence (TE = 2.98 ms, TI = 1000 ms, flip angle = 8 deg, 176 slices with 1 mm × 1 mm × 1 mm isometric voxels) with a 16-channel head coil. The TR for this sequence varied between 1650 and 1910. Acquisition parameters did not differ by group membership, nor were they associated with scan quality; thus, all scans are considered together. Cortical reconstruction and volumetric segmentation, and subsequent analysis of PFC gray matter, were performed with the FreeSurfer (http://surfer.nmr.mgh.harvard.edu). Additional technical details of these procedures are described elsewhere (Sheridan et al., 2012). PFC volume was calculated utilizing the following four subdivisions: middle frontal

Table 2. *Ns* for group x alleles (continuous variable 0–3) for Wechsler Intelligence Scale for Children, Spatial Working Memory and Stockings of Cambridge.

		Alleles			
		0 *n* (%)	1 *n* (%)	2 *n* (%)	3 *n* (%)
Total, $N = 193$		15 (8)	114 (59)	56 (29)	8 (4)
Wechsler Intelligence Scale for Children	Ever institutionalized group	12 (11)	61 (58)	26 (25)	7 (7)
	Never institutionalized group	3 (3)	53 (61)	30 (35)	1 (1)
Spatial Working Memory/ Stockings of Cambridge	Ever institutionalized group	12 (12)	57 (58)	23 (23)	7 (7)
	Never institutionalized group	1 (2)	28 (60)	17 (36)	1 (2)

Table 3. *Ns* for group x alleles (low versus high) for magnetic resonance imaging.

		Alleles	
		Low (0–1) *n* (%)	High (2–3) *n* (%)
Magnetic resonance Imaging	Ever institutionalized group	32 (73)	12 (27)
	Never institutionalized group	10 (59)	7 (41)

gyrus (MFG), inferior frontal gyrus (IFG), anterior cingulate cortex (ACC), and the orbital frontal cortex (OFC).

Genotyping

DNA was extracted from buccal swabs. Genotyping for *BDNF* and *SIRT1* was performed using reverse-transcription-polymerase chain reaction (RT-PCR) and Taqman single nucleotide polymorphism (SNP) assays (*BDNF*-C__11592758_10 and *SIRT1*-C___3003909_10; Life Technologies, Carlsbad, CA, USA). PCR was performed in duplicate. *COMT* genotyping was performed using PCR and the following primers (Massat et al., 2005): 5′-ACT GTG GCT ACT CAG CTG TG-3′ and 5′-CCT TTT TCC AGG TCT GAC AA-3′. PCR was carried out in a 50 µl reaction with 10 pmol of each primer, 1.25 U unit of Ex TaqTM DNA Polymerase (TaKara Bio Inc., Otsu, Shiga, Japan), 1× Ex TaqTM Buffer, 200 µm dNTPs; 20 µl of the PCR was digested with Nla III (New England Biolabs, Ipswich, MA, USA) and size fractionated on a 4% agarose gel. Allele status was determined by fragment size. Ten percent of the samples were sequenced for confirmation of allele status. Repeat analysis was performed on 100% of the samples. Any ambiguities in amplification product or allele status were subsequently directly sequenced.

Presence of genotypes *BDNF Met* carriers, *COMT Met/Met* homozygotes, and *SIRT1 C* allele carriers were counted for each individual to create a genoset index with a range of 0–3 genotypes given the low prevalence of *BDNF Met* and *SIRT1 C* homozygotes. Distribution of alleles did not significantly differ between EIG and NIG groups (see Tables 2 and 3).

Data analytic plan

The presence of Hardy–Weinberg equilibrium for each genotype was determined with a χ^2 test for goodness of fit. Bivariate tests were used to confirm that there was no significant association between genotype and group or any demographic characteristics (i.e., sex and ethnicity) prior to analysis. Analyses were conducted to examine the independent effect of group, the independent effect of genoset, and the potential for a two-way group by genotype interaction for each of four outcomes (Full Scale IQ from the WISC; SWM strategy score and SOC problems solved in minimum moves; and PFC gray matter). For the first three outcomes, hierarchical linear regression analyses were performed using SPSS version 20 (IBM Corp.), in which a continuous genoset variable (range 0–3) and group (EIG versus NIG) were examined. Demographic variables (sex and ethnicity) were entered in Step 1 of each model, with the effect of either group or genoset entered in Step 2 to examine the independent effect of each variable over and above the effect from Step 1. In the interaction analyses, demographic variables were included in Step 1, genoset and group were included in Step 2, and the interaction term was included in Step 3. For tests in which PFC gray matter was the outcome variable, due to the significantly smaller sample size available with magnetic resonance imaging (MRI), data genotype was further grouped into "low" (0–1) and "high" (2–3). The covariates of birth weight and total intracranial volume were included as additional covariates in the model assessing PFC gray matter. For all outcomes a bootstrapping resampling procedure was used to decrease bias due to random sampling. This methodology was utilized to calculate 95% confidence intervals (CIs) given the small sample size. This process is equivalent to sampling with replacement from the empirical probability distribution function (Efron, 1979). Bootstrapping was performed using bias-corrected and accelerated (BCa) CIs, with 1000 bootstrap resamples (Efron & Tibshirani, 1993), such that for all analyses there is 95% confidence that the interval contains the true population parameter. Significant two-way interactions were probed by examining the effect of group (NIG versus EIG) at each end of the genoset variable, as well as the effect of genoset within each group.

Results

All genotypes were in Hardy–Weinberg equilibrium (*BDNF* $X^2 = 0.83$, $p = 0.36$; *COMT* $X^2 = 0.27$, $p = 0.60$; *SIRT1* $X^2 = 0.64$, $p = 0.42$). There was no association between genotype and group or genotype and demographic characteristics (i.e., sex and ethnicity) ($ps > .05$). Means, standard deviations, and correlations for the four outcome variables can be found in Table 4.

IQ

Hierarchical ordinary least squares (OLS) regression was used to examine the independent effects of both group and genotype on

Table 4. Descriptive statistics and correlations for outcome variables.

Measure	1	2	3	4	*n*	*M*	*SD*	*Range*
1. Intelligence quotient	–	–.33***	.40***	.30**	193	88.43	19.10	40–137
2. Spatial Working Memory		–	–.23**	.03	147	38.91	3.36	26–47
3. Stockings of Cambridge			–	.21†	147	5.79	1.95	0–10
4. Prefrontal cortex total gray matter				–	61	67.29	2.66	62–74

Note: †$p < .10$; **$p < .01$; ***$p < .001$. For Spatial Working Memory strategy lower score is indicative of a more efficient search strategy.

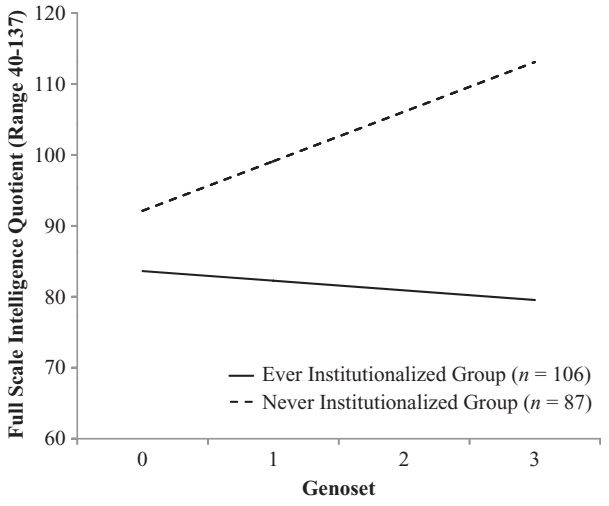

Figure 1. Wechsler Intelligence Scale for Children full-scale intelligence quotient for genoset score by group (ever institutionalized group versus never institutionalized group).

full-scale IQ, as well as examining the potential interaction of these variables.

EIG versus NIG group direct effects. Consistent with our previous report (Fox et al., 2011), in the subset of children with genetic data, a significant direct effect of group was found on the full-scale IQ composite score ($t(188) = 7.70$, B = 19.36 [95%CI: 14.49, 24.41], $p < .001$, $\Delta R^2 = .20$).

Genotype direct effects. There was no significant direct association for genoset on full-scale IQ ($t(188) = 0.89$, B = 1.73 [95%CI: –1.79, 5.64], $p = .35$, $\Delta R^2 = .004$).

EIG versus NIG group x gene interaction. A significant interaction was identified between group x genotype and full-scale IQ ($t(186) = 2.33$, $p = .021$, $\Delta R^2 = .02$), such that at low susceptibility (genoset score = 0), there was no significant difference between the EIG and NIG (B = 8.49, [95%CI: –1.36, 18.08], $p = .12$). However, at high susceptibility (genoset score = 3), a significant group difference was observed (B = 33.52 [95%CI: 21.05, 45.34], $p < .001$), where the NIG demonstrated an estimated average IQ score 33 points higher than EIG participants with the high susceptibility genoset (see Figure 1). We then examined the effect of genoset within each group separately. Genoset was a significant predictor of full-scale IQ within the NIG group (B = 7.64 [95%CI: 1.93, 13.81], $p = .012$), but not within the EIG group (B = –1.85 [95%CI: –5.35, 1.53], $p = .34$).

Spatial Working Memory

EIG versus NIG group direct effects. A direct effect of group was detected for SWM strategy ($t(141) = –2.93$, B = –1.75 [95%CI: –3.10, –0.55], $p = .008$, $\Delta R^2 = .06$), such that the NIG had significantly lower strategy scores on this task (i.e., better performance).

Genotype direct effects. No significant direct association was identified between genoset and SWM strategy ($t(141) = –1.40$, B = –0.54 [95%CI: –1.22, 0.15], $p = .14$, $\Delta R^2 = .01$).

EIG versus NIG group x gene interaction. A significant interaction was identified between group x genoset and SWM strategy score ($t(139) = –2.56$, $p = .042$, $\Delta R^2 = .04$). Posthoc analyses revealed that, in individuals with the low susceptibility genotype (genoset score = 0), there was no significant difference between the EIG and NIG (B = 1.37, [95%CI: –1.20, 4.49], $p = .37$). However, at high susceptibility (genoset score = 3), a significant group difference was observed (B = –5.51, [95%CI: –9.89, –1.97], $p = .012$), with the NIG demonstrating lower SWM scores (better performance) than EIG participants (see Figure 2(a)). The effect of genoset was examined within each group. Genoset was a significant predictor of SWM strategy score within the NIG group (B = –2.89 [95%CI: –5.47, –0.51], $p = .028$), but not within the EIG group (B = 0.04 [95%CI: –0.73, 0.87], $p = .38$).

Strategy and Spatial Planning (SOC)

EIG versus NIG group direct effects. No significant direct effect of group was found for SOC problems solved in minimum moves ($t(141) = 0.60$, B = 0.22 [95%CI: –0.40, 0.94], $p = .50$, $\Delta R^2 = .002$).

Genotype direct effects. A direct effect of genoset on SOC problems solved in minimum moves was found ($t(141) = –1.85$, B = –0.41 [95%CI: –0.82, 0.02], $p < .05$, $\Delta R^2 = .02$), such that higher genoset score was associated with lower number of problems solved (i.e., poorer performance).

EIG versus NIG group x gene interaction. A trend for an interaction was found using problems solved in minimum moves from the SOC task ($t(139) = 1.64$, $p = .051$, $\Delta R^2 = .02$). Posthoc analyses revealed that, at low susceptibility (genoset score = 0), there was no significant difference between the EIG and NIG (B = –0.92, [95%CI: –2.44, 0.53, $p = .21$). However, at high susceptibility (genoset score = 3), a significant effect for group was observed (B = 1.74, [95%CI: 0.23, 3.67], $p = .025$), with the NIG demonstrating higher SOC scores (better performance) than EIG participants (see Figure 2(b)). The effect of genoset was again examined within each group. Genoset was not a significant predictor of SOC score

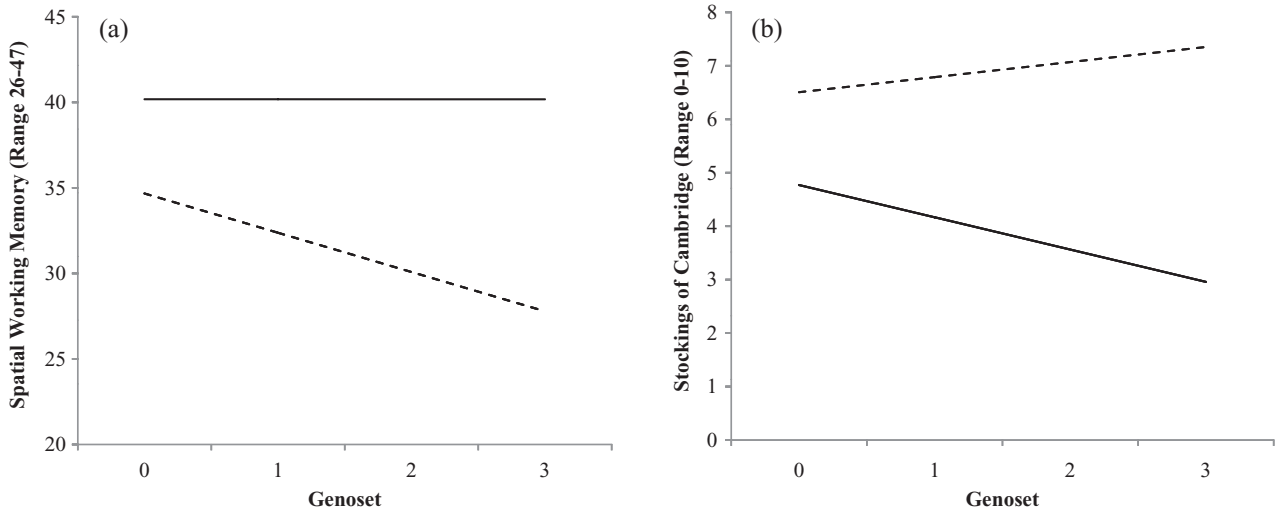

Figure 2. Cambridge Automated Neurocognitive Testing Battery (CANTAB). (a) Spatial Working Memory strategy for genoset score by group (ever institutionalized group versus never institutionalized group). Note that for Spatial Working Memory strategy lower score is indicative of a more efficient search strategy. (b) Stockings of Cambridge problems solved for genoset score by group (ever institutionalized group versus never institutionalized group).

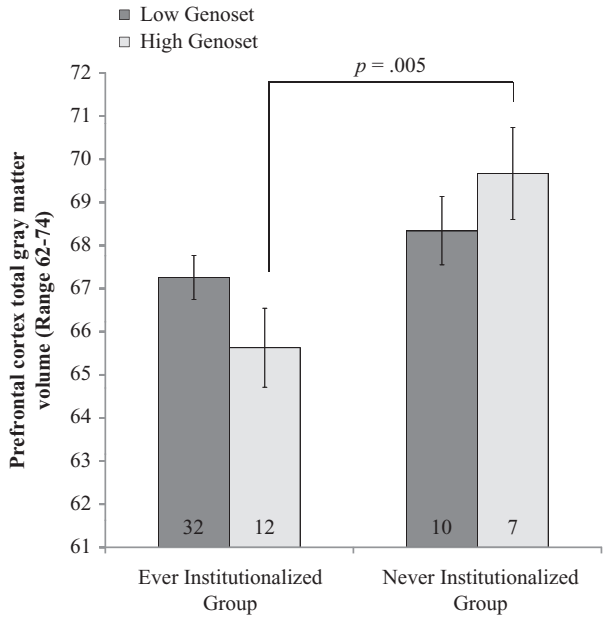

Figure 3. Prefrontal cortex total grey matter volume for 2–3 alleles versus 0–1 alleles for ever institutionalized group and never institutionalized group groups.

within the NIG group (B = 0.25, [95%CI: –0.70, 1.27, p = .57). There was, however, a significant effect of genoset within the EIG group (B = –0.61, [95%CI: –1.13, –0.13, p = .016).

PFC gray matter volume

We examined total PFC volume after controlling for the effects of sex, ethnicity, total intracranial volume, and birth weight.

EIG versus NIG group direct effects. There was a significant direct effect of group for PFC volume (t(54) = 2.39, B = 1.90, [95%CI: 0.37, 3.54], p = .013, ΔR^2 = .08), such that the NIG had significantly greater PFC volume than the EIG.

Genotype direct effects. No direct effect of genotype was found for PFC volume (t(54) = –0.27, B = –0.20, [95%CI: –1.78, 1.47], p = .79, ΔR^2 = .001).

EIG versus NIG group x gene interaction. A significant genotype x group interaction was found for PFC volume (t(52) = 1.97, B = 2.96, [95%CI: 0.59, 5.27], p = .029, ΔR^2 = .05). Posthoc analyses revealed that, at low susceptibility (genoset score = 0–1), there was no significant difference between the EIG and NIG (B = 1.08, [95%CI: –0.39, 2.75, p = .18). However, at high susceptibility (genoset score = 2–3), a significant effect for group was observed (B = 4.04, [95%CI: 1.56, 6.74], p = .005), with the NIG demonstrating significantly greater PFC brain volume than EIG participants (see Figure 3). The effect of genoset was examined within each group. Genoset did not reach statistical significance in the prediction of PFC volume within the NIG group (B = 1.31, [95%CI: –0.37, 2.73, p = .28) or the EIG group (B = –1.59, [95%CI: –3.71, 0.59, p = .10).

Discussion

This study demonstrates that an a priori biologically informed multilocus genoset containing *BDNF-COMT-SIRT1* interacted with early institutional rearing in the prediction of IQ, SWM, SOC, and PFC volume. Although we hypothesized that the effect of this genoset would operate in a manner consistent with differential susceptibility, our results demonstrate a complex interaction where vantage susceptibility, diathesis stress, and differential susceptibility are implicated for different outcomes. Specifically, we demonstrated that, for IQ and SWM, there is no difference based on environment in the non-susceptible children. Genetically defined susceptible children fared the best (compared to non-susceptible individuals) in the positive environment, but fared similar to children with other alleles in the negative environment, consistent with vantage sensitivity theory (Pluess & Belsky, 2013). Conversely, for SOC, susceptible children performed

the worst (compared to non-susceptible individuals) in the negative environment, but fared similar to other alleles in the positive environment, implying diathesis stress (Zuckerman, 1999), the effect opposite to "vantage sensitivity," recently described by Pluess and Belsky (2013). Finally, for PFC total grey matter volume, although group differences did not meet statistical significance, the susceptible children have the lowest PFC volume within the negative environment and the highest (compared to non-susceptibility individuals) in the positive environment most consistent with differential susceptibility (Belsky et al., 2013; Roisman et al., 2012).

The data presented here offer a novel extension of previous research in institutionalized children. Specifically, while significant group differences between PI children and never institutionalized children have been demonstrated (Fox et al., 2011; Sheridan et al., 2012), our results suggest that these group differences may be driven by dramatic differences in a subset of children, rather than an overall group effect. Taken together our data suggest that the dopaminergic pathway associated with cortical plasticity, defined by the *BDNF-COMT-SIRT1* genoset, is critical for susceptibility to early caregiving context. High susceptibility children may demonstrate tremendous benefit from educational and behavioral interventions targeting this pathway.

Rodent models of early life stress have highlighted the lasting impact across a range of neurodevelopmental measures, including dendritic complexity (Pascual & Zamora-Leon, 2007), attention (Colorado, Shumake, Conejo, Gonzalez-Pardo, & Gonzalez-Lima, 2006), and memory (Aisa, Tordera, Lasheras, Del Rio, & Ramirez, 2007). Individual genetic differences, particularly in genes associated with regulation of neurodevelopment and synaptic plasticity, may modulate the negative impact of early extreme environments (Makinodan, Rosen, Ito, & Corfas, 2012). Examining these same pathways in human studies is expected to provide greater mechanistic insight.

While models using genosets are complicated, they are likely more reflective of the complex nature of molecular genetic interactions in the CNS. Independently, genetic variation within *BDNF*, *COMT*, and *SIRT1* has been associated with cognitive function (Kuningas et al., 2007; Schulz-Heik et al., 2011; Sheldrick et al., 2008; Yamada, Mizuno, & Nabeshima, 2002), neurodevelopmental plasticity (Berton et al., 2006; Deltheil et al., 2008; Gao et al., 2010; Lu, 2003; Michan et al., 2010; Pencea et al., 2001), and PFC gray matter volume (Ho et al., 2006; Honea et al., 2009; Pezawas et al., 2004). Despite these numerous studies, there has been a lack of consensus as to which genotype confers a "positive" outcome (Barnett, Scoriels, & Munafo, 2008; Colzato, Waszak, Nieuwenhuis, Posthuma, & Hommel, 2010; Egan et al., 2003; Gong et al., 2009; Hariri et al., 2003; Ho et al., 2006; Nederhof et al., 2010). This suggests that specific alleles are neither "good" nor "bad," but rather the relative advantage or disadvantage of each allele is contingent upon environmental conditions, developmental stage, other genetic factors, and potentially the specific outcome or phenotype. This interactive developmentally sensitive model is consistent with the known biological complexity and offers insight into the high frequency of many putative "risk" alleles in the general population (Petryshen et al., 2010).

Multiple studies have documented epistatic effects between *BDNF*, *COMT*, and *SIRT1* (Gao et al., 2010; Witte et al., 2012). Specifically, *BDNF* exerts complex modulation of dendritic and axonal growth in neurons of the CNS, which is indexed by both the *BDNF Val66Met* polymorphism (Tan et al., 2011) and *SIRT1* expression, via a miR-134 mediated mechanism (Gao et al., 2010). The co-modulation of *SIRT1* and *BDNF* by miR-134a suggest both overlapping function and concurrent regulation (Yamakuchi, 2012). An additive impact on dopamine neurotransmission, cognitive function, and cortical plasticity has been demonstrated with *BDNF* and *COMT* variants (Park et al., 2011; Trotman et al., 2010; Witte et al., 2012). Our genoset finding suggests that *SIRT1* modulated *BDNF* regulation, in response to early adversity, results in altered neurocognitive development that is further moderated by altered dopamine bioavailability indexed by the *COMT-Met* variant. Coupled with the existing molecular evidence of overlapping function, pathway analysis suggesting their interaction, and previous literature documenting additive effects, our results provide evidence that these genes interact to influence neurocognitive outcomes in an experience-dependent manner. The use of this biologically informed multilocus profile allows for known polymorphisms of *BDNF*, *COMT*, and *SIRT1* to be collectively harnessed to represent function across a specific neural system, that is, dopaminergic neurotransmission in the PFC (Bogdan et al., 2012).

The PFC is characterized by significant growth across early development with a peak expected volume at age 11–12. Subsequently, there is an expected decrease during adolescence consistent with pruning and apoptosis driving by experience-dependent use (Teffer & Semendeferi, 2012). This trajectory of PFC volume is highly correlated with age-appropriate changes in cognition and behavior, particularly for PFC-related tasks such as working memory (Teffer & Semendeferi, 2012). In the context of our study, in which PFC volume was studied in children prior to the adolescent pruning stage, larger PFC gray matter volumes likely represent better growth (neurogenesis, synaptogenesis, dendritic proliferation and arborization, etc.), and therefore the most positive outcome. However, observations made after 12 years of age, and particularly between the ages of 12 and 20, must consider the expected developmental trajectory of PFC development. In older individuals, it may be that smaller PFC volumes or ratio differences between gray and white matter represent the most positive outcome.

Several limitations exist. Grouping of genotypes for the genoset was performed a priori, based on evidence in the literature that suggests that *BDNF Met/**, *COMT Met/Met*, and *SIRT1 C/** genotypes have increased synaptic plasticity and cognitive flexibility. However, multiple additional genes and genetic variants could have also been examined. Given this limitation these results still represent an important proof-of-concept for future studies of genetic differential susceptibility. The relatively small sample size of our study, coupled with differing sample size between outcomes is another limitation. A few potentially relevant covariates, such as prenatal factors, socioeconomic status (SES), and physical growth rates are not available in this cohort. We acknowledge that there are likely growth differences between previously institutionalized and never-institutionalized groups that may further contribute to the impact of the genoset, which we have previously reported on for this cohort (Johnson et al., 2010; Rotoli, Grignol, Hu, Merchenthaler, & Dudas, 2011). In this study, we are unable to assess the potential interaction between genoset and growth due to sample size limitations; however, we anticipate that this would not impact the findings within the EIG and the NIG due to the initial study design. Controlling for birth weight did not impact our results and no association between genoset and birth weight was found. Further, the relationship between growth and EF measures does not preclude the independent, or the interactive, impact of genoset. Although we acknowledge this is a relatively small sample size it represents one of the largest studies available with well-characterized extreme differences in early caregiving. To address sample size limitations,

BCa 95% CIs were used to adjust for both bias and skewness in the bootstrap distribution (Efron, 1987). Although bootstrapping is most accurate with large sample sizes, the use of this method has been recommended for use in smaller samples (e.g., $n = 30$) (Singh & Xie, 2008). The smaller sample size in the MRI data required that genotype be collapsed into "high" and "low" susceptibility as opposed to the continuous 0–3 measure used with other outcomes. Despite these limitations, we believe that the genoset association, across two distinct neurocognitive measures (WISC and CANTAB), and PFC gray matter (i.e., the genoset associated with higher cognitive function is also associated with increased gray matter) is compelling and unlikely the result of spurious g x e findings.

These results offer novel evidence for a model of differential susceptibility, guided by extant preclinical and translational findings, and leveraging the existing molecular evidence for g x e interactions. Our results suggest that individual differences in the response to extremes of early caregiving may be, in part, dictated by complex genotype interactions. Given the known molecular and neurobiological functions of our selected genes, our results provide preliminary evidence that alterations in synaptic plasticity and dopaminergic neurotransmission may be part of the underlying mechanism linking negative neurocognitive consequences and early adverse caregiving.

Future research should consider the role of other genetic and epigenetic mechanisms that may modulate the relation between synaptic plasticity and dopaminergic function. Harnessing the use of both genetic databases and the current literature will allow for the expansion of identified genosets to encompass more comprehensive genetic pathways. For example, additional genes, identified by the ConsensusPathDB using the *BDNF-COMT-SIRT1* genoset as a seed, are also linked to neurodevelopment and synaptic plasticity. Specifically, *ESR1* is associated with neuronal differentiation (Lovén et al., 2010), *NOS3* is linked to regulation of neurotransmitter release and development of synaptic plasticity (Huang & Lo, 1998), and *TAF1* is related to cell division and proliferation (Jambaldorj, Makino, Munkhbat, & Tamiya, 2012). These genes may provide novel avenues of research to further describe the mechanisms underlying vantage sensitivity, diathesis stress, and differential susceptibility in relation to neurobiological outcomes and early caregiving environments. Replication of these findings in larger studies of institutional children and other high-risk populations is needed. Finally, longitudinal studies are necessary to develop an understanding of the relation between this genoset and negative early life experiences across development.

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