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A novel differential susceptibility gene: *CHRNA4* and moderation of the effect of maltreatment on child personality

Rachael G. Grazioplene,[1] Colin G. DeYoung,[1] Fred A. Rogosch,[2] and Dante Cicchetti[3]

[1]Department of Psychology, University of Minnesota, Minneapolis, MN; [2]University of Rochester, Mt. Hope Family Center, 187 Edinburgh Street, Rochester, NY; [3]University of Minnesota – Institute of Child Development, Minneapolis, MN, USA

Background: The differential susceptibility hypothesis states that some genetic variants that confer risk in adverse environments are beneficial in normal or nurturing environments. The cholinergic system is promising as a source of susceptibility genes because of its involvement in learning and neural plasticity. The cholinergic receptor gene *CHRNA4* has been linked to characteristics related to the personality traits Neuroticism and Openness/Intellect. **Methods:** The effects of interaction between *CHRNA4* genotype and maltreatment status on child personality were examined in a well matched sample of 339 maltreated and 275 non-maltreated children (aged 8–13 years). **Results:** Variation in *CHRNA4* interacted with childhood maltreatment to predict personality in a manner indicating differential susceptibility. The interaction of *CHRNA4* and maltreatment status predicted Neuroticism and Openness/Intellect. Maltreated children with the rs1044396 T/T genotype scored highest on Neuroticism and showed no effect of genotype on Openness/Intellect. Non-maltreated children with this genotype scored lowest on Neuroticism and highest on Openness/Intellect. **Conclusion:** Variation in *CHRNA4* appears to contribute to personality by affecting degree of developmental sensitivity to both normal and adverse environments. **Keywords:** Personality, genetics, CHRNA4, differential susceptibility, neuroticism, openness/intellect.

Introduction

Many studies have been conducted to find genetic variants that confer risk for disorders or maladaptive traits. Evidence has been found for many such effects, but failures to replicate have also been common (Chanock et al., 2007; Duncan & Keller, 2011). One explanation offered for the difficulty in finding these effects reliably is the importance of gene by environment interactions, in which a particular genotype confers risk only under harsh environmental conditions (e.g. Caspi et al., 2002). A more nuanced perspective suggests that genes conferring risk in harsh environments may confer benefits in normal or nurturing environments (Belsky & Pluess, 2009). This *differential susceptibility* hypothesis is appealing in part because it offers one explanation as to why genetic variants associated with negative outcomes would remain in the gene pool. Differential susceptibility implies that some genotypes render individuals more likely to be influenced, for better or for worse, by their environments. Ample evidence for differential susceptibility has been found using several genes in the dopaminergic and serotonergic systems (Belsky et al., 2009; Belsky & Pluess, 2009). This study took a theoretically guided approach to identifying a novel differential susceptibility gene, examining a biological system crucially involved in sensitivity to environmental conditions.

The cholinergic system is an excellent candidate for genes producing differential susceptibility because it is strongly involved in neural plasticity and learning. The neurotransmitter acetylcholine is released in novel environments and during task-related attention shifting and associative learning. Acetylcholine plays a broad role in modulating cortical responsiveness to the contents of attention (Sarter, Hasselmo, Bruno, & Givens, 2005). Its release lowers the threshold for firing in neurons involved in forming new associations with the contents of the external world (especially thalamocortical pathways) while at the same time selectively suppressing intracortical communication pathways that might bias interpretation of novel events (Dani & Bertrand, 2007; Sarter et al., 2005). Artificial stimulation of acetylcholine release increases experience-dependent neural plasticity (Bakin & Weinberger, 1996), and depletion of acetylcholine suppresses this effect (Baskerville, Schweitzer, & Herron, 1997).

Inputs that activate the cholinergic system can be broadly categorized as 'expected uncertainty,' which characterizes contexts where uncertainty exists, but is anticipated (Yu & Dayan, 2005). This is in contrast to unexpected uncertainty, or strong violation of expectations, which appears to be related primarily to the neurotransmitter norepinephrine (Yu & Dayan, 2005). In other words, the cholinergic system

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is most active in situations when the individual can predict that learning is possible.

Because acetylcholine induces neural plasticity and is associated with learning from the environment, genetic variation in the cholinergic system is likely to be associated with differential susceptibility – that is, with individual differences in susceptibility to environmental influences. In people with more active cholinergic systems, increased influence from harsh environments may lead to poor outcomes, but increased influence from normal or nurturing environments may lead to better outcomes.

Existing research allows us to form a hypothesis about what aspects of personality are likely to be affected by cholinergic genes, namely those related to automatic responses to uncertainty, such as anxiety, curiosity, and attention. Unpredictable and novel contexts, which generate uncertainty, function simultaneously as threats and incentive rewards (McNaughton & Gray, 2000; Peterson & Flanders, 2002). When the significance of an environment or stimulus is not certain, both caution and exploration are adaptive. Thus, traits related to both positive and negative responses to uncertainty or novelty are most likely to be affected by cholinergic genes, and the harshness of the environment during development is likely to determine whether individuals tend to find expected uncertainty more threatening than promising, or *vice versa*.

We utilized these observations to predict the effects of childhood maltreatment and variation in cholinergic genes on the Big Five personality traits: Extraversion, Neuroticism, Conscientiousness, Agreeableness, and Openness/Intellect. The Big Five constitute the most widely used and well validated taxonomy of personality in adulthood (John, Naumann, & Soto, 2008) and appear to provide an effective model of childhood personality as well (Caspi & Shiner, 2006). Studies of twins indicate that the Big Five are strongly genetically influenced, with heritability estimates ranging from .40 to .80, depending on trait and method (Riemann, Angleitner, & Strelau, 1997). In this study, we examined the effects of genetic variation on the Big Five, in children who were maltreated and those in a closely matched comparison group. Maltreatment has been associated with differences in every Big Five dimension except Extraversion (Rogosch & Cicchetti, 2004). We hypothesized that cholinergic genetic variation would moderate the effects of maltreatment on Neuroticism and Openness/Intellect because these are the Big Five traits most linked to anxiety, curiosity, and response to uncertainty and novelty.

We examined the polymorphism rs1044396 in the nicotinic acetylcholine receptor alpha-4 subunit gene (*CHRNA4*), which produces a component of one major acetylcholine receptor. This single nucleotide polymorphism (SNP), in which one letter of the genetic code is changed, consists of a C → T transposition in the exonic region of *CHRNA4*. Although it does not alter the amino acid sequence coded by the gene, the repeated associations of this SNP with behavioral and neural characteristics (e.g. Espeseth, Sneve, Rootwelt, & Laeng, 2010; Winterer et al., 2007) suggest it is in linkage disequilibrium with some functional variation or has some direct effect on transcription. In keeping with our hypothesis, previous studies have linked rs1044396 to Neuroticism and to cognitive functions related to Openness/Intellect.

Neuroticism reflects the tendency to experience negative affect and related cognitive processes, including anxiety, depression, irritability, and self-consciousness. It has been linked to sensitivity to threat and punishment both psychologically and biologically (DeYoung & Gray, 2009), and it has been linked specfically to feeling threatened by uncertainty (McNaughton & Gray, 2000; Hirsh & Inzlicht, 2008). Animal models have implicated *CHRNA4* in anxiety, and, in a healthy human population, the C/C genotype of rs1044396 was associated with higher Neuroticism than genotypes containing the T allele (Markett, Montag, & Reuter, 2011).

Openness/Intellect is probably best described in terms of cognitive exploration of both inner and outer experience, and curiosity is central to this trait (DeYoung, Grazioplene, & Peterson, 2012; DeYoung, Peterson, & Higgins, 2005). Individuals high in Openness/Intellect tend to be imaginative, artistic, intellectual, and perceptive, suggesting an underlying tendency to seek out, explore, and utilize novel information. The compound label for this trait reflects the fact that it involves both Openness to Experience, which reflects engagement with sensory information, and Intellect, which reflects engagement with abstract information (Johnson, 1994; DeYoung, Shamosh, Green, Braver, & Gray, 2009; DeYoung et al., 2012). Openness/Intellect is the only Big Five trait that is related to tests of attention and working memory capacity (DeYoung et al., 2005, 2009), and variation in *CHRNA4* rs1044396 has been implicated in attentional function and working memory (Espeseth et al., 2010; Greenwood et al., 2009; Markett, Montag, Walter, & Reuter, 2010; Parasuraman, Greenwood, Kumar, & Fossella, 2005). Because these cognitive functions have been hypothesized to be integral components of the cognitive substrate of Openness/Intellect (DeYoung et al., 2005, 2009, 2012), *CHRNA4* is likely to be an important genetic influence on this personality trait.

The hypothesis that variation in rs1044396 moderates the effects of maltreatment on both Neuroticism and Openness/Intellect was tested in a large sample of children enrolled in a week-long day camp research program. Roughly half of these children were selected because they had been maltreated, as determined from records of the Department of Human Services (DHS), whereas the other half were from the same socioeconomic background, but were carefully screened to exclude any maltreated

children. Although an ideal situation for examining differential susceptibility would involve a more detailed measure of variability in parental nurturing in the non-maltreated group, the present sample nonetheless provides a good test of the differential susceptibility hypothesis because differences in environment were clearly defined, rigorously assessed, and dramatic (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011). Furthermore, all children were assessed in the context of a novel, information-rich camp environment likely to be ideally suited to observation of individual differences associated with cholinergic function.

We did not expect *CHRNA4* to moderate the effects of maltreatment on Conscientiousness or Agreeableness. Regarding the last of the Big Five, Extraversion, we formulated no strong hypothesis. No existing evidence directly links *CHRNA4* to Extraversion or related characteristics; however, Extraversion is related to the tendencies to engage in approach behavior and to experience positive affect and is linked to the biological substrates of reward (DeYoung & Gray, 2009). Whereas Openness/Intellect is associated with cognitive exploration, Extraversion reflects the tendency to explore the world behaviorally. *CHRNA4* might influence Extraversion because acetylcholine is heavily involved in modulating striatal dopamine function (Miwa, Freedman, & Lester, 2011), which governs sensitivity to cues of reward and appears to be related to Extraversion (DeYoung & Gray, 2009). One study found no association between rs1044396 and Extraversion (Markett et al., 2011); however, they examined only main effects, and thus could not have detected gene × environment interaction effects like those we examine here.

Methods

Participants

Participants were 614 children (age range = 8–13 years, $M = 11.3$, $SD = 1.0$), who were recruited from an urban setting in upstate New York to participate in a weeklong day camp program. A total of 339 of these children had been maltreated (169 girls, 170 boys), and 275 had not (142 girls, 133 boys). The sample was racially and ethnically diverse, and was categorized to allow separation of groups of mixed race/ethnicity. The sample was 60% Black, 11% Hispanic, 10% White, 2.5% Hispanic/Black, 3.5% Hispanic/White, 5.5% Black/White, and 4% other. (All results remained the same if we used the simpler racial and ethnic categorization system employed in previous research on this sample; DeYoung, Cicchetti, and Rogosch, 2011; DeYoung, Cicchetti, Rogosch, Gray, Eastman, and Grigorenko 2011). The camp program was designed for comparison of developmental processes and functioning in maltreated and non-maltreated children. All participants came from low-income homes (Cicchetti & Manly, 1990). In the recruitment process, a liaison from the DHS contacted families with a child meeting research criteria, provided information about the camp and associated research, and asked families for written permission to have their names released to project staff. (Due to confidentiality, the DHS liaison was not able to provide information regarding families who were not interested in participation.) Subsequently, parents of all participating children provided informed consent for their child's participation, as well as consent for examination of any DHS records associated with the family; children provided assent. Children attended the camp free of charge and received small prizes for completing research measures; mothers received compensation ($25) for completing a research interview. The procedures in this investigation were approved by the Research Subjects Review Board of the University of Rochester.

Children in the maltreated group were recruited based on DHS records indicating they had experienced maltreatment. Those in the non-maltreated (comparison) group did not have records of maltreatment; these children were additionally screened through checks of the child abuse registry as well as through interviews with their mothers (utilizing the Maternal Maltreatment Classification Interview; Cicchetti, Toth, & Manly, 2003) to verify lack of DHS involvement and absence of maltreatment experiences. Children in the comparison group were well matched in age, socioeconomic status, and race. To avoid inclusion of unidentified maltreatment in the comparison group, additional screening excluded families who received preventive services through DHS due to concerns over risk for maltreatment.

Children attended the program for a week and participated in research assessments. While at camp, children were assigned to groups of eight (four maltreated, four non-maltreated) same-age and same-gender peers. Each group was led by three trained camp counselors, who were unaware of the maltreatment status of children and the hypotheses of the study. Camp lasted 7 hr/day for 5 days, providing 35 hr of interaction between children and counselors.

Maltreatment

Descriptions of maltreatment in DHS records were used to identify, for each child, the presence of sexual abuse, physical abuse, neglect, and/or emotional maltreatment. Trained raters coded DHS records using the operational criteria of the Maltreatment Classification System (Barnett, Manly, & Cicchetti, 1993), a well validated approach for classifying maltreatment experiences. Among the maltreated children, 8.6% had experienced sexual abuse, 28.6% physical abuse, 78.5% neglect, and 52.2% emotional maltreatment; most children (59.1%) had experienced more than one type of maltreatment.

Personality

The Big Five personality traits were assessed using two instruments: the Big Five scales derived from the California Child Q-sort (CCQ; John, Caspi, Robins, Moffitt, & Stouthamer-Loeber, 1994) and a set of 46 trait descriptive adjectives (TDA) designed for assessment of the Big Five in children (Hagekull & Bohlin, 1998).

The CCQ comprises 100 personality descriptive items that are sorted according to a fixed distribution into nine categories, representing the degree to which each is characteristic of the child. The TDA comprises 46 items rated on a 5-point Likert scale. Two adult camp counselors completed each of these two instruments after 35 hr of extensive observation and interaction with participants. Counselors were trained in use of the instruments, but were unaware of research hypotheses and maltreatment status. Interrater agreement was high, with the average intraclass correlation among pairs of raters ranging from .85 to .87 for the CCQ and from .74 to .89 for the TDA scales. Ratings for each item by each of the two raters were averaged before deriving scale scores for each instrument.

Big Five scores from the CCQ and TDA were standardized separately to combine scores across the two instruments. The standardized scores were then averaged, restandardized, and recentered by adding 1 (recentering was performed for clarity of graphical representation). Composite scores from these two inventories were very reliable, with Cronbach's Alphas as follows: Extraversion: .95 (18 items), Agreeableness: .96 (25 items), Conscientiousness: .91 (18 items), Neuroticism: .90 (20 items), Openness/Intellect: .75 (10 items). (The lower Alpha for Openness/Intellect is attributable to its relatively fewer items.) Three items (one each from Agreeableness, Conscientiousness, and Openness/Intellect) were excluded from the calculation of trait scores because their correlations with the scale total (calculated without the item in question) were near zero and their inclusion reduced Cronbach's Alpha. Scores calculated without these items correlated at .99 or higher with scores including them.

Neuroticism

Items in the Neuroticism scale from the CCQ were, 'Is fearful and anxious'; 'Tends to brood and ruminate and worry'; 'Tends to become rigidly repetitive or immobilized under stress'; 'Can recoup or recover after stressful experiences' (reversed); 'Tends to go to pieces under stress'; 'Seeks reassurance from others about his/her worth'; 'Has bodily symptoms as a function of tension and conflict'; 'Becomes anxious if the environment is unpredictable or poorly structured'; 'Appears to feel unworthy, thinks of self as bad'; and 'Is easily offended, sensitive to ridicule or criticism.' Items in the Neuroticism scale from the TDA were 'nervous,' 'tense,' 'anxious,' 'worries about things,' 'fearful,' 'relaxed' (reversed), 'content' (reversed), 'self-confident' (reversed), 'oversensitive,' and 'calm and stable' (reversed).

Openness/intellect

Items from the Openness/Intellect scale of the CCQ were 'Is curious and exploring; eager for new experiences'; 'Appears to have high intellectual capacity'; 'Is verbally fluent'; 'Becomes strongly involved in what (s)he does'; 'Is creative in perception, thought, work, or play'; 'Has an active fantasy life'. TDA Openness/Intellect items were, 'imaginative,' 'curious,' 'creative,' and 'tries new activities.'

Genotyping

DNA was collected from all children using the Buccal Amp Kit (Epicentre, Cat. No. BQ0901SSC; Madison, WI, USA) and amplified using the Repli-g kit (Qiagen, Catalog No. 150043; Valencia, CA, USA) as per the kit instructions. DNA was whole-genome amplified to ensure the availability of data over the long term for this valuable sample. Amplified samples were then diluted to a working concentration and genotyped using an assay for SNP rs1044396 purchased from Applied Biosystems, Inc. (ABI). Individual allele determinations were made using TaqMan Genotyping Master Mix (Applied Biosystems, Catalog 4371357; Foster City, CA, USA) with amplification in an ABI 9700 thermal cycler and analyzing the endpoint fluorescence using a Tecan M200. No other polymorphisms in the cholinergic system were genotyped. Genotypes in other neurotransmitter systems have been examined in this sample (DeYoung, Cicchetti, and Rogosch, 2011; DeYoung, Cicchetti, Rogosch, Gray, et al. 2011); these genotypes were not included in our primary analysis, but we did conduct a secondary analysis to test whether the effects of *CHRNA4* were independent of the previously identified effects of other genes.

If a genotype was unable to be determined after the first run, then it was repeated up to four times. If the null result persisted, then the whole-genome amplification reaction was repeated along with subsequent genotyping until a genotype could be confidently assigned to a participant. The resultant genotyping data were subjected to quadratic discriminant analysis using JMP statistical software from SAS. Samples with a predicted probability of 0.95 or less were repeated. All DNA samples were genotyped in duplicate for quality control. In addition, human DNA from cell lines was purchased from Coriell Cell Repositories for all representative genotypes in duplicate and genotypes confirmed by sequencing using DTCS chemistry on an ABI 3130x1. The call rate for rs1044396 for CHRNA4 was 100%. There were no missing results. Rs1044396 did not differ significantly from Hardy-Weinberg equilibrium $\chi^2_{(1)} = 1.77$, $p = .77$.

Analysis

To test gene by environment interaction effects for each of the Big Five, we conducted a single MANCOVA with five criterion variables to control for multiple comparisons. *CHRNA4* genotype, maltreatment status, gender, and race were entered as fixed factors, and age was included as a continuous covariate. In addition, the interaction of genotype × maltreatment status was entered as the effect of interest for our hypothesis.

Results

Table 1 shows allele frequencies, comparing maltreated and non-maltreated children, by gender and race. The maltreated and non-maltreated groups did not differ by genotype, which indicates absence of gene-environment correlation, $\chi^2_{(1,\ N = 614)} = 1.10$, $p = .30$. In other words, *CHRNA4* genotype did not influence the likelihood that children would be maltreated. Gender was also unrelated to genotype,

Table 1 CHRNA4 rs1044396 genotype frequencies (% of total *N*) by gender and race/ethnicity

	Maltreated (*N* = 339)			Nonmaltreated (*N* = 275)		
	C/C	C/T	T/T	C/C	C/T	T/T
Gender						
Female	99 (16.1)	62 (10.1)	8 (1.3)	84 (13.7)	46 (7.5)	12 (2.0)
Male	112 (18.2)	52 (8.3)	8 (1.1)	79 (12.9)	44 (7.2)	10 (1.6)
Race/ethnicity						
Black	166 (27)	43 (7.0)	1 (0.2)	126 (20.5)	44 (7.2)	3 (0.5)
White	10 (1.6)	23 (3.7)	8 (1.3)	0 (0)	11 (1.8)	12 (2)
Hispanic	11 (1.8)	15 (2.4)	3 (0.5)	22 (3.6)	19 (3.1)	1 (0.2)
Hispanic–Black	7 (1.1)	5 (0.8)	0 (0)	5 (0.8)	4 (0.7)	2 (0.3)
Hispanic–White	1 (0.2)	7 (1.1)	1 (0.2)	1 (0.2)	3 (0.5)	2 (0.3)
Black–White	10 (1.6)	13 (2.1)	1 (0.2)	4 (0.7)	6 (1.0)	1 (0.2)
Others	6 (1.0)	7 (1.1)	2 (0.3)	4 (0.7)	2 (0.3)	0 (0)

$\chi^2_{(1,\ N = 614)} = 1.13$, $p = .29$; race was significantly associated with *CHRNA4* genotype, $\chi^2_{(3,\ N = 614)} = 150.1$, $p < 0.01$, making it important to control for race in all analyses to account for potential population stratification.

Table 2 shows means and standard deviations for the Big Five in each group. As reported in previous work, maltreated children in this sample exhibited higher Neuroticism and lower Agreeableness, Conscientiousness, and Openness/Intellect than non-maltreated children (DeYoung, Cicchetti, Rogosch, Gray, et al., 2011).

Results of MANCOVA are shown in Table 3 for Neuroticism, Openness/Intellect, and Extraversion. Significant gene × environment interaction effects were present for Neuroticism and Openness/Intellect (Figure 1). Individuals who were maltreated and possessed two copies of the T allele had the highest levels of Neuroticism, whereas non-maltreated individuals with that genotype had the lowest levels of Neuroticism (Figure 1A). Non-maltreated individuals with the T/T genotype also had the highest levels of Openness/Intellect (Figure 1B). Simple effects analysis of Neuroticism revealed a significant difference between maltreated and non-maltreated children in the T/T genotype group, $t_{(35)} = 3.26$, $p = .003$, but no significant difference in Neuroticism in the C/T group, $t_{(201)} = 1.48$, $p = .25$, or in the C/C group, $t_{(372)} = 1.34$, $p = .18$. The same pattern of simple effects was observed for Openness/Intellect when comparing maltreated to non-maltreated groups in each genotype, T/T: $t_{(35)} = 3.04$, $p = .005$; C/T: $t_{(201)} = 1.09$, $p = .28$; C/C: $t_{(372)} = .72$, $p = .47$. The interaction effect was not significant for Extraversion ($p = .23$), nor was there any main effect of *CHRNA4* genotype on Extraversion, after removing the interaction term from the model, $p = .68$. As expected, there was no effect of *CHRNA4* genotype on Conscientiousness or Agreeableness, either as a main effect or in interaction with maltreatment, all $p > .22$.

DeYoung, Cicchetti, and Rogosch (2011) demonstrated that variation in the corticotropin-releasing hormone receptor 1 gene (*CRHR1*), a component of the stress-response system, moderated the effect of

Table 2 Means and standard deviations of the Big Five in maltreated and non-maltreated children

	Maltreated (*N* = 339)		Nonmaltreated (*N* = 275)				
	M	*SD*	*M*	*SD*	$t_{(612)}$	*p*	*d*
Neuroticism	1.10	1.03	0.88	0.95	2.70	.01	0.22
Extraversion	1.02	1.01	0.97	0.98	0.66	.51	0.05
Agreeableness	0.83	1.02	1.21	0.94	−4.72	<.001	0.39
Conscientiousness	0.82	1.02	1.22	0.94	−4.95	<.001	0.39
Openness/Intellect	0.92	0.97	1.10	1.02	−2.13	.03	0.17

Table 3 Analysis of Variance: Effects of CHRNA4 genotype and maltreatment on Neuroticism, Openness/Intellect, and Extraversion

	Neuroticism				Openness/Intellect				Extraversion			
	F	*df*	*p*	η^2	*F*	*df*	*p*	η^2	*F*	*df*	*p*	η^2
Age	3.32	1	.13	.004	0.08	1	.77	<.001	2.5	1	.11	.004
Gender	0.04	1	.85	<.001	0.38	1	.54	.001	3.23	1	.07	.005
Race	1.97	6	.07	.019	2.74	6	.01	.011	0.36	6	.91	.004
Maltreatment	15.12	1	<.001	.025	9.61	1	<.001	.021	0.85	1	.36	.001
CHRNA4	0.20	2	.82	.001	0.34	2	.71	.001	0.77	2	.46	.003
Maltreatment × *CHRNA4*	4.45	2	.01	.015	4.23	2	.02	.014	1.59	2	.21	.005

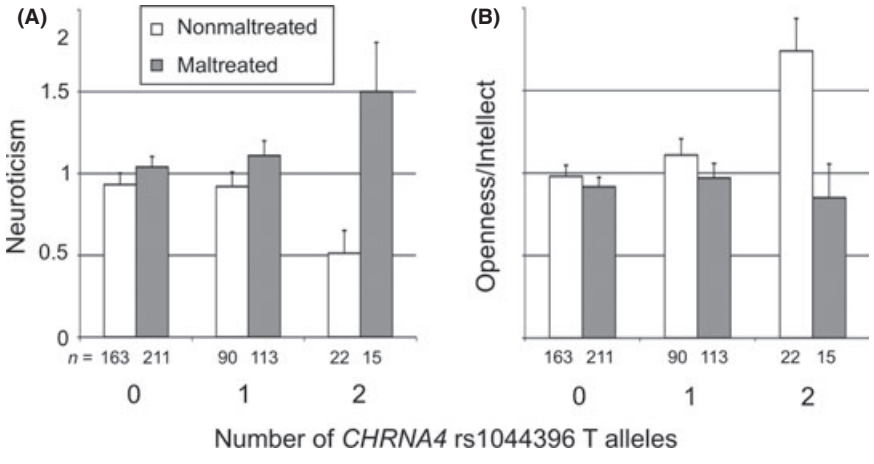

Figure 1 Levels of Neuroticism and Openness/Intellect associated with *CHRNA4* genotype for maltreated and non-maltreated children

maltreatment on Neuroticism. DeYoung and colleagues (DeYoung, Cicchetti, and Rogosch, 2011; DeYoung, Cicchetti, Rogosch, Gray, et al., 2011) demonstrated that variations in the dopamine D4 receptor gene (*DRD4*) and the catechol-*O*-methyltransferase gene (*COMT*) had main effects on Openness/Intellect. To test for any influence of these previous findings on the findings of the present study, we ran an additional model in which *CRHR1*, *DRD4*, and *COMT* genotypes, and their interactions with maltreatment, were all entered into the same MANCOVA that we used to test the effect of *CHRNA4*. The effects reported in this study remained significant when controlling for the previously demonstrated effects of other genes, and those other genes predicted variance in Neuroticism and Openness/Intellect independently of the effects of *CHRNA4*. (Further information regarding these latter analyses is available from the corresponding author upon request.)

Discussion

Results confirmed our hypothesis that variation in *CHRNA4* is associated with differential susceptibility to environmental influences. The polymorphism rs1044396 moderated the association between childhood maltreatment and childhood personality. When compared to children with at least one copy of the C allele, individuals with the T/T genotype at rs1044396 appeared to be more susceptible to the conditions of their rearing environment. They were higher in Neuroticism when exposed to maltreatment, but lower in Neuroticism and higher in Openness/Intellect when not maltreated. This gene × environment interaction effect was independent of age, race, and gender, and it explained approximately 1% of the variance in both Openness/Intellect and Neuroticism. This is the first study to examine differential susceptibility effects for cholinergic genes, which are plausible because of the importance of acetylcholine in learning and neural plasticity.

Effect sizes around 1% or less are to be expected for prediction of complex traits by single polymorphisms (Ioannidis, Trikalinos, & Khoury, 2006). Genetic research increasingly indicates that most complex traits are massively polygenic, influenced by large numbers of common genetic variants (e.g. Davies et al., 2011). Any given polymorphism, therefore, is likely to account for only a small fraction of trait variance. Our results are consistent with these observations, given that the effect of *CHRNA4* was independent of previously reported effects (in this sample) on Openness/Intellect and Neuroticism by *DRD4*, *COMT*, and *CRHR1* (DeYoung, Cicchetti, and Rogosch, 2011; DeYoung, Cicchetti, Rogosch, Gray, et al., 2011). It would appear, therefore, that genes in both the cholinergic and dopaminergic systems influence Openness/Intellect, and that genes in both the cholinergic and corticotropin systems influence Neuroticism.

Direct effects of rs1044396 variation have previously been reported for individual differences in cognitive function and Neuroticism (Espeseth et al., 2010; Greenwood et al., 2009; Markett et al., 2011; Parasuraman et al., 2005); this study suggests that detection of such associations may be facilitated by considering environmental conditions during development. Neuroticism was previously found to be lower for the T/T than the C/C genotype (Markett et al., 2011). We found a similar effect for children who had not been maltreated, whereas for children who had been maltreated the T/T genotype was associated with higher Neuroticism. The effect of genotype on Neuroticism observed in the study by Markett et al. suggests that the sample they analyzed, which consisted primarily of students, was more similar to our non-maltreated than our maltreated group, which would not be surprising given that they are university students. Thus, in populations with normal rearing environments, the T allele may be beneficial, encouraging lower levels of Neuroticism and higher levels of Openness/Intellect. This pattern of personality traits suggests decreased anxiety and increased curiosity and cognitive engagement in response to situations containing expected uncertainty, where learning is likely to be potentiated by acetylcholine.

Acetylcholine is increasingly appreciated for its role in the etiology of mental illnesses such as schizophrenia and affective disorders (Miwa et al., 2011). As childhood maltreatment is a known risk factor for psychiatric illness, the present findings may be of clinical importance for early intervention and treatment of mental illness in children who have been maltreated (Cicchetti & Valentino, 2006; Cicchetti & Rogosch, 2001). Genetic markers for variation in cholinergic function could potentially be used in tailoring pharmacological and therapeutic interventions (Kirchheiner et al., 2004). The present results are particularly relevant for clinical phenomena because Neuroticism is the major personality risk factor for most forms of psychopathology, including anxiety and schizophrenia-spectrum disorders (Griffith et al., 2010).

Although the association of Neuroticism and Openness/Intellect with *CHRNA4* genotype and childhood maltreatment fits well with the emerging picture of acetylcholine function in development and disease, this research is not without limitations. The T/T genotype of rs1044396, which represents the 'susceptible' group in the present analyses and hence drives the interaction effect, is relatively infrequent; the small size of this group in this study makes replication an important goal of future research. In addition, the functional implications of variation at rs1044396 are not known; thus, it is not possible to determine whether the T allele increases or decreases acetylcholine binding.

Limitations exist for our non-genetic measures as well, including those of personality, race, and environment. The personality measures available in this sample assessed the Big Five only, without breaking those broad traits down into narrower facets. Additional research would be necessary to detect whether *CHRNA4* has different effects on subtraits within Neuroticism and Openness/Intellect. Controlling for race using self-reported race and ethnicity is less desirable than the use of direct genetic markers, especially for the relatively heterogeneous group identifying as 'Hispanic' (Caballero, 2011). We attempted to capture some of this heterogeneity by creating separate categories for those of mixed race/ethnicity, but the lack of genetic markers for race remains a limitation. Finally, we acknowledge that inclusion of an assessment of parental nurturing in the non-maltreated group would have enabled a more thorough test of differential susceptibility. Because 'normal' parenting is likely to vary widely in quality, our data do not shed light on the effects of CHRNA4 on personality in average relative to more nurturing rearing environments. Given this limitation, it is compelling that we nonetheless found a differential susceptibility effect. Our ability to detect this effect was probably due in part to the rigor with which maltreatment was assessed and to the dramatic difference between the environments of maltreated and non-maltreated children.

Another important question for future research is to address whether the associations observed in a child sample would carry through into adulthood. Evidence from twin studies indicates that many behavioral phenotypes become more heritable over the course of late adolescence and young adulthood (Bergen, Gardner, & Kendler, 2007). Because children who possess genetic variants associated with differential susceptibility are likely to be more malleable to environmental circumstances, they may undergo substantial personality change across the course of later development.

Conclusion

One important objective of research on childhood maltreatment is to understand the mechanisms by which abuse and neglect alter mental functioning in the course of development. The present findings suggest that genetic variation in the cholinergic system alters the degree to which children are influenced by their environments. The cholinergic system was identified as a likely candidate for differential susceptibility genes because of its role in learning and neural plasticity. Children with the T/T genotype of *CHRNA4* rs1044396 who reside in harsh environments may be more likely to learn anxious and fearful responses (associated with Neuroticism) to situations with increased uncertainty. However, children with this same genotype may be more likely to exhibit curiosity and cognitive engagement (associated with Openness/Intellect) in response to uncertainty if they have been reared in normal or nurturing environments. We acknowledge that the relatively low frequency of the T/T genotype encourages a cautious interpretation of these results, but we emphasize that the finding that *CHRNA4* genotype and maltreatment status interact to influence childhood personality adds to a growing body of evidence suggesting that some genetic variants confer differential susceptibility to environmental influence. Although the T/T genotype may be maladaptive in the presence of extreme environmental stressors, it may in fact contribute to the optimal developmental outcomes when combined with normal or nurturing rearing conditions.

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Correspondence

Rachael G. Grazioplene, Department of Psychology, University of Minnesota, Minneapolis, MN 55455, USA; Email: graz0029@umn.edu

Key points

- The *differential susceptibility* hypothesis posits that some genetic polymorphisms that confer risk in harsh environments are beneficial (linked to positive outcomes) in normal or nurturing environments.
- Understanding individual differences in susceptibility to environmental conditions is a crucial step in understanding how early maltreatment leads to risk for psychopathology.
- The present results indicate that genetic variation in the cholinergic system interacts with childhood maltreatment to affect personality (Openness/Intellect and Neuroticism) in a pattern indicating differential susceptibility associated with the polymorphism rs1044396 in the *CHRNA4* gene.
- When examining genetic and environmental correlates of clinical and nonclinical traits, future research should consider the role of variation in genes likely to influence individual differences in learning and neural plasticity.

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