

A Common Polymorphism in a Williams Syndrome Gene Predicts Amygdala Reactivity and Extraversion in Healthy Adults

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ABSTRACT

BACKGROUND: Williams syndrome (WS), a genetic disorder resulting from hemizygous microdeletion of chromosome 7q11.23, has emerged as a model for identifying the genetic architecture of socioemotional behavior. Common polymorphisms in *GTF2I*, which is found within the WS microdeletion, have been associated with reduced social anxiety in the general population. Identifying neural phenotypes affected by these polymorphisms would help advance our understanding not only of this specific genetic association but also of the broader neurogenetic mechanisms of variability in socioemotional behavior.

METHODS: Through an ongoing parent protocol, the Duke Neurogenetics Study, we measured threat-related amygdala reactivity to fearful and angry facial expressions using functional magnetic resonance imaging, assessed trait personality using the Revised NEO Personality Inventory, and imputed *GTF2I* rs13227433 from saliva-derived DNA using custom Illumina arrays. Participants included 808 non-Hispanic Caucasian, African American, and Asian university students.

RESULTS: The *GTF2I* rs13227433 AA genotype, previously associated with lower social anxiety, predicted decreased threat-related amygdala reactivity. An indirect effect of *GTF2I* genotype on the warmth facet of extraversion was mediated by decreased threat-related amygdala reactivity in women but not men.

CONCLUSIONS: A common polymorphism in the WS gene *GTF2I* associated with reduced social anxiety predicts decreased threat-related amygdala reactivity, which mediates an association between genotype and increased warmth in women. These results are consistent with reduced threat-related amygdala reactivity in WS and suggest that common variation in *GTF2I* contributes to broader variability in socioemotional brain function and behavior, with implications for understanding the neurogenetic bases of WS as well as social anxiety.

Keywords: Amygdala, Emotion, Extraversion, fMRI, *GTF2I*, Williams syndrome

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Hemizygous microdeletion of ~25 genes on chromosome 7q11.23 causes Williams syndrome (WS), a developmental disorder characterized by a unique profile of social and cognitive phenotypes, including hypersociability and increased approach to strangers (1). Systematic investigation of the genes within the WS microdeletion is underway in an effort to better understand the genetic architecture of the disorder specifically and of socioemotional behavior broadly (2,3). Clinical and preclinical studies have highlighted the importance of the WS gene *GTF2I*, which encodes for general transcription factor III (*GTF2I*) implicated in transcriptional regulation of a wide range of genes. For instance, in one case study, the microdeletion of this region was partial and spared *GTF2I*. In contrast to the social profile typically seen in WS, the individual in this case did not exhibit hypersociability and instead was rated by her parents as much less likely to approach strangers compared with individuals with the full WS microdeletion (4,5). A case study for a different individual

in which partial deletion spared *GTF2I* and the gene encoding general transcription factor III repeat domain-containing protein 1 (*GTF2IRD1*) revealed that this individual was categorized as a typically developing control subject based on multivariate pattern classification analysis with gray matter structure, implicating these genes more broadly in gray matter neuroanatomic abnormalities in WS (6).

More recent research has extended the characterization of *GTF2I* to the general population by examining the association between common single nucleotide polymorphisms (SNPs) in this region and variation in social and cognitive phenotypes. Specifically, two *GTF2I* SNPs in high linkage disequilibrium, rs13227433 and rs4717907, have been associated with individual differences on a composite WS profile score, including reduced communication abilities as rated on the Autism Spectrum Quotient scale, as well as reduced social anxiety (7). Moreover, *Gtf2i*-deficient mice evidenced increased social interaction but not alterations in learning and memory

or general anxiety, suggesting that deletion of *GTF2I* may play a specific role in the social phenotype of WS (8).

In the present study, we employ an imaging genetics strategy to examine the effect of common variation in imputed *GTF2I* genotypes on a systems-level neural phenotype, threat-related amygdala reactivity, associated with socioemotional behavior broadly and the hypersociability observed in WS specifically (9–11). We further test if this neural phenotype mediates associations between *GTF2I* genotype and extraversion, a personality trait that encompasses increased sociability (12). One previous study reported a positive correlation between extraversion and amygdala reactivity to happy but not threat-related (i.e., angry and fearful) facial expressions (13). However, this study was limited by a small sample size that likely reduced power to detect significant effects and prevented the examination of potential moderators such as sex. We expect that lower threat-related amygdala reactivity to fearful and angry facial expressions will predict higher extraversion for several reasons. First, WS is consistently associated with reduced amygdala reactivity to threat-related facial expressions (9,10). Second, consistent with its role in hypersociability, decreased amygdala reactivity to fearful facial expressions predicts individual differences in social approach toward strangers in individuals with WS (11). Third, mirroring the findings in WS, social anxiety, which is negatively correlated with extraversion (12,14), is consistently associated with relatively increased threat-related amygdala reactivity (15). Based on this evidence, we hypothesized that the *GTF2I* rs13227433 A allele, which has been linked to reduced social anxiety, would be associated with relatively decreased threat-related amygdala reactivity. Moreover, we hypothesized that such genotype-related differences in threat-related amygdala reactivity would indirectly link rs13227433 genotype to variability in extraversion.

METHODS AND MATERIALS

Participants

Participants included 808 young adult university students 18–22 years old who completed the ongoing Duke Neurogenetics Study (DNS) as of March 2, 2015 (Table 1). All procedures were approved by the Duke University Medical Center, and participants provided informed consent before participating in the study. Recruitment and exclusion criteria have been described in detail elsewhere (16–18). Diagnosis of any past or current DSM-IV Axis I disorder or select Axis II disorders (antisocial personality disorder and borderline personality disorder), assessed with the electronic Mini International Neuropsychiatric Interview (19) and Structured Clinical Interview for the DSM-IV subtests (20), was not an exclusion criterion, as the DNS seeks to establish broad variability in multiple behavioral phenotypes related to psychopathology. Consistent with epidemiologic data and the dimensional nature of psychopathology, 157 participants (19%) in the final sample reported here met criteria for at least one current or past Axis I disorder, including 101 with substance use disorders, 39 with major depressive disorder, 7 with bipolar disorder, 13 with bipolar disorder not otherwise specified, and

Table 1. Participant Characteristics as a Function of *GTF2I* rs13227433 Genotype and Race

	C Carrier	AA	Group Difference
Caucasian	<i>n</i> = 162	<i>n</i> = 265	
Age, years, mean (SD)	19.8 (1.3)	19.7 (1.2)	$t_{425} = .15, p = .88$
Sex (% female)	54%	52%	$\chi^2_1 = .20, p = .65$
Diagnosis ^a	23%	22%	$\chi^2_1 = .22, p = .64$
African American	<i>n</i> = 28	<i>n</i> = 82	
Age, years, mean (SD)	19.6 (1.1)	19.7 (1.2)	$t_{108} = -.48, p = .63$
Sex (% female)	61%	74%	$\chi^2_1 = 1.89, p = .17$
Diagnosis ^a	21%	21%	$\chi^2_1 = .01, p = .94$
Asian	<i>n</i> = 57	<i>n</i> = 214	
Age, years, mean (SD)	19.3 (1.2)	19.7 (1.3)	$t_{269} = -1.96, p = .05$
Sex (% female)	42%	59%	$\chi^2_1 = .512, p = .47$
Diagnosis ^a	16%	14%	$\chi^2_1 = .12, p = .74$
Total	<i>n</i> = 247	<i>n</i> = 561	
Age, years, mean (SD)	19.6 (1.3)	19.7 (1.3)	$t_{806} = -.86, p = .39$
Sex (% female)	52%	58%	$\chi^2_1 = 2.27, p = .13$
Diagnosis ^a	21%	19%	$\chi^2_1 = .93, p = .33$

^aDiagnosis is the percentage of participants in each group meeting criteria for at least one current or past psychiatric diagnosis.

36 with anxiety disorders (see Table S1 in Supplement 1 for full breakdown of diagnoses). Analyses were restricted to non-Hispanic Caucasian (*n* = 427), African American (*n* = 110), and Asian (*n* = 271) participants, as these were the largest racial/ethnic subgroups available within the DNS (see Supplement 1 for analyses by subgroup).

Amygdala Reactivity Paradigm

Amygdala reactivity to threat was assessed using an emotional face-matching challenge paradigm shown to consistently elicit robust amygdala reactivity in this and in previous samples (17,18). The paradigm version used in the DNS consists of four blocks of a face-processing task interleaved with five blocks of a sensorimotor control task. During task blocks, participants view a trio of faces and match one of two faces (bottom) identical to a target face (top). Each trial in the face-matching blocks lasts for 4 seconds with a variable interstimulus interval of 2–6 seconds (mean = 4 seconds), for a total block length of 48 seconds. In the control blocks, each of the six shape trios is presented for 4 seconds with a fixed interstimulus interval of 2 seconds for a total block length of 36 seconds. Total task time is 390 seconds.

Blood Oxygen Level-Dependent Functional Magnetic Resonance Imaging Data Acquisition, Preprocessing, and Quality Assurance

Functional magnetic resonance imaging (fMRI) of participants was performed using a research-dedicated GE MR750 3-tesla scanner (GE Healthcare, Marlborough, Massachusetts) at the Duke-UNC Brain Imaging and Analysis Center. A series of 34 interleaved axial functional slices aligned with the anterior commissure–posterior commissure plane were acquired for full-brain coverage using an inverse-spiral pulse sequence to reduce susceptibility artifact (repetition time = 2,000 ms; echo

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time = 30 ms; flip angle = 60°; field of view = 240 mm; 3.75 mm × 3.75 mm × 4 mm voxels; interslice skip = 0). The fMRI data were processed in SPM8 software using the standard preprocessing stream used in previously published research from the DNS (17,18), including realigning images to the first volume in the time series to correct for head motion, spatially normalizing images into a standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model (final resolution of functional images = 2-mm isotropic voxels), and smoothing set at 6-mm full width at half maximum.

Data Quality Assurance

Artifact Detection Tools software (The Gabrieli Lab at MIT, Cambridge, Massachusetts) (http://www.nitrc.org/projects/artifact_detect) was used to create regressors of no interest for 1) volumes exhibiting significant mean volume signal intensity variation (i.e., within volume mean signal greater or less than 4 standard deviations of mean signal of all volumes in time series) and 2) individual volumes where scan-to-scan movement exceeded 2 mm translation or 2° rotation in any direction. Quality control criteria for inclusion of a participant's imaging data were <5% volumes exceed artifact detection criteria for motion or signal intensity outliers, ≥90% coverage of signal within the anatomically defined bilateral amygdala region of interest, and accuracy ≥75% on the matching task performed during scanning. Within the current sample of 808 genotyped participants, 734 had fMRI data meeting quality assurance criteria.

Blood Oxygen Level-Dependent fMRI Data Analysis

The general linear model of SPM8 was used to conduct fMRI data analyses. Following preprocessing, linear contrasts employing canonical hemodynamic response functions were used to estimate main effects of expression for each individual. Individual contrast images were then entered in second-level random effects models to determine mean condition-specific regional responses using one-sample *t* tests. We extracted parameter estimates from functional clusters within anatomically defined amygdala regions of interest (Automated Anatomical Labeling atlas) (21) at $p < .05$ familywise error corrected across the search volumes for the contrast of angry and fearful blocks > control blocks. We did not have a priori hypotheses regarding laterality because WS and symptoms of hypersociability have been associated with altered activity in both the left (11) and the right (10) amygdala, and a meta-analysis found evidence for heightened left amygdala reactivity to facial expressions in individuals with social anxiety disorder relative to control subjects but reduced right amygdala reactivity in individuals with WS relative to control subjects (15). Therefore, we tested each separately and corrected for multiple comparisons using a false discovery rate (FDR) correction (i.e., the Benjamini-Hochberg procedure).

Genotyping

Genotyping was conducted by 23andMe, Inc. (Mountain View, California). Genomic DNA from all participants was isolated from buccal cells derived from Oragene DNA self-collection kits (DNA Genotek Inc., Kanata, Ontario, Canada) customized for 23andMe. DNA extraction and genotyping were performed at the

National Genetics Institute (Los Angeles, California), a Clinical Laboratories Improvement Amendment-certified clinical laboratory and subsidiary of Laboratory Corporation of America. One of two different Illumina arrays with custom content was used to provide genome-wide SNP data, HumanOmniExpress or HumanOmniExpress-24 (Illumina, Inc., San Diego, California) (22–24). Because neither SNP rs4717907 nor rs13227433 previously linked to social behavior was present on these arrays, genotypes at each locus were imputed using available SNP data.

Genotype imputation was performed on all DNS participants with genome-wide chip data using the prephasing/imputation stepwise approach implemented in SHAPEIT/IMPUTE2 (25,26). Imputation was run separately for participants genotyped on the Illumina HumanOmniExpress ($n = 728$) and the Illumina HumanOmniExpress-24 ($n = 246$) arrays using biallelic SNPs only, the default value for effective size of the population (20,000), and chunk sizes of 3 Mb and 5 Mb for the respective arrays. Within each array batch, genotyped SNPs used for imputation were required to have missingness <.02, Hardy-Weinberg equilibrium $p > 10^{-6}$, and minor allele frequency >.01. The imputation reference set consisted of 2504 phased haplotypes from the full 1000 Genomes Project Phase 3 data set (May 2013, >70 million variants, release “v5a”). Imputed SNPs were retained if they had high imputation quality (Info >.9), low missingness (<5%), and minor allele frequency >.01.

Imputed SNP data were available for 808 participants for rs13227433 and for 805 participants for rs4717907 (data for 3 participants did not meet quality assurance criteria for this SNP). Data for these imputed SNPs were perfectly correlated given that they are in high linkage disequilibrium; to maximize participants included, analyses were conducted with rs13227433. Results remain as reported when excluding the three participants with low imputation quality for rs4717907. The imputation quality for rs13227433 was .992 (HumanOmniExpress) and .981 (HumanOmniExpress-24). Moreover, we observed high levels of linkage disequilibrium ($R^2 = .98$) between imputed rs13227433 genotype and a proxy SNP (rs6964833; HapMap 3: CEU+TSI, JPT+CHB, GIH $R^2 = 1.00$, YRI $R^2 = .90$) that was genotyped in a portion of the sample ($n = 617$). We further tested the reliability of results obtained using imputed SNP data by analyzing data using the genotyped proxy SNP rs6964833; results remain as reported with the imputed SNP data from the full sample (Supplement 1).

Genotype distribution did not deviate from Hardy-Weinberg equilibrium across our entire sample ($\chi^2_1 = .89$, $p = .345$) or within Caucasian ($\chi^2_1 = .39$, $p = .532$), African American ($\chi^2_1 = .001$, $p = .975$), or Asian ($\chi^2_1 = .04$, $p = .841$) subgroups. A few participants were homozygous for the minor C allele (Caucasian, $n = 22$; African American, $n = 2$; Asian, $n = 3$); therefore, we created a binary variable grouping participants as A allele homozygotes or C allele carriers (see Supplementary Analyses in Supplement 1 supporting this grouping). Genotype was dummy-coded so that the AA genotype [previously associated with a higher WS profile score and lower social anxiety (7)] was the group of interest (C allele carriers = 0; AA = 1). Population stratification reflecting genetic heterogeneity associated with ancestry was examined using identity by state analysis in PLINK of the whole-genome SNPs, extracting the first four multidimensional scaling components for inclusion as covariates in all genotype analyses (27).

Extraversion

As a component of the DNS, all participants completed the Revised NEO Personality Inventory (28,29). Here we focus on the extraversion scale because it encompasses several pro-social behaviors, including warmth, gregariousness, assertiveness, activity, excitement seeking, and positive emotions.

Covariates

As described earlier, four multidimensional scaling components were derived from identity by state analysis and included as covariates in the path from rs13227433 genotype to amygdala reactivity to control for possible genetic heterogeneity associated with ancestry. In addition, age and sex were included as covariates on all paths. In preliminary analyses, we found that past or current DSM-IV diagnosis (dummy-coded, 0 = no diagnosis, 1 = past or current diagnosis) did not moderate the effects of interest (all $p > .05$); therefore, we included diagnosis as a binary covariate on all paths but did not separate the groups for our main analyses. Finally, to test whether amygdala reactivity specifically predicted extraversion above and beyond broader anxiety, we also included as a covariate on the brain to behavior path scores on the trait version of the State-Trait Anxiety Inventory (30).

Statistical Analyses

Our main aim was to construct a mediation model to test whether threat-related amygdala reactivity mediates the association between rs13227433 genotype and extraversion. Before this, we performed analyses to determine the best-fitting parameters for each path. For the gene to brain path (*GTF2I* to amygdala reactivity), we tested the effect of rs13227433 genotype on left and right amygdala reactivity controlling for age, sex, diagnosis, and the multidimensional scaling components. Next, given observed sex differences in extraversion (31), we further examined whether sex moderated links between *GTF2I* and amygdala reactivity (excluding sex as a covariate). Moderation was assessed using χ^2 difference tests; if constraining parameter estimates to be equal across groups leads to a significant decrease in model fit, one can conclude the effect is significantly moderated by sex. For the brain to behavior path (amygdala reactivity to extraversion), we conducted regressions examining associations between left and right amygdala reactivity and total Revised NEO Personality Inventory extraversion scores. We first modeled the effect in the full sample including age, sex, diagnosis, and trait anxiety as covariates. We next conducted a multigroup analysis to examine whether this path varied across sex. Lastly, following observations of a significant association between amygdala reactivity and extraversion, post hoc testing examined which facets (i.e., warmth, gregariousness, assertiveness, activity, excitement seeking, positive emotions) were primarily driving this association. All analyses were conducted with Mplus Version 7 software (32), which uses full information maximum likelihood estimation to provide unbiased estimates in the presence of missing data.

Indirect Effects Model

After model parameters were determined for each path, we constructed a mediation model to examine the indirect effect

of rs13227433 genotype on extraversion, mediated by threat-related amygdala reactivity. We also modeled the direct effect of genotype on behavior by including it as a predictor in the second path. To provide a measure of general effect size for the indirect effect, we report the product of coefficients $\alpha\beta$ statistic (also called a Sobel test). However, given well-documented distributional assumptions that are generally not met in that test, we also provide bootstrapped confidence intervals that use a Monte Carlo simulation (1000 draws), which do not assume normality of the distribution of indirect effects and provide better power to detect the indirect effect.

RESULTS

Threat-Related Amygdala Reactivity

Consistent with prior research, the angry and fearful blocks > control blocks contrast elicited significant threat-related amygdala reactivity in the left hemisphere ($t_{733} = 25.7, p < .001$, peak coordinates $-22, -6, -18$) and right hemisphere ($t_{733} = 29.5, p < .001$, peak coordinates $28, -4, -20$).

GTF2I Genotype to Amygdala Reactivity

The AA genotype of rs13227433 predicted decreased bilateral amygdala reactivity across the entire sample (left amygdala, $B = -.10, SE = .03, \beta = -.26, p = .005$, FDR-corrected $p = .01, \Delta r^2 = .01$; right amygdala, $B = -.06, SE = .03, \beta = -.19, p = .029$, FDR-corrected $p = .029, \Delta r^2 = .01$) (Table S2 in Supplement 1 and Figure 1). Multigroup analyses indicated that this effect was not moderated by sex for the left ($\Delta\chi^2_1 = .40, p = .527$) or right ($\Delta\chi^2_1 = .01, p = .920$) amygdala.

Amygdala Reactivity to Extraversion

The effect of left amygdala reactivity predicting extraversion was not significant in the full sample ($B = -1.36, SE = 1.84, \beta = -.03, p = .457, \Delta r^2 = .001$). However, a multigroup analysis

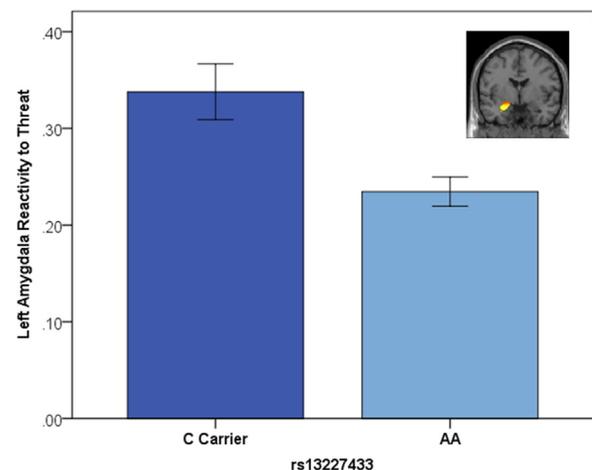


Figure 1. *GTF2I* rs13227433 genotype predicts left amygdala reactivity to threat. Parameter estimates for reactivity to threat were extracted from a functional cluster within the anatomically defined left amygdala region of interest for the contrast of angry and fearful blocks > control blocks. Error bars = 1 SE.

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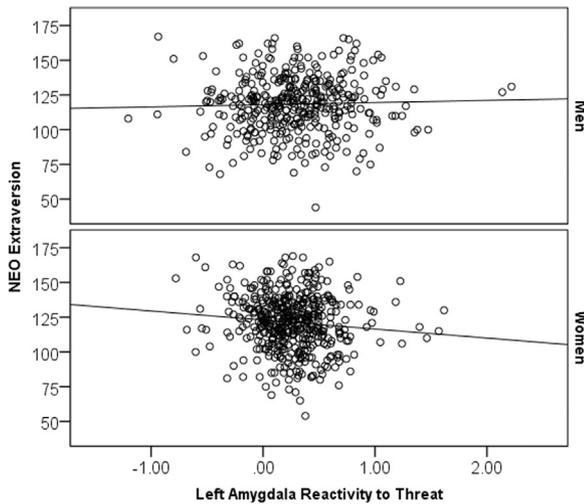


Figure 2. Decreased amygdala reactivity to threat predicts higher extraversion in women. Parameter estimates for reactivity to threat were extracted from a functional cluster within the anatomically defined left amygdala region of interest for the contrast of angry and fearful blocks > control blocks. NEO, Revised NEO Personality Inventory.

indicated that this effect was moderated by sex ($\Delta\chi^2_1 = 3.83, p = .050$). In women, reduced left amygdala reactivity predicted higher extraversion ($B = -5.59, SE = 2.73, \beta = -.09, p = .041, \Delta r^2 = .01$) (Table S2 in Supplement 1 and Figure 2), but in men this effect was not significant ($B = 1.66, SE = 2.44, \beta = .04, p = .498, \Delta r^2 = .001$). Right amygdala reactivity did not significantly predict extraversion in the full sample ($B = -.84, SE = 2.25, \beta = -.01, p = .708, \Delta r^2 = .001$), and this effect was not moderated by sex. Finally, we examined whether the effect of left amygdala reactivity in women was specific to extraversion subscales and found specificity for the warmth facet ($B = -1.78, SE = .56, \beta = -.13, p = .001, FDR\text{-corrected } p = .024, \Delta r^2 = .02$; all other facets $p > .05$). This effect survived FDR correction for the 6 subscales \times 2 hemispheres \times 2 groups (men, women) tested.

Indirect Effects Model

For the final mediation model, we examined the effect of rs13227433 genotype on extraversion, mediated by left amygdala reactivity. The gene to brain parameters were constrained to be equal across sex, but the brain to behavior parameters were allowed to vary given evidence for moderation by sex. The model had an excellent fit ($\chi^2_{17} = 16.84, p = .465$, root mean square error of approximation = .00, comparative fit index = 1.0, standardized root mean square residual = .016). As reported earlier, the AA genotype of rs13227433 predicted decreased left amygdala reactivity to threat ($B = -.10, SE = .03, p = .001$). Moreover, in women, decreased left amygdala reactivity predicted higher scores on extraversion ($B = -5.69, SE = 2.85, p = .046$). Finally, there was a significant indirect effect of rs13227433 genotype on extraversion in women, mediated by decreased left amygdala reactivity to threat ($\alpha\beta = .58, SE = .32, 95\% \text{ bias-corrected confidence interval } [.07, 1.32]$). Because the association between amygdala reactivity and extraversion was driven by the facet of warmth, we also tested this as an outcome in the mediation model. The indirect effect of rs13227433 on warmth mediated by left amygdala reactivity was also significant in women ($\alpha\beta = .19, SE = .08, 95\% \text{ bias-corrected confidence interval } [.06, .39]$) (Figure 3).

DISCUSSION

We provide novel results that common variation in the WS gene *GTF2I* previously associated with reduced social anxiety predicts threat-related amygdala reactivity. We further demonstrate that decreased threat-related amygdala reactivity partially mediates the association between *GTF2I* genotype and the personality trait of warmth in women but not men. Collectively, our results suggest that the effect of common variation in *GTF2I* on sociability may be mediated by reduced amygdala reactivity to threat and may extend dimensionally to normative populations and the personality dimension of warmth.

Although our current results, along with the results of Crespi and Hurd (7), are consistent with the hypersocial

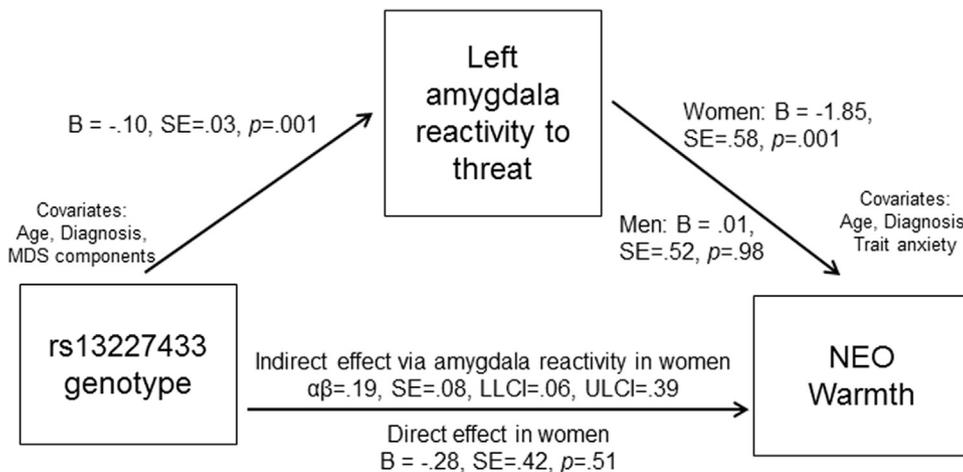


Figure 3. Full mediation model testing indirect effect of rs13227433 genotype on warmth, mediated by left amygdala reactivity to threat. Parameters for the gene to brain path were constrained to be equal for men and women. The direct effect was modeled by including genotype as a predictor in the regression for warmth, in addition to the mediator (threat-related amygdala reactivity) and covariates. $\alpha\beta$, indirect effect; LLCI, 95% lower limit confidence interval; MDS, multidimensional scaling; NEO, Revised NEO Personality Inventory; ULCI, 95% upper limit confidence interval.

phenotype of WS, the molecular mechanisms through which these associations occur is unclear. One intriguing possible molecular pathway through which *GTF2I* rs13227433 genotype may affect neural and behavioral socioemotional phenotypes is serotonin signaling, which plays an important role in modulating corticolimbic circuit function, including amygdala reactivity (33–35). The *GTF2I* protein regulates the transcription of the ligand-gated ion channel serotonin receptor 3A (*HTR3A*) (36), which is expressed in the amygdala (37). Thus, the observed neural circuit and behavioral effects of *GTF2I* rs13227433 may reflect differential transcriptional regulation of serotonin-related fast depolarization of neurons in the amygdala (38). Given the wide range of genes regulated by *GTF2I*, this is only one of several molecular signaling pathways (e.g., pathways involved in dendritic spine formation and synaptic plasticity) that could be considered in future research. Moreover, given evidence of abnormalities in the oxytocin pathway in WS (39) and the modulatory role of oxytocin on amygdala function (40), future research is warranted to test whether genes associated with the oxytocin signaling pathway may also be regulated by *GTF2I*.

This is the first study to our knowledge to identify an association between amygdala reactivity to threat-related facial expressions and extraversion in the general population and as such will require replication in future research. The association between threat-related amygdala reactivity and extraversion was significant only for women. Although many factors may have contributed to this finding, prior research demonstrated sex differences in the functional connectivity of the amygdala, including increased functional connectivity between the left amygdala and several prefrontal regions in women compared with men (41). Therefore, sex differences in the association between amygdala reactivity and extraversion may partly reflect sex differences in how the amygdala relays information to prefrontal cortical regions. It is also notable that this association was specific to warmth, given that increased warmth (e.g., hugging of strangers) is part of the characteristic hypersociability phenotype of WS, and was less robust for other subscales related to activity or energy levels, such as excitement seeking or positive emotions. Individuals with relatively decreased threat-related amygdala reactivity may be less inhibited and more likely to seek out social interactions, explaining their higher levels of extraversion and, specifically, warmth. The specificity of effects to the left amygdala was not hypothesized a priori but may reflect the finding that the left amygdala evidences more sustained responses to threatening faces, whereas the right amygdala habituates more quickly (42), such that the sustained signal in the left amygdala may have produced more robust effects. Prior research indicates that individuals with WS evidence greater amygdala reactivity to happy facial expressions relative to control subjects (10) and that in the general population greater amygdala reactivity to positive (e.g., happy) faces is also associated with higher levels of extraversion (13). We were unable to test this possibility in the present study because our fMRI paradigm does not include happy facial expressions. This possibility represents an important future direction for research to elucidate further the neural mechanisms mediating the association between common variation in the *GTF2I* gene and extraversion.

In addition to the lack of happy facial expressions, the lack of threatening nonsocial stimuli is a limitation of the present study. Individuals with WS evidence low levels of social anxiety but high levels of nonsocial anxiety, coupled with heightened amygdala reactivity to nonsocial, threatening scenes (9). With the current paradigm, we were unable to examine whether the effect of *GTF2I* rs13227433 genotype was specific to threatening faces or whether this would generalize to amygdala reactivity to threatening, nonsocial stimuli. Moreover, as neither rs4717907 nor rs13227433 were present on the Illumina arrays, we imputed these genotypes based on available genetic data. However, results were similar when using a genotyped proxy SNP in high linkage disequilibrium with rs4717907 and rs13227433 (Supplementary Analyses in Supplement 1), increasing confidence in our results and the quality of imputation. An additional limitation of the present study is that the DNS battery did not include a measure specific to social anxiety or a measure of social abilities similar to the Autism Spectrum Quotient used in prior research examining these *GTF2I* genotypes (7), and so we were unable to construct a WS profile similar to that examined by Crespi and Hurd. However, given that extraversion is negatively correlated with social anxiety (12), individuals who reported relatively higher levels of extraversion in the DNS likely also had low social anxiety. Moreover, by controlling for trait anxiety in our model, we were able to demonstrate that amygdala reactivity predicts individual differences in extraversion above and beyond broader anxiety and thus likely reflects socioemotional characteristics. Finally, although effects were statistically significant, effect sizes were in the modest range for both paths. However, these effect sizes are consistent with prior imaging genetics research (43,44) and likely reflect the complex and polygenic nature of variability in both brain and behavior.

In conclusion, our results help shed light on the role of common variation in *GTF2I* in the emergence of individual differences in neural circuit function associated with prosocial behaviors and suggest that a similar neural pathway may be implicated in the distinct social phenotype characteristic of WS, including hypersociability and increased approach of strangers. These results also have implications for identifying a potential genetic risk marker for social anxiety disorder, given that low levels of extraversion are associated with high social anxiety (12,14). As such, our results provide a foundation for pursuing the molecular mechanisms through which variation in *GTF2I* may impact gene transcription, affect amygdala function, and influence individual differences in socioemotional behavior.

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