Variation in the Williams syndrome GTF2I gene and anxiety proneness interactively affect prefrontal cortical response to aversive stimuli

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Characterizing the molecular mechanisms underlying the heritability of complex behavioral traits such as human anxiety remains a challenging endeavor for behavioral neuroscience. Copy-number variation (CNV) in the general transcription factor gene, GTF2I, located in the 7q11.23 chromosomal region that is hemideleted in Williams syndrome and duplicated in the 7q11.23 duplication syndrome (Dup7), is associated with gene-dose-dependent anxiety in mouse models and in both Williams syndrome and Dup7. Because of this recent preclinical and clinical identification of a genetic influence on anxiety, we examined whether sequence variation in GTF2I, specifically the single-nucleotide polymorphism rs2527367, interacts with trait and state anxiety to collectively impact neural response to anxiety-laden social stimuli. Two hundred and sixty healthy adults completed the Tridimensional Personality Questionnaire Harm Avoidance (HA) subscale, a trait measure of anxiety proneness, and underwent functional magnetic resonance imaging (fMRI) while matching aversive (fearful or angry) facial identity. We found an interaction between GTF2I allelic variations and HA that affects brain response: in individuals homozygous for the major allele, there was no correlation between HA and whole-brain response to aversive cues, whereas in heterozygotes and individuals homozygous for the minor allele, there was a positive correlation between HA sub-scores and a selective dorsolateral prefrontal cortex (DLPFC) responsivity during the processing of aversive stimuli. These results demonstrate that sequence variation in the GTF2I gene influences the relationship between trait anxiety and brain response to aversive social cues in healthy individuals, supporting a role for this neurogenetic mechanism in anxiety.

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INTRODUCTION

Although anxiety disorders are among the most frequent psychopathological presentations and are highly heritable, their clinical heterogeneity and underlying pathophysiological complexities pose a challenge for studying the neurogenetic origins of these conditions. A promising approach that could have therapeutic implications is to characterize neurogenetic mechanisms related to known copy-number variations (CNVs) associated with clinically relevant phenotypes. In the context of anxiety, one such strategic target is the general transcription factor gene, GTF2I (Gene ID: 2969), GTF2I (located approximately between base pair 74,657,664 and 74,760,691 on chromosome 7) is one of a set of genes that, in the rare disorder Williams syndrome (MIM 194050), is hemizygotically deleted from chromosomal location 7q11.23, thereby leaving only one remaining copy of affected genes. Interestingly, individuals with the more recently discovered and even rarer 7q11.23 duplication syndrome (Dup7q11.23; MIM 609757) have three copies of this same set of genes. With one copy of the genes, individuals with Williams syndrome show hypersociability together with very little social anxiety, whereas persons with the duplication syndrome are highly socially anxious and even have autistic spectrum symptoms. Particular importance of GTF2I is suggested by the fact that mouse models constructed to specifically have one, two or three copies of GTF2I show relationships between anxiety and GTF2I copy number that parallel observations in the human hemideletion and duplication syndromes: compared with mouse pups with one or two GTF2I copies, pups with additional GTF2I copies exhibited significantly increased maternal separation-induced anxiety as measured by ultrasonic vocalizations. In another study, mice with GTF2I haploinsufficiency showed increased social interaction with unfamiliar mice and did not show typical social habituation processes, again consistent with the Williams syndrome personality.

A potential molecular mechanism that may contribute to this pattern of GTF2I copy-number-related anxiety phenotype in both model animals and humans is suggested by the role of GTF2I protein in the induction of c-fos, the latter being a behaviorally relevant marker for neural activity. C-Fos expression is functionally influenced by GTF2I expression in a GTF2I gene-dosage-dependent manner, in line with recent evidence of anxiolytic-induced changes in brain c-fos expression in mice with different GTF2I...
CNVs.14,15 GTF2I can also bind to promotor elements of other genes,13 some of which are known to functionally impact major neurotransmitter systems with anxiogenicity and antidepressant effects, such as the glutamategic and monoamnergic systems.

These data point to a gene-dosage-sensitive relationship between GTF2I CNVs and socially relevant anxiety in both mice10,12 and humans,10,11 and thereby suggest that GTF2I functionality may have implications for understanding neurobiological mechanisms that could contribute to the heritable component of anxiety. However, the neural mechanism by which GTF2I alterations might impact neurobehavioral correlates of anxiety phenotypes in clinical cohorts and in the general population remains to be elucidated.

Here, in a large cohort of healthy humans studied with functional magnetic resonance imaging (fMRI) and the Tridimensional Personality Questionnaire, we examined the effect of sequence variation in this gene, specifically choosing rs2527367, a common intronic GTF2I single-nucleotide polymorphism (SNP) that is in very high linkage disequilibrium with many other GTF2I SNPs between chromosomal location 73706683 and 73777987. Our choice of this high linkage disequilibrium tag SNP obviates the need to investigate a number of related GTF2I SNPs, thus minimizing the number of statistical procedures necessary to test our hypothesis. Because genes code for molecular processes that act on neurons and neural systems,15 which, in turn, are the intermediaries of genetic effects on complex behaviors,16 brain phenotypes may be more proximately related to genes than are behaviors.15,16 Indeed, the effect size of risk genes at the level of neural structure and function has been demonstrated to be considerably higher than what has been found for association of the same gene variants with neuropsychiatric diagnosis.15 Therefore, we reasoned that, particularly in light of the fact that sequence variation is generally less altering of a gene’s function than is a CNV, the mechanism by which the effects of a GTF2I SNP interact with trait anxiety in the general population would be more robust and best experimentally observed in a neurofunctional circuit activated by processing aversive stimuli. We applied this reasoning to test the hypothesis that GTF2I sequence variation affects the relationship between trait anxiety proneness and behaviorally relevant regional brain responsivity to anxiety-laden social stimuli.

MATERIALS AND METHODS

Participants

A sample of 260 healthy participants (mean age = 30.00 ± 9.12 years, range 18–54; and mean intelligence quotient (IQ) = 110.30 ± 8.59, range 86–132; 141 females) participated in the study after giving written informed consent in accordance with NIH institutional review board–approved procedures. To minimize population stratification artifacts, only Caucasians of self-identified European descent were included. Each participant completed history, physical and laboratory tests to assess for medical conditions that could affect the brain. Those with current or past medical or neuropsychiatric conditions (the latter as determined by the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders Version IV carried out by qualified raters), including alcohol or drug abuse or head trauma, were excluded, as were those with abnormalities on structural MRI.

Genotyping

The standard Taqman allelic Discrimination 5’ exonuclease assay was applied to DNA from peripheral blood. SNP probe and primer sets were obtained from Applied Biosystems (Foster City, CA, USA). GTF2I sequences were amplified using allele-specific Taqman fluorescent probes, and PCR was performed in an ABI (Applied Biosystems) 9700 thermal cycler. The amplified fluorescent amplicon was read in an ABI 7900 sequence detection system. Genotype accuracy was assessed by re-genotyping within a subsample, and reproducibility was routinely > 99%.

Of the 260 participants of self-identified European ancestry, 115 were homozygous for the major GTF2I rs2527367 allele (t/t), 118 were heterozygotes (t/c) and 27 were homozygous for the minor allele (c/c). Given that our sample includes individuals of northern, eastern, western and southern European origin, the allelic frequency of t = 348 (66.92% of our sample) and c = 172 (33.07% of our sample) does not represent any specific HAPMAP population.

Trait anxiety scores

The Tridimensional Personality Questionnaire Harm Avoidance (HA) subscale, a trait measure of anxiety proneness that specifically taps into characterological dispositions to excessive worrying, shyness and pessimism, as well as general fearfulness, doubtfulness and fatigability,14 was administered to all 260 participants. In the current study, HA scores were used (a) for the purpose of assessing the effects of GTF2I genotype on trait anxiety scores, and (b) as covariates of interest in the analysis of GTF2I genotypic effects on neural response to aversive affective stimuli during fMRI.

Aversive face-viewing task

This well-established fMRI paradigm consists of two experimental conditions: an emotional face identity-matching condition and a low-level sensorimotor control task.13 Each sensorimotor and emotion block consisted of six 5-s duration trials. Each trial involved the presentation of two images in the lower panel and one image in the upper panel. For sensorimotor trials, the two lower images were shapes, and the upper panel image was identical to one of the shapes in the lower panel. For emotional face trials, the lower panel consisted of two faces, one angry and one afraid, derived from a standard set of pictures of facial affect;12 the upper panel consisted of one of the two faces shown in the lower panel. Subjects responded using button presses (left or right) to indicate which lower panel item matched the item in the upper panel, a matching parameter that was chosen with the goal of keeping the processing of the aversive emotional content of the faces implicit.13 In addition to promoting implicit processing of aversive social stimuli, keeping in mind that implicit behavioral processes are likely more proximate to trait measures such as HA than explicit behavioral processes, this fMRI paradigm taps into the neural substrates of innate socially relevant threat perception,20 and has been shown to be sensitive to genetic effects.21,22,23 Stimuli were displayed through a back-projection system and participants’ responses were recorded through a fiber-optic connection.

Image acquisition

fMRI was performed on a 3-Tesla scanner (General Electric, Milwaukee, WI, USA) equipped with a real-time functional imaging upgrade and a standard head coil. We acquired two experimental runs containing blocks of emotional and sensorimotor trials using the following acquisition parameters: Echo planar imaging BOLD fMRI, 24 axial slices (4 mm thick, 1 mm gap) encompassing the whole cerebrum and the majority of the cerebellum (TR/TE = 2000/20 ms, 170 volumes for each subject, FOV = 24 cm, matrix = 64 × 64).

Image processing and analysis

Using Statistical Parametric Mapping software version 8 (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), we preprocessed (realignment, coregistration, normalization and 8-mm smoothing) and applied a first-level one-sample test, to compare BOLD reactivity during blocks of aversive face processing with that during shape processing (aversive faces > shapes). In a second-level analysis, the results of the main-effect-of-task analysis were thresholded at P < 0.001 to provide a mask within which to assess the relationship between the behavioral (HA) scores and BOLD response to aversive social cues using a voxel-wise regression. This approach was used to ensure that the results of our next step, identifying HA-related BOLD findings, were restricted to task-relevant areas. We then performed a multiple regression, this time including HA scores as the covariate of interest, and IQ, age and sex as control variables, and thresholded the results at P < 0.05. The results of this analysis were similarly used as a mask for the next step, which was aimed to assess the main hypothesis of this paper that is as follows: sequence variation in the GTF2I gene affects the relationship between HA and neural response to processing aversive stimuli. We then performed a full factorial analysis by including GTF2I genotype as a group factor, and HA scores as predictors, whereas age, sex and IQ were included as control variables.
RESULTS

GTF2I genotype is not associated with behavioral or demographic measures

To control for potential confounding effects, we first assessed the association between GTF2I genotype and the demographic measures (sex and age) and found no relationship between genotype and sex (F = 0.497, P = 0.609) or age (F = 0.986, P = 0.381). We then assessed the gene–behavior relationship and found no direct effect of GTF2I sequence variation on participants’ IQs (F = 0.089, P = 0.915), HA scores (F = 0.76, P = 0.469) or face identity-matching performance (F = 0.773, P = 0.463), together suggesting a lack of potential behavioral confounds in our study.

Aversive face processing recruits frontolimbic and occipital BOLD response

As reported previously,18,24 we found a main effect of the task response. Aversive face processing recruits frontolimbic and occipital BOLD responses in the bilateral amygdala, parahippocampal gyrus, as well as in a right dorsolateral prefrontal cortex (DLPFC).

Harm avoidance scores are associated with BOLD response to aversive face processing

To examine the effects of HA scores on whole-brain response, we conducted a simple regression analysis using SPM8 by including HA scores in the model as predictors and age, sex and IQ as control variables, and we limited our search area to the region defined by the brain response to aversive faces. We found that the right DLPFC locus responding to the main effect of task described above (centered on the Montreal Neurological Institute (MNI) coordinates x, y, z = 51, 30, 12) was modulated by individual differences in HA tendencies at P < 0.05 (Figure 1b), such that the higher an individual’s trait measure of anxiety, the greater their DLPFC response to aversive stimuli.

GTF2I genotype affects the relationship between HA and DLPFC BOLD response to aversive faces

Although a direct assessment of a relationship between GTF2I sequence variation and BOLD response to aversive cues yielded no significant findings as expected, a multiple regression analysis using a full factorial design with GTF2I sequence variation as a group factor and HA scores as predictors (along with age, sex and IQ as control variables) demonstrated that the relationship between HA scores and BOLD signal differed as a function of GTF2I genotype within the DLPFC region defined by the HA-by-task interaction analysis. Specifically, an F-test assessing the interaction between HA and GTF2I variation identified a genotype-dependent differential relationship between HA and the BOLD signal (P = 0.001; cluster-level statistics) at MNI x, y, z = 51, 30, 12 within the same DLPFC locus that has been previously implicated in regulating top-down guidance of attention and thought.25 and in choice-related decision making26 and shown here to be correlated with HA scores (see Figure 1a) across the entire group (Figure 1c). Performing the same analysis by using a mask created from task-negative response data (regions showing negative BOLD signal to face processing), we found no interaction between GTF2I variation and HA scores, suggesting relative regional specificity of our DLPFC findings.

Finally, we performed a post hoc graphical exploration to delineate the nature of this genotype-dependent differential relationship between HA and DLPFC responsivity. From the one-sample t-test (main effect of task), we extracted the raw BOLD signals from the DLPFC locus shown to be commonly active in (1) the one-sample t-test of the main effect of task, (2) the HA-associated brain response and (3) the F-test of GTF2I rs2527367-HA interactive response. To better understand the F-test results, we plotted and tested the relationship between HA scores and DLPFC BOLD responses (MNI x, y, z = 51, 30, 12) for each genotype group separately. This post hoc visualization revealed a genotype-related association such that individuals homozygous for the major allele (t/t variants) showed no association between percent BOLD signal changes in DLPFC and HA scores (R = −0.122,
by HA scores and their interaction with cluster centered on MNI coordinates HA, harm avoidance; MNI, Montreal Neurological Institute. GTF2I in frontal and limbic circuitry in facilitating the regulation of homozygous for the on right-DLPFC responsivity to aversive social stimuli: homozygous individual between DLPFC response and HA scores, such that the greater an distance of the DLPFC aversive social stimuli. c/c and GTF2I our study on this particular gene. With a neuroimaging experi- DLPFC to the striatum via the posterior cingulate.28 In addition, behavior more generally, a recent study showed that HA was regulatory role of the DLPFC and related circuitry in emotional show more.10 Despite these intriguing clinical observations, the role of individual 7q11.23 genes in the etiology of anxiety remains to be defined. Guided by convergent findings of gene-dose dependent anxiety in mouse models constructed to have CNVs specifically in GTF2I10—findings that parallel observations in the human hemideletion and duplication syndromes10—we focused our study on this particular gene. With a neuroimaging experi- in a large adult cohort of 260 healthy participants, we demonstrated an interactive effect between GTF2I rs2527367 genotype and the degree of a trait measure of anxiety proneness on right-DLPFC responsivity to aversive social stimuli: homozygous carriers of the major allele (t/t) showed no association between HA scores and DLPFC response to aversive social cues, whereas heterozygotes and homozygote carriers of the minor allele (t/c and c/c, respectively) instead displayed a positive association between DLPFC response and HA scores, such that the greater an individual’s propensity for anxiety proneness as measured with HA scores, the greater an individual c carrier’s DLPFC response to aversive social stimuli. Considerable neurobiological evidence supports the importance of the DLPFC’s top–down inhibitory influence on related frontal and limbic circuitry in facilitating the regulation of cognition and emotion.27 Of interest to our findings and to the regulatory role of the DLPFC and related circuitry in emotional behavior more generally, a recent study showed that HA was positively associated with white matter fiber connectivity from the DLPFC to the striatum via the posterior cingulate.28 In addition, the magnitude of amygdala resting-state functional connectivity with the dorsal region of the frontal cortex was shown to be predictive of HA tendency in healthy female individuals.29 The importance of the DLPFC in emotional regulation has also been supported by several findings in clinical populations. First, our group previously demonstrated aberrant functional interaction between the amygdala and frontal regions including the DLPFC in adult Williams syndrome patients during aversive face processing.7 Second, within this same neuroanatomical circuitry, dysregulated amygdala connectivity with DLPFC was selectively observed in generalized social anxiety disorder patients during perception of threatening information, and, moreover, the strength of the amygdala–DLPFC connectivity was related to measures on the Liebowitz Social Anxiety Scale.30 Another study using fMRI to measure resting functional connectivity in children with anxiety disorders as well as in anesthetized young monkeys identified a relationship between functional connectivity and early-life anxiety that appeared to be evolutionarily conserved: across primate species, reduced functional connectivity between the DLPFC and the central nucleus of the amygdala was found to be predictive of increased measures of anxiety.31 As childhood anxiety, including separation anxiety, predicts the eventual emergence of adult anxiety,32–34 these findings, as well as the separation anxiety findings in children with 7q11.23 CNVs including GTF2I10 are directly relevant to our brain-imaging findings in adults. Despite the strong evidence implicating DLPFC-associated circuitry in anxiety, a molecular framework for these system-level dynamics in the context of anxiety has not been delineated. Our current results regarding an effect of GTF2I genotype extend previous systems-level brain circuitry findings by identifying one potential molecular candidate that may contribute to biasing the response of relevant neural circuitry to aversive stimuli. The GTF2I gene has been identified as a transcriptional factor that binds to promoter elements of many relevant genes,13 making it a candidate effector gene that may influence other genes of relevance in the etiology of anxiety, as well as molecular pathways that may be therapeutic targets. For example, it has recently been shown that serotonergic-targeting drugs exhibited a GTF2I gene-dosage-dependent effect, whereby the anxiolytic effects of these drugs were found to be reduced as a function of the GTF2I gene copy-number increase in a mouse model.14 In summary, our results support the hypothesis that GTF2I sequence variation impacts neural correlates of anxiety-related behavior in normal controls, demonstrating a genetic influence on the response repertoire of the DLPFC in processing aversive social cognition. In addition to the implications of our findings of DLPFC-

Figure 2. Post hoc visualization plots showing the association between HA scores and DLPFC BOLD response for each GTF2I allelic variant separately. (a) Correlation plot showing no association between HA scores and DLPFC BOLD response to aversive social stimuli in individuals homozygous for the GTF2I major t/t allele. (b, c) Correlation plots show a robust positive association between HA scores and DLPFC response in GTF2I heterozygous t/c carriers and those homozygous for the minor c/c alleles, respectively. Signal values were extracted from the DLPFC cluster centered on MNI coordinates x, y, z = 51, 30, 12 representing the maximal voxel in the DLPFC where BOLD response was also predicted by HA scores and their interaction with GTF2I allelic variation, as shown in Figure 1a (upper right image). DLPFC, dorsolateral prefrontal cortex; HA, harm avoidance; MNI, Montreal Neurological Institute.

P = 0.192, two tailed; Figure 2a), whereas heterozygotes (t/c variants) showed a positive association between DLPFC response and HA (R = 0.234, P = 0.011, two tailed; Figure 2b), and individuals homozygous for the minor allele (c/c variants) showed a more robust positive association between signal change in DLPFC response to aversive cues and HA scores (R = 0.505, P = 0.007, two tailed; Figure 2c).
associated circuitry in anxiety proneness, our current results uncovered a putative neurogenetic mechanism that may, in part, account for the individual difference in behaviorally relevant anxiety traits across the normal population and thereby inform the search for genetic contributors to anxiety disorders. Nonetheless, the correlational nature of our findings in healthy individuals cannot infer causality in anxiety per se. Furthermore, additional research examining the influence of GTF2I sequence variation and CNV in cell and animal models of disease, as well as in patient populations with contrasting 7q11.23 CNVs is necessary to better understand the full impact of GTF2I and related genetic involvement in human anxiety. At a healthy population level, the present findings may suggest an intermediate brain phenotype that could contribute to a better mechanistic understanding of the role of GTF2I in anxiety and may provide information to guide the search for new pharmacotherapeutic targets.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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