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Preliminary evidence of association between EFHC2, a gene implicated in fear recognition, and harm avoidance

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ABSTRACT

Genetic variation at the EF-hand domain containing 2 gene (*EFHC2*) locus has been associated with fear recognition in Turner syndrome. The aim of this study was to examine whether *EFHC2* variants are associated with non-syndromic anxiety-related traits [harm avoidance (HA) and behavioral inhibition (BI)] and with panic disorder (PD). Our sample comprised 127 PD patients and 132 controls without psychiatric disorder. We genotyped nine SNPs within the *EFHC2* locus and used PLINK to perform association analyses. An intronic SNP (rs1562875) was associated with HA (permuted $p = 0.031$) accounting alone for over 3% of variance in this trait. This same SNP was nominally, but not empirically, associated with BI ($r^2 = 0.022$; nominal $p = 0.022$) and PD (OR = 2.64; nominal $p = 0.009$). The same association was found in a subsample of only females. In sum, we observed evidence of association between a variant in *EFHC2*, a gene previously associated with the processing of fear and social threat, and HA. Larger studies are warranted to confirm this association.

Anxiety-proneness is known to be influenced by genes [13]. One mechanism by which genes may affect anxiety-proneness is through altering sensitivity to fear-related stimuli in limbic areas of the brain. [4,5,12]. Studies of women with X-monosomy (Turner syndrome) have implicated X-linked genes in the mediation of fear recognition and threat detection [10]. Recently, Weiss et al. [18] reported that variants in the X chromosome gene EF-hand domain containing 2 (*EFHC2*) were associated with differences in fear recognition among women with Turner syndrome. *EFHC2* is a novel transcript predicted to contain a calcium-binding domain, which could have diverse neuronal functions related to normal social cognitive competence [18]. There is a high homology between *EFHC2* and *EFHC1* which has been shown to facilitate apoptosis and influence neurotransmission [2,14].

In light of this, we examined whether *EFHC2* variants are also associated with non-syndromic anxiety-related traits that have been shown to reflect altered fear recognition and that have been associated with enhanced amygdala reactivity to fearful faces, i.e. harm avoidance (HA) [4] and behavioral inhibition (BI) [8]. We also examined whether *EFHC2* is related to panic disorder (PD), a disorder in which reactivity to fearful stimuli has also been implicated [5].

Our sample comprised 127 Caucasian PD patients (75.5% females, mean age 39.05 ± 10.12 years) recruited from an outpatient anxiety disorder clinic and 132 Caucasian employees from Hospital de Clínicas de Porto Alegre (73.5% females mean age 36.93 ± 9.75 years) with no psychiatric diagnosis who were recruited as controls. The mean age of PD onset was 31 (S.D. = 10.7) years, and mean of PD duration of 7.8 year (S.D. = 8.3). PD comorbidities included agoraphobia (89%), major depression (29%), generalized anxiety disorder (37%), social anxiety disorder (16%), and dysthymia (14%). Blood was collected from participants for DNA extraction. Institutional review board approval was obtained from the ethics committee in accordance with the Declaration of Helsinki and all subjects provided informed consent.

The Mini International Neuropsychiatry Interview—Brazilian version was used to confirm psychiatric diagnoses after a psychiatric interview in both patients and controls. All subjects were evaluated for the anxiety-related traits (HA and BI). HA was assessed with the Cloninger's Temperament and Character Inventory [1]. HA

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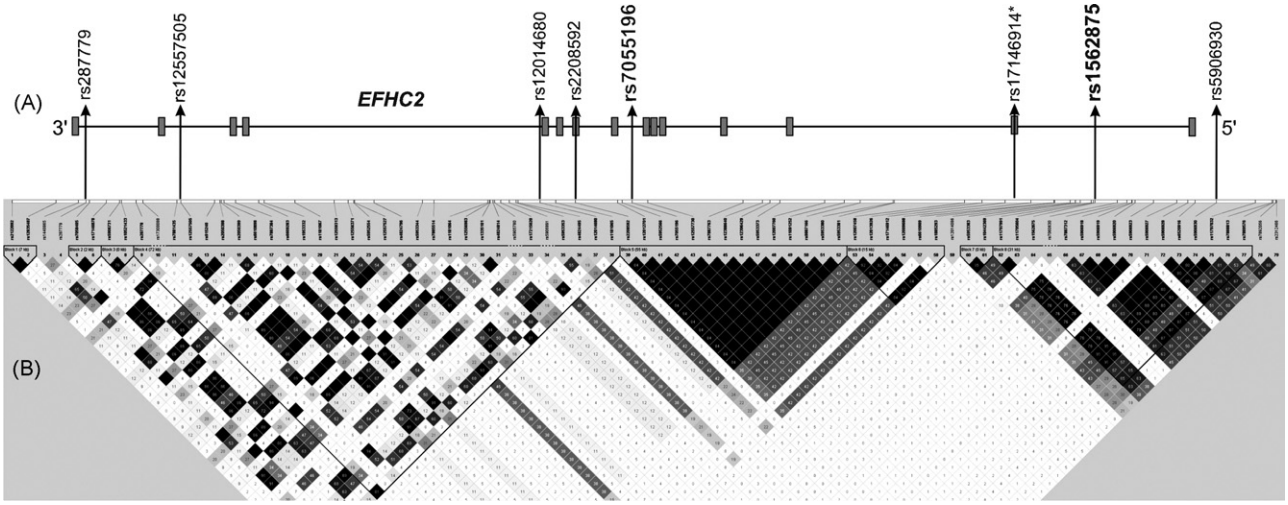

Fig. 1. Location of single nucleotide polymorphisms (SNPs) that span the *EFHC2* gene. (A) Genomic layout of *EFHC2*, with the approximate positions of genotyped SNPs indicated by arrows (gray blocks represents *exon*, bold SNP indicates significant association with harm avoidance and gray SNP indicates previous association with altered fear recognition in Turner syndrome according to Weiss et al. [18]. Linkage disequilibrium (LD) plot of *EFHC2* generated by Haploview (http://www.broad.mit.edu/mpg/haploview/) showing r^2 statistics (black = high, white = low) according to HAPMAP Project (CEU/ Release 21; http://www.hapmap.org). * Not genotyped in HAPM AP (Release 21).

is described as a heritable tendency to inhibit or stop behaviors in response to signals of aversive stimuli in order to avoid punishment. The Self-Report Scale of Behavioral Inhibition—Retrospective Version [7] was used to assess childhood BI, a temperamental risk factor for panic and phobic anxiety disorders [11].

Weiss et al. [18] performed a dense mapping of the X-chromosome corresponding to 5 Mb of Xp11.3–4 using 242 single nucleotide polymorphisms (SNPs) and found an association between *EFHC2* and fear recognition. Nine SNPs previously genotyped by Weiss et al. [18] were selected within the *EFHC2* gene and 10 kB flanking region to represent the three main blocks of linkage disequilibrium (LD) in the region (Fig. 1). Genotyping of SNPs was performed according to Weiss et al. [18] with minor modifications. Markers were retained only if they met the following quality control criteria: >90% genotype call rate, minor allele frequency (MAF) >5%, Hardy–Weinberg equilibrium (HWE) $p > 0.001$ [6]. One SNP was excluded due to departure from HWE.

We used PLINK [6] to perform the basic single SNP association test for alleles in all sample and only in females. For each SNP, we calculated empirical p values by permutation ($N = 10{,}000$ permutations). The overall level of significance adopted was $\alpha = 0.05$.

When analyzed the whole sample ($n = 259$), single marker tests of eight EFHC2 SNPs and HA showed one nominally significant association at rs1562875 that remained significant in the permuted analysis (permuted $p = 0.031$). In the analysis of BI, rs1562875 again showed nominal association ($p = 0.022$), but did not remain significant in the permuted analysis ($p = 0.137$; Table 1). In the PD analysis, the same SNP (rs1562875) was associated with risk of disorder (minor [A] allele frequency = 10.7% cases vs. 4.3% controls; OR 2.64, CI95% 1.23–5.66, $p = 0.009$). However this association did not remain significant in the permuted analysis ($p = 0.059$). In the female subgroup ($n = 193$), rs1562875 was associated with HA and remained significant in the permuted analysis (permuted $p = 0.02$), and also showed nominal association with BI and PD that did not remain significant in the permuted analysis (permuted $p = 0.12$ and 0.12, respectively). Multiple logistic regression for the PD phenotype, controlling for HA and/or BI was not informative because of high colinearity among the three phenotypes (tolerance values $<1 - r^2$).

This is the first study to examine an association between non-syndromic anxiety traits and *EFHC2*, a gene previously associated with fear recognition [18]. We observed modest evidence that the minor (A) allele of rs1562875, a SNP in intron 1, is associated with higher scores in HA and nominally significant evidence of association with BI and PD that did not remain significant by permutation testing. The same finding was found in the analysis performed only in females. Spurious association due to multiple testing of three phenotypes should be considered although, as these phenotypes are correlated [12,17], Bonferroni correction would be overly conservative.

Table 1
Allelic association between individual SNPs in EFHC2 with behavioral inhibition and harm avoidance in whole sample ($n = 259$).

SNP characteristic					Behavioral inhibition				Harm avoidance			
SNP	Role	Alleles	MAF	HWE p	β	r^2	p-Value (asymptotic)	p-Value corrected	β	r^2	p-Value (asymptotic)	p-Value corrected
rs287779	Intron	A/**G**	0.24	0.54	−0.059	0.005	0.267	0.849	−0.237	<0.001	0.690	0.999
rs12557505	Intron	**A**/T	0.10	0.70	0.066	0.002	0.410	0.961	0.888	0.004	0.309	0.894
rs12014680	Intron	**A**/G	0.18	0.47	0.081	0.006	0.210	0.761	1.091	0.009	0.119	0.536
rs2208592	Exon	G/**T**	0.12	0.53	0.151	0.015	0.055	0.301	1.154	0.007	0.178	0.698
rs7055196	Intron	A/**G**	0.18	0.48	0.089	0.007	0.170	0.681	0.032	<0.001	0.964	1.000
rs17146914	Exon	**C**/T	0.09	1	0.204	0.019	0.031*	0.184	1.232	0.005	0.236	0.800
rs1562875	Intron	**A**/T	0.08	1	0.206	0.021	0.022*	0.137	2.798	0.031	0.005*	0.032*
rs5906930	Promoter	**C**/G	0.40	0.54	0.011	<0.001	0.814	1.000	−0.206	<0.001	0.694	0.999

Abbreviations: SNP, single nucleotide polymorphism; EFHC2, EF-hand domain containing 2; MAF, minimal allele frequency; HWE, Hardy–Weinberg equilibrium; β, regression coefficient; r^2, regression r-squared; p, Wald-test asymptotic p-value. Statistic tests: Wald test.
Note: Bold indicates minor allele.
* $p < 0.05$.

EFHC2 may be associated with anxiety by interfering in fear processing related to amygdala function. Skuse [10] imply that X-linked genes are essential for binding somatic responses to the cognitive appraisal of emotional stimuli. Turner syndrome (X-monosomy) is associated with several deficits related to the impaired cognitive response to the presentation of a fearful face (i.e. the inability to accurately classify the emotion). This deficit was associated with a lack of correlation between activity in the left amygdala and the fusiform cortex. This finding led Weiss et al. [18] to perform fine mapping in a critical region and identify association between *EFHC2* and fear recognition in two Turner Syndrome samples, although Zinn et al. [19] could not replicate this finding. In addition, Most et al. [3] showed that amygdala activity was correlated with the personality variable harm avoidance. Pillay et al. [5] showed that PD patients produced more activation of the amygdala than controls in response to fearful faces using fMRI. Of note, however, rs7055196, the SNP most highly associated with fear recognition in Turner Syndrome, is not in linkage disequilibrium with rs1562875 ($r^2 < .5$) and is not known to have functional significance. We did not observe any evidence of association between anxiety phenotypes and rs7055196 (Fig. 1).

Our results should be interpreted in light of some limitations. Most importantly, the small sample size means that our analyses may be subject to Type II error. In addition, although the association with harm avoidance remained significant after permutation, we did not correct for the examination of three phenotypes. Of note, however, these phenotypes (harm avoidance, BI, and PD) are not independent, so Bonferroni correction would be overly conservative. Finally, rs1562875 is an intronic SNP and its functional significance, if any, is unknown. It is possible that the association we observed is more directly related to an untyped causal SNP in LD with rs1562875. Moreover, genome-wide association (GWA) studies [9] have recently reported that the heritability of neuroticism, a phenotype highly associated with anxiety, probably arises from many loci each explaining much less than 1% of variance. This study, as well as other GWA studies [15,16], which could detect small genetic effects, have not reported any association with *EFHC2*.

In sum, we observed modest evidence of association between a variant in *EFHC2*, a gene previously associated with the processing of fear and social threat, and harm avoidance. An intronic SNP (rs1562875) was associated with HA accounting alone for over 3% of variance in this trait. However, further studies are needed to confirm our finding and examine additional SNPs at the *EFHC2* locus.

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