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

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A R T I C L E   I N F O

Article history:
Received 14 November 2008
Received in revised form 13 January 2009
Accepted 14 January 2009

Keywords:
Harm-avoidance
Panic
EFHC2

A B S T R A C T

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 disorders. We genotyped nine SNPs within the EFHC2 locus and used PLINK to perform association analyses. An intronic SNP (rs1562875) was associated with HA (permutated p = 0.031) accounting alone for over 3% of variance in this trait. This same SNP was nominally, but not empirically, associated with BI (p = 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.

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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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doi:10.1016/j.neulet.2009.01.036
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 α=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–r²).

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

<table>
<thead>
<tr>
<th>SNP characteristic</th>
<th>Behavioral inhibition</th>
<th>Harm avoidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP</td>
<td>Role</td>
<td>Alleles</td>
</tr>
<tr>
<td>rs287779 Intron</td>
<td>A/G</td>
<td>0.24</td>
</tr>
<tr>
<td>rs12557505 Intron</td>
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<td>0.10</td>
</tr>
<tr>
<td>rs12014680 Intron</td>
<td>A/G</td>
<td>0.18</td>
</tr>
<tr>
<td>rs2808592 Exon</td>
<td>C/T</td>
<td>0.12</td>
</tr>
<tr>
<td>rs7055196 Exon</td>
<td>A/G</td>
<td>0.18</td>
</tr>
<tr>
<td>rs17146914 Exon</td>
<td>C/T</td>
<td>0.09</td>
</tr>
<tr>
<td>rs1562875 Intron</td>
<td>A/T</td>
<td>0.08</td>
</tr>
<tr>
<td>rs5906930 Promoter</td>
<td>C/G</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**Abbreviations:** SNP, single nucleotide polymorphism; EFHC2, EF-hand domain containing 2; MAF, minimal allele frequency; HWE, Hardy–Weinberg equilibrium; β, regression coefficient; r², 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 (r² < .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 and 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 (r² < .5) and is not known to have functional significance. We did not observe any evidence of association between anxiety phenotypes and rs7055196 (Fig. 1).

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.

Acknowledgement

Funding was provided by Fundação de Incentivo à Pesquisa, CAPES, CNPq, Rose and Eugene Kleiner Family Foundation and Ruth L. Kirschstein National Research Service Award. Authors thank Dr. Luis Augusto Rohde for his contribution.

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