BRIEF REPORT

Association study between the -62A/T *NFKBIL1* polymorphism and obsessive-compulsive disorder

Estudo de associação entre o polimorfismo -62A/T *NFKBIL1* e o transtorno obsessivo-compulsivo

Quirino Cordeiro,[1] Carolina Cappi,[1] Aline Santos Sampaio,[1] Selma Aliotti Palácios,[2] Carlos Alberto de Bragança Pereira,[3] Roseli Gedanke Shavitt,[1] Eurípedes Constantino Miguel,[1] Luiza Guilherme,[2] Ana Gabriela Hounie[1]

Abstract

***Objective**: Evidence from family and molecular genetic studies support the hypothesis of involvement of immunologic mechanisms in the pathophysiology of obsessive-compulsive disorder. The nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 (NFKBIL1) has been suggested as a modulator of the immunological system. Given the importance of NFKBIL1 in the immunological response, the present study investigated the -62A/T polymorphism (rs2071592), located in the promoter region of its gene* (NFKBIL1)*, as a genetic risk factor for the development of obsessive-compulsive disorder. **Method:** The -62A/T* NFKBIL1 *polymorphism was investigated in a sample of 111 patients who met DSM-IV criteria for obsessive-compulsive disorder and 272 healthy age- and gender-matched controls. **Results**: There were no differences in genotypic distributions between patients and controls (χ^2 = 0.98; 2 d.f.; p = 0.61). **Discussion:** Despite these negative findings, more comprehensive polymorphism coverage within the* NFKBIL1 *is warranted in larger samples. Populations with different ethnic backgrounds should also be studied. **Conclusion:** The results of the present investigation do not provide evidence for the association between the -62A/T* NFKBIL1 *polymorphism and obsessive-compulsive disorder in this Brazilian sample.*

***Descriptors:** Case-control studies; Patients; Polymorphism genetic; Immune system diseases; Obsessive-compulsive disorder*

Resumo

***Objetivo:** Evidências advindas de estudos de família e de genética molecular têm dado suporte à hipótese do envolvimento de mecanismos imunológicos na fisiopatologia do transtorno obsessivo-compulsivo. Tem sido sugerido que o potencializador do fator nuclear do polipeptídeo* kappa light *em células-B inibidoras-*like *1 (NFKBIL1) é um modulador do sistema imunológico. Dada a importância do NFKBIL1 na resposta imunológica, o presente estudo investigou o polimorfismo -62A/T (rs2071592), localizado na região promotora de seu gene, como fator de risco genético para o desenvolvimento do transtorno obsessivo-compulsivo. **Método:** O polimorfismo -62A/T do gene do NFKBIL1 foi investigado em uma amostra de 111 pacientes com o diagnóstico de transtorno obsessivo-compulsivo, de acordo com os critérios do DSM-IV, e 272 controles saudáveis emparelhados por idade e gênero. **Resultados:** Não houve diferenças na distribuição genotípica entre pacientes e controles (χ^2 = 0,98; 2 d.f.; p = 0,61). **Discussão:** Apesar dos resultados negativos, estudos compreendendo mais polimorfismos no gene do NFKBIL, em amostras maiores, são necessários. Populações com diferentes origens étnicas também precisam ser avaliadas. **Conclusão:** Os resultados da presente investigação não evidenciam associação entre o polimorfismo -62A/T do gene do NFKBIL1 e o transtorno obsessivo-compulsivo nesta amostra brasileira.*

***Descritores**: Estudos de casos e controles; Pacientes; Polimorfismo genético; Doenças do sistema imune; Transtorno obsessivo-compulsivo*

[1] Department and Institute of Psychiatry, Universidade de São Paulo (USP) Medical School, São Paulo (SP), Brazil
[2] Heart Institute (InCor) and Clinical Immunology and Allergy, Department of Clinical Medicine, Universidade de São Paulo (USP) Medical School, São Paulo (SP), Brazil
[3] Department of Statistics, Institute of Mathematics and Statistics, Universidade de São Paulo (USP), São Paulo (SP), Brazil

Correspondence
Ana Gabriela Hounie
Instituto de Psiquiatria do Hospital das Clínicas
Rua Doutor Ovídio Pires de Campos, 785, 3º andar, CEAPESQ,
sala 07 - Cerqueira César
05403-010 São Paulo, SP, Brasil
Phone: (+55 11) 3069-6972 Fax: (+55 11) 3069-7896
E-mail: anah@protoc.com.br

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Introduction

Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by obsessions (intrusive and recurrent thoughts, images or impulses) and compulsions (repetitive behaviors or mental acts usually performed to relieve the discomfort raised by the obsessions). OCD prevalence ranges from 2 to 3% in the general population, with equal gender distribution.[1] The etiology of the disorder remains unknown, although twin and family studies and segregation analyses have provided consistent evidence that OCD presents a strong genetic component.[2,3] Family studies have shown an increased prevalence of OCD among first-degree relatives of probands with OCD, suggesting that the risk for the development of the disorder is 3-12 times greater than that of first-degree relatives of control subjects.[2] Twin studies have found higher concordance between monozygotic twin pairs when compared to dizygotic ones, suggesting, in children, a genetic influence in the range of 45% to 65%, and in adults, ranging from 27% to 47%.[3]

OCD is a clinically heterogeneous disorder, with several possible subtypes.[4] It has been hypothesized that certain forms of OCD may be associated with autoimmune disorders triggered by streptococcal infections [e.g., rheumatic fever (RF) and Sydenham's chorea (SC)].[4] Children who develop acute OCD after a group A streptococcal infection have been described with the acronym PANDAS, for "pediatric autoimmune neuropsychiatric disorders after streptococcal infection". These children develop OCD and tics, but do not manifest symptoms of RF or SC.[5]

Investigations have found higher prevalence of obsessive-compulsive symptoms[6,7] and OCD[8] in patients with immunological diseases such as RF with or without SC. Family studies have reported that obsessive-compulsive spectrum disorders aggregate in first-degree relatives of RF probands.[9] Recently, two polymorphisms of the promoter region of the tumor necrosis factor alpha gene *(TNF-α)* have been associated with RF. The same polymorphisms were associated with OCD in a previous study, using the same sample of this present investigation. This is an interesting finding, since *TNF-α* is a pro- inflammatory cytokine involved in RF and several other autoimmune diseases.[10,11] Imbalance in cytokine production can result in the loss of appropriate immune responses and excessive inflammation, contributing to the severity of infection and progression of autoimmunity. The autoimmune hypothesis for neuropsychiatric disorders warrants studies focusing on cytokine genes.

Chromosome 6 has emerged as an interesting region for genetic studies of immunological system because it hosts the human major histocompatibility complex (MHC) class III region that harbors a wide variety of immune response genes. Susceptibility locus for many different immunological diseases has been found in this chromosomal region, such as multiple sclerosis, type 1 diabetes, chronic Chagas' cardiomyopathy, rheumatoid arthritis, hepatitis C virus-associated dilated cardiomyopathy.[12] The nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 1 (NFKBIL1), homologous to the IKB family of proteins that regulate the NFKB family of transcription factors, is suggested as a putative inhibitor of NFKB, modulating the immunological system, and its gene is located in the MHC region, between the HLA-B-associated transcript 1 (*BAT1*) and the *TNF*-β and *TNF*-α cluster on chromosome 6p21.3. Okamoto et al. have identified a diallelic polymorphism in the promoter region (adenine/thymine: -62A/T) of the NFKBIL1 gene (*NFKBIL1*), that represented the second rheumatoid arthritis susceptibility locus within the HLA region.[13] The -62A/T *NFKBIL1* polymorphism seems to be functional. In a transient transfection of luciferase reporter assay, constructs that incorporate the -62A versus -62T sequences expressed lower levels of transcription of -62A allele compared with the -62T sequences.[14] However, if the lower or higher transcription rate has clinical implications is yet to be clarified.

To evaluate the hypothesis of the involvement of other immune system related genes on OCD risk, it was performed an investigation with the -62A/T *NFKBIL1* polymorphism (rs2071592) in 111 DSM-IV OCD patients and 276 healthy controls as to evaluate its possible association with OCD. As far as we know, this is the first study investigating this polymorphism as a genetic risk factor for OCD.

Method

1. Sample

1) Patients: 111 participants who met the Diagnostic and Statistical Manual of Mental Disorders - 4th edition (DSM-IV) criteria for OCD were recruited from the Obsessive-Compulsive Spectrum Disorders Program at the Department of Psychiatry, Universidade de São Paulo Medical School. Patients underwent structured psychiatric evaluation with Structured Clinical Interview for DSM-IV (SCID-I)[15] and additional modules for DSM-IV tic and impulse control disorders.[7] More details about the evaluation of the present OCD sample can be found in Hounie et al.[11] and Miguel et al.[16]

2) Controls: Unselected 272 age and sex matched healthy controls (unrelated bone marrow donors) were obtained from the Immunology Laboratory at the Heart Institute (InCor), Department of Clinical Medicine, Universidade de São Paulo Medical School.

All participants provided written informed consent. Ethical approval of the study was obtained from the Ethics Committee at the Hospital das Clínicas, Universidade de São Paulo Medical School (CAPPesq).

2. Genotyping

Fifteen milliliters of venous blood were collected from each subject and genomic DNA was extracted from lymphocytes. The investigated polymorphism was analyzed as follows: the polymorphism was identified by polymerase chain reaction followed by restriction fragment length polymorphism (*PCR-RFLP*). The following pair of primers flanking the polymorphisms was used for amplification: Forward 5′-CACAGTTCACTTCCGTCCTCCAGC-3′ and Reverse 5 -CTGTGTTTAAGAAGCTCGG-3′. The underlined nucleotide denotes the introduction of a mismatch nucleotide for the creation of restriction site for PvuII. As to generate the polymerase chain reactions (PCR) products, the region of interest was amplified with primers. Ten microliters of PCR product were digested with 6 units of PvuII (New England Biolabs) in a final volume of 30 μL that contained 3 μL of 10× enzyme buffer. The resulting fragments were size-separated by electrophoresis in a 4% agarose gel. The fragment was cleaved into 23 bp and 84 bp while the -62A allele remains uncleaved by PvuII (107 bp).[12] In order to avoid errors, genotyping was read by two independent trained research technicians. In case of disagreement, the process of genotyping was repeated.

3. Statistical analysis

We used standard statistical tests to compare the groups: t-test for continuous variables and chi-square for categorical data. We used the Excel program to test for polymorphism frequency differences and the Hardy-Weinberg equilibrium. Analyses were performed comparing the polymorphism frequencies between the groups of patients and controls, and subsequent analyses were also conducted

in order to compare these groups according to gender and presence of tics. The significance values adopted for all analyses were the standard 0.01, 0.05 and 0.1 significance levels to decide about highly, medium and low significant differences.

Results

We found no significant differences between the groups regarding gender and age: 46% (n = 51/111) of females in the OCD group and 56,5% (n = 152/269) in the control group (χ^2 = 1.39, p = 0.49) and 30.8 years (sd = 12.63; 95% CI 28.34-32.27) for the OCD group and 28.5 years (sd = 13.6; 95%CI 26.69-30.32) for controls (t = -1.44; sd = 13.3; p = 0.15), respectively. There were 3 missing values for gender and 57 missing values for age in the control group while on the OCD group there were 10 missing values for age. The genotypic distributions were in Hardy-Weinberg equilibrium in patients (p = 0.96) and controls (p = 0.65). We found no differences between OCD patients and controls regarding the genotypic distributions (χ^2 = 0.98; 2 d.f.; p = 0.61) (Table 1). When the analyses were performed according to gender and presence of tics, differences were not find as well (Gender: 1- females: χ^2 = 0.04; 2 d.f.; p = 0.97; 2-males: χ^2 = 1.61; 2 d.f.; p = 0.44; Presence of tics: χ^2 = 1.34; 2 d.f.; p = 0.51). Considering that the homogeneity of the genotypic proportions between the groups of patients and controls was not rejected (H: p1 = q1, p2 = q2 and p3 = q3), and for this reason any function of these proportions could not be also rejected (H´: p1+0.5p2 = q2+0.5q2), there was no need to conduct the allelic analysis (a function of the genotypic proportions); nonetheless, the allelic frequencies may be found in Table 1.

Table 1 - Distributions of the -62A/T *NFKBIL1* genotypic and allelic frequencies in OCD patients and controls

	OCD n (%)	Controls n (%)	χ^2	p value
Genotype				
A/A	11 (9.92)	37 (13.60)	0.98	0.61
A/T	50 (45.04)	117 (43.01)		
T/T	50 (45.04)	118 (43.39)		
Total	111 (100)	272 (100)		
Allele				
A	72 (32.43)	191 (35.11)		
T	150 (67.57)	353 (64.88)		
Total	222 (100)	544 (100)		

Discussion

In face of the evidence for the involvement of the immunological system in the pathophysiology of OCD, investigations with polymorphisms associated with the immune response in OCD patients are warranted. In the present investigation, we tested the association of the -62A/T *NFKBIL1* polymorphism and OCD. We have found no evidence of genotypic association between OCD and the investigated polymorphism.

The most important limitation of the present study is related to the ethnic admixture that could lead to population stratification bias. The allelic frequency can be significantly different between ethnical populations reducing the power to detect associations. In order to handle such a problem, we can try to identify the ethnical origin of the participants and create more homogenous samples. However, Parra et al.[17] and later Pimenta et al.[18] have recently published studies that used ancestry-informative markers to conclude that in Brazil, at an individual level, phenotypic information, as determined by physical evaluation, was a poor predictor of genomic ancestry, better estimated by molecular markers. This particular genetic admixture condition makes it more difficult to achieve ethnic matching in case-controls studies. The fact that in the present study patients and controls were in Hardy-Weinberg equilibrium may be an indication that population stratification was not necessarily a problem. Nevertheless, we are aware that ethnic admixture may have been a limitation of the present work.

A second limitation is the sample size. In the polygenic and multifactor model of the pathophysiology of OCD, it has been hypothesized that there may be several additive genes, each of them with a small contribution for the OCD phenotype.[19] Thus, the chance of detecting these putative genes may be reduced in relatively small samples. Our effort to overcome this limitation has been to calculate the power sample as 90%. Another point to be considered is that, in Psychiatry, environmental factors may be as important as the genetic ones, both contributing to the interaction that will be determinant. This interaction also varies according to the period of the neural development of each individual.[20] Hence, future prospective, systematic longitudinal studies evaluating environmental factors in individuals at risk are warranted especially to investigate the possible relation between infection (i.e., group A beta-hemolytic streptococcal infection), the immunological system and the development of OCD.[21]

Despite the negative results of the present investigation, future studies with *NFKBIL* polymorphisms are welcomed because of the interesting region where this gene is located. The *NFKBIL* is around 30 kb to the *TNF*-α at the telomeric end of the central MHC region.[12] As a previous study showed association between polymorphisms in the promoter region of the *TNF*-α and OCD in the same sample of the present study[11] and knowing that this chromosomal region presents tight linkage disequilibrium,[12] other polymorphisms of the *NFKBIL* may be an interesting focus for future investigation.

In conclusion, the results of this investigation do not provide evidence for the association between the -62A/T *NFKBIL1* polymorphism and OCD in this Brazilian sample. More comprehensive polymorphism coverage within the *NFKBIL1* region should be conducted in larger samples, with the inclusion of comparisons in potential subgroups (early- *vs*. late-onset OCD, or tic-related OCD), as well as investigations in samples with different ethnic backgrounds.

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Disclosures

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
Quirino Cordeiro	Hospital João Evangelista	-	-	-	-	-	-
Carolina Cappi	IPq-FMUSP	-	-	-	-	-	-
Aline Santos Sampaio	-	CAPES	-	-	-	Johnson & Johnson*	-
Selma Aliotti Palácios	INCOR	-	-	-	-	-	-
Carlos Alberto de Bragança Pereira	IME USP	CNPq	-	-	-	-	-
Roseli Gedanke Shavitt	IPq-FMUSP	FAPESP	-	-	-	-	-
Eurípedes Constantino Miguel	Ipq-FMUSP	CNPq FAPESP	-	-	-	-	-
Luiza Guilherme	INCOR	CNPq FAPESP	-	-	-	-	-
Ana Gabriela Hounie	IPq-FMUSP	CNPQ	-	-	-	-	-

** Modest*
*** Significant*
**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*
Note: IPq-FMUSP = Psychiatry Institute of the School of Medicine of the Universidade de São Paulo; INCOR = Instituto do Coração; IME USP = Instituto de Matemática e Estatística of the Universidade de São Paulo; CAPES = Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; CNPq = Conselho Nacional de Desenvolvimento Científico e Tecnológico; FAPESP = Fundação de Amparo à Pesquisa do Estado de São Paulo.
For more information, see Instructions for authors.

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