BRIEF REPORT

Molecular genetic case-control women investigation from the first Brazilian high-risk study on functional psychosis

Investigação genético-molecular do tipo caso-controle em uma amostra de mulheres portadoras de psicose funcional do primeiro estudo de alto-risco brasileiro

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Abstract

Objective: *Data from epidemiological studies have demonstrated that genetics is an important risk factor for psychosis. The present study is part of a larger project, pioneer in Brazil, which has been conducted by other researchers who intend to follow a high-risk population (children) for the development of schizophrenia and bipolar disorder. In this first phase of the project, the objective was to investigate the distribution of four candidate genetic polymorphisms for functional psychosis (Ser9Gly DRD3, 5HTTLPR, the VNTR 3'-UTR SLC6A3 and Val66Met BDNF) in a case-control sample.* ***Method:*** *A total of 105 women (58 with schizophrenia and 47 with bipolar disorder) and 62 gender-matched controls were investigated.* ***Results:*** *Allele and genotype distributions of all identified functional polymorphisms did not differ statistically between cases and controls.* ***Conclusions:*** *These results suggest that the investigated polymorphisms were not related to susceptibility to functional psychoses in our Brazilian sample. These findings need to be validated in larger and independent studies.*

Descriptors: *Schizophrenia; Bipolar disorder; DAT1 gene; SLC6A3 gene; DRD3 gene; Brain-derived neurotrophic factor gene*

Resumo

Objetivo: *Estudos epidemiológicos demonstram que alterações genéticas são fatores de risco importantes para o desenvolvimento de psicose. O presente estudo é parte um projeto maior, pioneiro no Brasil, realizado com mais pesquisadores, que pretende seguir uma população de alto risco genético para o desenvolvimento de esquizofrenia e transtorno bipolar. Nesta primeira fase, o objetivo foi investigar a distribuição de quatro polimorfismos genéticos candidatos no desenvolvimento de psicose funcional (Ser9Gly DRD3, 5HTTLPR, o VNTR 3'-UTR SLC6A3 e Val66Met BDNF) em uma amostra caso-controle.* ***Método:*** *Um total de 105 mulheres (58 esquizofrenia e 47 transtorno bipolar) e 62 controles sem diagnóstico psiquiátrico foi investigado.* ***Resultados****: Nenhuma diferença estatisticamente significante foi observada nas distribuições alélicas e genotípicas entre os grupos investigados.* ***Conclusões:*** *Os resultados sugerem que estes polimorfismos não estavam relacionados à suscetibilidade para psicose funcional nesta amostra brasileira estudada. Esses achados precisam ser validados em estudos maiores e independentes.*

Descritores: *Esquizofrenia; Transtorno bipolar; Gene DAT1; Gene SLC6A3; Gene DRD3; Gene do fator neurotrófico derivado do encéfalo*

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Introduction

The diagnostic difference between schizophrenia and bipolar disorder has been for more than a century based on differences in phenomenology and outcome.[1] However, the distinction or even the real existence of both diagnostic categories has been progressively challenged. The accumulated evidence against it has come from a great number of studies such as follow-up, family, twin and neurobiological investigations for both disorders.[2]

Interestingly, both schizophrenia and bipolar disorders present a good number of studies demonstrating the existence of an important genetic factor for their development. The contribution of the genetic component for the susceptibility of schizophrenia or bipolar disorder is about 70% of the total variance for each disorder. Many molecular genetic studies have been carried out, and their results have indicated some common specific chromosomal areas or DNA markers for both phenotypes, suggesting a further support for revising the concept of these diagnostic categories.[3]

An alternative to validate a better phenotype for the investigation of putative genes for schizophrenia and bipolar disorder is the use of a broader concept called functional psychosis. However, this term contains not only the categories of schizophrenia and bipolar disorder, but also schizoaffective disorder and psychotic depression.[4]

It has been suggested that if a better phenotype (i.e. closer to the true clinical characterization associated with the underlying "disease genes") is used, the molecular genetic studies will identify these genes with certainty.[2]

One of the most common strategies in molecular genetics to identify genes associated with complex disorders has been the utilization of the association study design. This method investigates genes (usually selected a priori as a viable choice based on previous scientific results – they are also called "candidate genes") with small to moderate effect for the development of the phenotypes.[5]

The present research team is pioneer in Brazil and will follow children with a high genetic/biological risk for functional psychosis (their mothers have bipolar disorder or schizophrenia) and will compare them with children without such risk.[6] Although these children present high genetic/biological risk for the development of psychotic disorders, they do not present such disorders at the moment. Therefore, the aim of the present study was to investigate the role of polymorphisms from candidate genes for the development of psychosis in the mother sample, in a case-control approach, in order to replicate its results in future investigations with the sample of children.

The polymorphisms were investigated in four important genes, dopamine receptor subtype 3 gene (DRD3), the brain-derived neurotrophic factor gene (BDNF) and the serotonin and dopamine transporters genes (5HTT and DAT1/SLC6A3, respectively) in the mothers and compare them with a non-psychotic gender-matched group.

One representative functional polymorphism from each gene was chosen, the serine-to-glycine amino acid substitution at position 9 (Ser9Gly) in the N-terminal domain of the DRD3 (rs6280); a functional 44-bp insertion/deletion polymorphism (5HTTLPR) in the promoter region of the 5HTT; a variable number of tandem repeats (VNTR) polymorphism in the 3'-untranslated region of the SLC6A3; a non-conservative substitution from valine to methionine at amino acid position 66 (Val66Met) in the pro-domain of BDNF (rs6265).

These genes have been usually investigated in studies on schizophrenia and bipolar disorder as separated phenotypes. However, studies with the dimensional or continuum approach in functional psychosis phenotype have been uncommon.

A meta-analysis with the Ser9Gly DRD3 polymorphism accomplished by Jönsson et al.[7] has found a relative risk of homozygosis (Ser/Ser and Gly/Gly), with odds ratio varying from 1.08 to 1.10 for the development of schizophrenia. On the other hand, association studies on bipolar disorder and the Ser9Gly DRD3 polymorphism have not found positive results.[8]

The studies with 5HTT have found inconsistent findings regarding the association between the 5HTTLPR polymorphism and functional disorder. However, Cho et al.[9] have found a significant pooled odds ratios of 1.12 for 5-HTTLPR as risk factor for bipolar disorder. 5HTTLPR has been considered functionally biallelic, even though other genetic variations were known. 5HTTLPR consists of varying numbers of copies of a 20–23-basis-pairs imperfect repeat sequence. Substantial interpopulation variation occurs. Rare alleles contain up to 20 copies of the repeat.[9] On the other hand, none of these additional alleles was found to be functional. Therefore, it has been possible to use different methods in the genotype exploratory data analysis in the study of this polymorphism. However, recently a third functional allele of this genetic variant was discovered, which is an A > G single nucleotide polymorphism within the first of two extra repeats that characterize the "insertion" allele.[10] As such discovery occurred after the beginning of the present work, unfortunately the genetic analysis of this paper did not take into account the existence of this new allele.

Although some studies have found positive association between the VNTR 9/9 and 10/10 polymorphisms of DAT1 and risk for schizophrenia,[11,12] most of them have not supported these findings.[13] Other studies have found positive association with negative symptoms,[14] loss of dopaminergic neurons and/or decrease in the expression of DAT1 in patients with chronic schizophrenia.[15] Finally, in a meta-analysis with 659 schizophrenic and 563 controls, even though there was over 90% of power to detect a significant odds ratio as small as 1.3, no association was observed.[16]

In a recent meta-analysis, there was no evidence of association of the Val66Met polymorphism of BDNF and increased risk for schizophrenia (2,955 patients and 4,035 controls) or bipolar disorder (3,143 patients and 6,347 controls), with pooled odds ratios of 1.00 ($p = 0.944$) and 0.95 ($p = 0.161$).[17] Xu et al.,[18] in their population-based study and meta-analysis, have demonstrated that the Val66Met BDNF polymorphism should not play major roles in the susceptibility for schizophrenia in either Caucasian or Asian populations.

Method

The present study was approved by the Ethics Committee of the Universidade de São Paulo Medical School (process: 030/05). After explanations on the research and approval by the participants, all of them provided written informed consent. This is a cross-sectional study and part of a larger investigation with mothers and children with high genetic/biological risk for schizophrenia and bipolar disorder. In the present study allele and genotype frequencies from the investigated polymorphisms were compared between the groups of women with schizophrenia and bipolar disorder with children between 6 and 18 years old (case sample), originating from the Institute of Psychiatry of the Hospital das Clínicas of the Universidade de São Paulo Medical School, and women without such psychiatric disorders (control sample) from the Gynecologic Clinic of the same university with same-age children. Individuals unable to answer the questionnaire of evaluation, to understand the

term of informed consent, and to refuse the donation of blood for genetic analysis were excluded from the study. For the participants in the investigation, blinded interviews were administered using the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (SCID-I) and a sample of blood was collected for genetic analysis.[19]

The deoxyribonucleic acid (DNA) extraction was performed using the "salting out" method described by Miller et al.[20] and the amplification of all polymorphisms studied was performed using polymerase chain reaction (PCR). A different PCR condition was used for each polymorphism: VNTR SLC6A3 by Vandenbergh et al.;[21] DRD3 Ser9Gly by Lannfelt et al.;[22] 5HTTLPR by Heils et al.;[23] BDNF Val66Met by Rozen & Skaletsky.[24]

The groups were compared using Pearson's chi-square test or Fisher's exact test for categorical variables using the Statistical Program for Social Sciences (SPSS). The polymorphism frequency differences were investigated with Pearson's chi-square test using the Clump v1.9. The significance value adopted was 0.05. The Hardy-Weinberg equilibrium was tested using the HWE Program.[25]

Results

The total number of individuals investigated was 105 women with functional psychosis (58 with schizophrenia and 47 with bipolar disorder). These patients were compared with 62 women (mothers) without functional psychosis. The mean age was 40.27 (SD ± 6.32) (no statistical difference between the groups).

The power of the sample, based on 105 patients and 62 controls, disorder prevalence rate of 3% in the general population (schizophrenia and bipolar disorder), allelic frequency close to 30%, multiplicative model with the genotype relative risk = 1.7, and significance level of 0.05, was lower than 70%. Despite that, underpowered non-significant study of possible real associations with modest genetic effects can reasonably account for much of the variability in replication and can contribute for meta-analysis (in cases of modest genetic effects that would be hard to replicate in small studies, meta-analysis may be interesting because it avoids type I errors, false positive).

There were no significant deviations from the Hardy-Weinberg equilibrium in any of the samples for the polymorphisms studied. Case-control analysis provided no differences in genotypic distributions, as well as homozygosis between psychosis and control samples (Table 1). Considering that the homogeneity of the genotypic proportions between the groups of patients and controls was not rejected (H: p1 = q1, p2 = q2 e p3 = q3), and for this reason any function of these proportions could be rejected as well (H': P1 + (1/2)p2 = q2 + (1/2)q2), it was not necessary to conduct the allelic analysis (a function of the genotypic proportions).

The DAT1 polymorphism presents many rare alleles (e.g.: 8 and 11). This increases the degrees of freedom of the test and reduces the power of the sample. However, no statistical difference was observed even when the analysis was performed again (Table 2).

Table 1 - Genotype distribution and allele frequencies of the Ser9Gly, SLC6A3, 5HTTLPR and BDNF polymorphisms in functional psychosis and control group

Polymorphisms	Cases	Controls	p
Ser9Gly DRD3	**N (%)**	**N (%)**	
Genotypes			
Ser/Ser	22 (21.35)	15 (24.59)	0.32
Ser/Gly	56 (54.68)	26 (42.62)	
Gly/Gly	25 (24.27)	20 (32.79)	
Total	103 (100)	61 (100)	
Homozygosis Ser9Gly			
SerSer+GlyGly	47 (45.63)	35 (57.37)	0.14
SerGly	56 (54.36)	26 (42.62)	
SLC6A3 VNTR			
Genotypes			
11 10	0	1 (1.92)	
11 9	0	1 (1.92)	
10 10	44 (51.76)	31 (59.62)	0.39
10 9	35 (41.17)	16 (30.77)	
10 8	1 (1.17)	0	
9 9	5 (5.88)	3 (5.77)	
Total	85 (100)	52 (100)	
Homozygosis VNTR			
9 9 /10 10	49 (57.64)	34 (65.38)	0.37
11 10/ 11 9/ 10 9 /10 8	36 (42.35)	18 (34.61)	
5HTTLPR L/S			
Genotypes			
LL	37 (37.37)	20 (33.33)	0.36
LS	45 (45.45)	24 (40.00)	
SS	17 (17.17)	16 (26.67)	
Total	99 (100)	60 (100)	
Homozygosis L/S			
LL+SS	54 (54.54)	36 (60.00)	0.50
LS	45 (45.45)	24 (40.00)	
Val66Met BDNF			
Genotypes			
CC	3 (2.88)	1 (1.64)	
CT	24 (23.07)	11 (18.03)	0.63
TT	77 (74.03)	49 (80.33)	
Total	104 (100)	61 (100)	
Homozygosis Val66Met			
CC + TT	80 (76.92)	50 (81.96)	0.44
CT	24 (23.07)	11 (18.03)	

Discussion

The present research investigated the allelic and genotypic distributions of polymorphisms from four candidate genes in 167 women (105 with psychosis and 62 controls) with the aim of studying their role as genetic susceptibility for functional psychosis. The results have not given support for the association of such polymorphisms as risk factors for the investigated phenotype.

The ethnic admixture of the Brazilian sample may be a bias in genetic association studies. To face such a problem, identification of the ethnic origin of the sample and homogenous samples may be of great help. However, in Brazil physical characteristics such as skin pigmentation, hair color and texture, shape of the nose and lips, are poor predictors of genomic ancestry,[26] which makes it difficult to achieve ethnic matching in case-control studies. Nevertheless, the fact that the present sample is in Hardy-Weinberg equilibrium indicates that the sample may not have important problems of population stratification.[27] Moreover, ethnic matching conducted using genetic markers was performed in part of our sample in a case-control study with cocaine dependence and the results showed that despite the ethnic admixture in Brazil the ethnic stratification was not a bias in that case.[28] In spite of that, we are aware that ethnic admixture may be a limitation for the present work.

The fact that a third recently discovered functional allele of the 5HTTLPR polymorphism has not been analyzed in the present

Table 2 - Distribution of the most frequent VNTR SLC6A3 polymorphisms in functional psychosis and control group

VNTR SLC6A3 Genotypes	Functional psychosis n (%)	Controls n (%)	p
10 10	44 (51.2)	31 (56.3)	0.097
10 9	35 (40.7)	16 (29.1)	
9 9	6 (7.0)	3 (5.5)	
Other genotypes	1 (0.1)	5 (9.1)	
TOTAL	86 (100)	55 (100)	

study, as mentioned before, may be related to the lack of association between such polymorphism and the investigated disorders. Another possible methodological limitation of the present study may be the sample size.

In a follow-up study it is possible to change the experimental drawing and the investigation techniques. Our group will also include the offspring of the patients from the main study to replicate the present findings, introduce larger number of participants and analyze the offspring (clinic and genetics) using the microarray technique.

The technique known as microarray is particularly powerful in providing a global view of gene expression patterns in biological samples. By the simultaneous determination of the expression levels of thousands of genes, microarrays allow researchers to compare the molecular behavior of different types of cells that have been exposed to pathological or experimental conditions.[29]

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Disclosures

Writting group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Spekear's honoraria	Ownership interest	Consultant/ Advisory board	Other
Renata Krelling	Clínica privada	---	---	---	---	---	---
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** 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: HC-FMUSP = Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo; PROGENE = Programa de Genética e Farmacogenética do Instituto de Psiquiatria do HC-FMUSP; 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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