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Nogo CAA 3′UTR Insertion polymorphism is not associated with Schizophrenia nor with bipolar disorder

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Abstract

The *Nogo* gene maps to 2p14-p13, a region consistently associated with schizophrenia and bipolar disorder. The association of a polymorphism in *Nogo* was previously investigated by two groups, with divergent results. In this report, using an alternative approach, we evaluated this same polymorphism in 725 individuals, including patients with schizophrenia, bipolar disorder, normal controls and non-human primate samples. Our results indicate that the polymorphism is not associated with any of these diseases, but has a remarkably biased distribution in ethnic groups. Genotyping of primate samples, suggest that this polymorphism is a recent event in human speciation.

Keywords: *Nogo*; Schizophrenia; Bipolar disorder; Polymorphism

1. Introduction

Nogo (Neurite Outgrowth inhibitor) is a myelin-associated protein that inhibits axonal regeneration in the central nervous system (Fournier et al., 2001), with important consequences over regenerative and repair processes of the central nervous system (Grand-Pre et al., 2000). The gene seems to be regulated during neural development and after lesion, indicating that its functions are beyond its neuronal growth inhibition activity in shaping the neuronal circuitry (Meier et al., 2003). *Nogo* is mapped in the human chromosome 2p14-p13, a region strongly associated

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with schizophrenia (SCZ) and bipolar disorder (BPD) (DeLisi et al., 2002; Lewis et al., 2003).

Nogo is an attractive candidate to be evaluated in SCZ and BPD, conditions that share some genetic predisposition. Nogo is a myelin-associated inhibitor, and it has been reported that oligodendrocyte and myelin dysfunction seem to be common to schizophrenia and bipolar disorder (Tkachev et al., 2003), whereas changes in white matter seen in brains of schizophrenic patients seem to be derived from a myelin-related dysfunction (Davis et al., 2003). *Nogo* also plays an important role during development of hippocampal connections (Mingorance et al., 2004), a brain structure that is central to the neuropathology and pathophysiology of schizophrenia (reviewed in Harrison, 2004) and bipolar disorder (Blumberg et al., 2003).

Novak et al., 2002, showed that *Nogo* mRNA is over expressed in the cortex of schizophrenic patients and also identified a polymorphic CAA insertion in the 3′UTR of the transcript, which was more prevalent in the SCZ group (21% in 81 patients) than in the controls (3% in 61 individuals; P=0.0022). Subsequently Covault et al. (2004) failed to replicate this association in 243 controls and 77 schizophrenic patients. However, this group observed a remarkable difference in the frequency of CAA insertion among African and European descendents.

Possible associations of this polymorphism and psychosis (SCZ and BPD), as well as with ethnic backgrounds, were investigated here using 725 human DNA samples. This sample set includes schizophrenia (N=181), bipolar disorder (N=98), controls (N=427) and a sub-set of specific ethnic groups (N=19). Our data agrees with the results of Covault et al. (2004), confirming the absence of association with SCZ, a marked ethnic bias and also do not indicate any association of this polymorphism with BPD. The genotyping results derived from primate-derived samples failed to reveal the CAA insertion, suggesting this to be a recent event in human speciation.

2. Materials and methods

2.1. Subjects and samples

A total of 181 schizophrenic (105 males and 76 females; mean age: 33.1±10.8) and 98 bipolar patients (26 males and 72 females; mean age: 44.7±13.7) were included; all derived from the Institute of Psychiatry, Hospitaldas Clínicas, FMUSP, Brazil. Diagnoses were made through structured interviewsbased on DSM-IV for SCZ and on DSM-III-R for BPD (as the collection of BPD samples started much earlier). Controls were composed of 427 individuals, from the blood bank of the same hospital, without previous history of personal or familial psychiatric disorders (249 males and 178 females; mean age: 33.2±10.9), together with a small subset of samples, included to reinforce the representation of the Asiatic group (9 samples from Kazakhstan) and to include some Native Americans (10 samples from San Martin, Peru). Patients and controls signed an informed consent form, previously approved by the ethics committee of our institution.

Clinical data assessed by Positive and Negative Syndrome Scale (PANSS—Kay et al., 1987), age of disease onset, schizophrenia subtype and familiar history of psychosis were obtained for schizophrenic patients and considered for endophenotype analysis. These clinical data were not available for bipolar patients.

The ancestry of the CAA insertion event was studied using DNA from old world (*Pan troglodytes* and *Papio hamadryas*) and new world (*Saimiri* sp., *Brachyteles arachnoides* and *Alouatta fusca*) primates. Samples from Old-world monkeys as well as *Saimiri* were obtained from Coriell Cell Repositories while the remaining came from the DNA Bank from the Department of General Biology of Universidade Federal de Minas Gerais.

2.2. DNA extraction, amplification and analysis

DNA was extracted from peripheral blood leukocytes of patients and controls, according to salting-out methods for protein precipitation and DNA isolation (Laitinen et al., 1994). PCR amplification of *Nogo* was carried out with the primers NOGOF 5-TCAACATGAAATGCCACACAA-3′ and NOGOR 5′-CAGTCAGTCTGTGCAATGAAA-3′. Fifty nanograms of genomic DNA were amplified in 10 μl reactions, containing 1.5 pmol of each primer, 1.5 mM $MgCl_2$, 125 μM of dNTPs and 0.75 U Taq DNA polymerase (Labtrade, Brazil). The two-step cycling protocol includes an initial DNA denaturation (94 °C

Table 1
Frequency of *Nogo* 3′UTR CAA insertion in controls and patients with Schizophrenia (SCZ) and Bipolar disorder (BPD)

Groups				Genotype frequencies[a]			Allelic frequencies[a]	
	Males	Females	Mean age	1.1	1.2	2.2	Allele 1	Allele 2
Controls (*n*=427)	249	178	33.2±10.9	40.5% (173/427)	43.3% (185/427)	16.2% (69/427)	62.2% (531/854)	37.8% (323/854)
SCZ (*n*=181)	105	76	33.1±10.8	40.9% (74/181)	47% (85/181)	12.1% (22/181)	64.4% (233/362)	35.6% (129/362)
BPD (*n*=98)	26	72	44.7±13.7	37.7% (37/98)	46.9% (46/98)	15.3% (15/98)	61.2% (120/196)	38.8% (76/196)

[a] 1,1: homozygous without the CAA insertion; 1,2: heterozygous; 2,2: homozygous for CAA insertion.

for 5 min) followed by 35 cycles of 94 °C for 45 s and 59 °C for 40 s. PCR products were subjected to electrophoresis and evaluated in 12% silver-stained polyacrylamide gels (Sanguinetti et al., 1994). The resulting bands have 63 bp or 60 bp according to the presence or absence of the CAA insertion.

2.3. Statistical analysis

The magnitude of association between alleles and psychiatric disorders was measured by the odds ratio (OR) and respective 95% confidence interval (CI) together with the chi-squares tests using the level of confidence of α=0.05.

3. Results

Genotype distributions for patients and controls were not deviated from the Hardy-Weinberg equilibrium. For schizophrenia, the genotype distributions of *Nogo* were not significantly associated with any of the clinical parameters, including age of disease onset, disease subtype, familial history of psychiatric disease or PANSS scores. Genotypic or allelic frequencies were not statistically different between patients and controls (Table 1), but showed to be distinct among the different ethnic groups studied (Table 2).

For the investigation of allelic ancestry, we amplified this gene in other primates using these same primers. No amplification results were obtained from *Nogo* gene in the new world macaques used. However, all eight alleles amplified for old-world animals corresponded to our allele 1, without the CAA insertion (data not shown).

4. Discussion

In our analyses, we evaluated the amplified bands in native silver-stained 12% polyacrylamide gel electrophoresis, a method that enabled a fast, inexpensive and clear distinction of the two alleles in a large number of samples. The high frequency of heterozygotes as well as the presence of heteroduplexes enabled the prompt and unequivocal genotype determination.

In the study of Novak et al. (2002) a strong association of the CAA insertion homozygous genotype and schizophrenia was suggested from the analysis of 142 individuals. This association was not replicated in the subsequent study of Covault et al. (2004), which investigated a total of 372 subjects. The

Table 2
Frequency of *Nogo* 3′UTR CAA insertion in controls and patients with schizophrenia (SCZ) according to the populational background

Nogo[a]	European-American		Mixed European-American		Afro-African-American	
	Control	SCZ	Control	SCZ	Control	SCZ
N	231	118	99	28	27	17
1.1	36.4% (84/231)	35.6% (42/118)	49.5% (49/99)	57.1% (16/28)	63% (17/27)	58.8% (10/17)
1.2	45.4% (105/231)	48.3% (57/118)	41.4% (41/99)	35.8% (10/28)	33.3% (9/27)	41.2% (7/17)
2.2	18.2% (42/231)	16.1% (19/118)	9.1% (9/99)	7.1% (2/28)	3.7% (1/27)	0% (0/17)
1	59.1% (273/462)	59.7% (141/236)	70.2% (139/198)	75% (42/56)	79.6% (43/54)	79.4% (27/34)
2	40.9% (189/462)	40.3% (95/236)	29.8% (59/198)	25% (14/56)	20.4% (11/54)	20.6% (7/34)

[a] 1,1: homozygous without the CAA insertion; 1,2: heterozygous; 2,2: homozygous for CAA insertion.

possible association of this polymorphism with SCZ or BPD was not verified in our study, using a total of 706 samples (181 SCZ, 98 BPD and 427 controls). The frequency of alleles and genotypes among the groups studied was statistically the same for controls as well as for SCZ and BPD samples (Table 1).

When we evaluated the genotypic data of individuals with available ethnic information (N=556), we observed a strong bias for a distinct genotypic frequency in the diverse ethnic groups. Our African-Americans showed the smaller frequency for CAA insertion (20.4% versus 40.7% in the European-American group—P=0.0001), similar to the findings of Covault et al., 2004 (respectively, 19.5% and 43.2%). It is also interesting to note that in our intermediate group of mixed Afro-European-Americans this allele showed the in-between frequency of 28.7%, as expected for a mixing of both groups. In our sample of 48 Asiatic-derived chromosomes, we saw the highest frequency of the CAA insertion, reaching 60.4% of the alleles (Table 2), while only 4 out of 20 chromosomes of Native Americans presented the insertion (data not shown). The discrepancy of our data and the original study of Novak et al., 2002 is likely to be a fruit of the small sampling of the previous study, as well as the ethnic discrepancy between their SCZ (that are close to that we found for Europeans) and controls (close to African derived chromosomes).

To have a glimpse on the ancestry of this polymorphic event, we evaluated non-human primate-derived DNAs, in a small set of samples. Amplification was achieved only for Old-world monkeys, suggesting a higher modification of this gene after the divergence of the Catarrhini and Platyrrhini groups. In all eight alleles amplified from old-world species the CAA insertion was absent, suggesting this to be a recent event in human speciation.

This study presents the largest sample of BPD, SCZ and controls genotyped for this *Nogo* polymorphism. After exhaustively crossing clinical and ethnographical data we failed to demonstrate any association of this *Nogo* polymorphism with SCZ or BPD.

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