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Research Report

Gender-specific association of insertion/deletion polymorphisms in the *nogo* gene and chronic schizophrenia

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Abstract

Nogo is a myelin-associated protein associated with neurite outgrowth and regeneration. A previous study has reported an association between an insertion/deletion polymorphism in schizophrenia. We tested for the distribution of the polymorphism and haplotypes of this and another insertion/deletion polymorphism in our population. We have also developed an assay combining allele-specific polymerase chain reaction (AS-PCR) and restriction fragment length polymorphism (RFLP) to simultaneously type these two insertion/deletion polymorphisms. There was a statistically significant difference at the allelic level for both the CAA (χ^2 = 4.378, *df* = 1, *P* value = 0.036) and TATC (χ^2 = 5.807, *df* = 1, *P* = 0.016) polymorphisms in the female subgroup, but not in males. With our genotyping method, we also determined the molecular haplotype. Within the female gender, odds ratio is at 1.57 (95% CI 1.05–2.37) for CAACAA-TATC and 1.40 (95% CI 0.55–3.60) for CAA-TATC, the two at-risk haplotypes. Odds ratio is 0.63 (95% CI 0.42–0.93) for the protective wildtype haplotype CAA-TATCTATC. Further study of these two polymorphisms to investigate functional significance and confirm gender-specific association should be carried out.

Theme: Disorders of the nervous system
Topic: Neuropsychiatric disorders

Keywords: Schizophrenia; Genetic polymorphism; Nogo; Chinese; Female

1. Introduction

It is well established that axons of the CNS of higher vertebrates such as man are limited in their capacity to regenerate and undergo repair after injury or assault. This lack of regeneration is associated with a number of proteins which inhibit the growth of the axons. These proteins include myelin-associated glycoprotein (MAG), reticulon 4 (RTN4 or Nogo), and oligodendrocyte-myelin glycoprotein (OMgp). Together with other growth and transcription factors, they regulate the regenerative and repair processes in the central nervous system by inhibiting the outgrowth of neurites and growth cones.

Nogo was first identified as a high molecular weight myelin inhibitor. The *nogo* gene with 14 exons spanning 75 kb is located on chromosome 2p13–14 [22]. It encodes three major transcripts corresponding to 3 protein variants, Nogo-A (1192 amino acid residues), Nogo-B (373 aa), and Nogo-C (199 aa) [2]. Additionally, there might be as many as 10 splice variants whose mRNAs have been documented although the existence of the proteins are still not clear [16]. The CNS-specific Nogo-A has a unique amino-terminal domain, while the 66-aa extracellular domain and the carboxyl-terminal are shared by all 3 variants. All three bind to a common Nogo receptor (NgR). Nogo-A is expressed at high levels in both oligodendrocytes and

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neurons [10]. IgM autoantibodies to the recombinant large N-terminal inhibitory domain of Nogo-A have been reported in patients with multiple sclerosis, acute inflammatory, and non-inflammatory neurological diseases [17].

The levels and distribution of Nogo mRNA have been shown to be altered in the cortex of patients with schizophrenia [15], making it an excellent candidate gene for the disorder as deficiencies in cortical functions are well documented in patients. In addition to functional significance, the *nogo* gene is also a positional candidate, being located within the region 2p12–15 for which linkage to schizophrenia families have been reported [1,4,13,19].

Novak et al. also reported an association between schizophrenia and the homozygous CAA insertion genotype in a small set of mostly Caucasoid samples [15]. In order to follow up on their observation, we investigated the frequencies of this triplet insertion and an additional tetranucleotide insertion/deletion polymorphism in the *nogo* gene in a larger set of controls and patients with schizophrenia. We have also developed simple PCR-RFLP and ARMS-PCR assays for molecular haplotype information.

2. Materials and methods

2.1. Subjects

Unrelated patients with schizophrenia (n = 363) were recruited from the in-patient wards for the study (270 males and 93 females with mean age 53.3 ± 10.5 years). They were assessed independently by two psychiatrists to have met the DSM-IV criteria for schizophrenia. Controls numbered 253 (124 males and 129 females aged 39.2 ± 16.9 years) were volunteers from hospital and institute research staff with no history of psychiatric disorders. All were ethnic Chinese which was defined as having both parents and all four grandparents who were Chinese. Participants gave informed consent and the study was approved by the Hospital Ethics Committee.

2.2. PCR amplification and restriction endonuclease analysis

Venous blood was collected in EDTA tubes and genomic DNA extracted using the QIAamp Blood Kit (Qiagen GmbH, Hilden, Germany). Target DNA was then amplified with two outer primers (5′-TTACCTGTCTTGACTGCC-3′ and 5′-TACAGCTTAAACCACAATGG-3′ according to Novak et al.) and two allele-specific inner primers (5′-TGAAATTGATGTTGTTGGA-3′ for CAA insertion and 5′-CACACATAGAACTCCAACAT-3′ for no CAA insertion). Polymerase chain reaction was carried out in a volume of 10 μl consisting of genomic DNA, 5% glycerol, 2 mM of each of the 4 deoxyribonucleotides, 1 pmol of each of the two outer primers and 10 pmol of each of the two inner primers, 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 1 unit of Taq polymerase (Promega Corporation, Madison, WI, USA). Amplicon sizes expected are 738 bp from the outer primers, 584 bp if the CAA insertion is present or 185 bp if there is no CAA insertion.

For genotyping of the TATC insertion/deletion polymorphism, the PCR products were incubated with the restriction enzyme *Eco*NI according to the manufacturer's recommendations (New England BioLabs, Beverly, MA, USA). Product sizes would be 649 bp, 495 bp, and 89 bp if the allele with only one copy of TATC is present. For the other allele TATCTATC, the fragments would not be cleaved and the products remain at 738 bp and 584 bp. (In both cases, the 158-bp amplified product from the wildtype CAA allele might also be present.) Amplified and restricted products were separated by agarose gel electrophoresis and photographed under ultraviolet light with ethidium bromide staining. Alleles and genotypes were scored manually by gene counting.

2.3. Statistical analysis

Chi-square analysis and Fisher's exact test were used for comparisons between groups and testing for conformation to Hardy–Weinberg Equilibrium. Odds ratio (OR) and respective 95% confidence intervals were reported to evaluate the effects of any difference between alleles, genotypes, and haplotypes. Probability values of 0.05 or less were regarded as statistically significant. In addition to molecular haplotyping, the 2 polymorphisms were also genotyped and scored separately. Frequencies of haplotypes were then inferred using the Expectation–Maximization (EM) algorithm. Statistical significance for the strength of linkage disequilibrium was obtained empirically through Monte Carlo permutation tests of 10,000 iterations. All statistical analysis was performed on the statistical software S-Plus Professional.

3. Results

Allele and genotype frequencies for the two polymorphisms for 363 patients with schizophrenia and 253 healthy controls are shown in Table 1. The observed genotype distribution for both polymorphisms did not deviate from those expected under Hardy–Weinberg equilibrium (CAA-controls: $\chi^2 = 0.787$, $P = 0.374$, CAA-cases: $\chi^2 = 0.047$, $P = 0.828$, TATC-controls: $\chi^2 = 1.729$, $P = 0.188$, TATC-cases: $\chi^2 = 1.680$, $P = 0.999$).

In accordance with the finding of Novak et al., the CAA insertion was found to be more common in the patients although the difference did not reach statistical significance. As there was significant difference in gender distribution between the control and patient groups, analysis was performed for males and females separately. There was no positive association for males. For females, there was a statistically significant association for both polymorphisms at the allelic level (CAA: OR 1.54 95% CI 1.03–2.32;