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Genetic and expression analyses of the STOP (*MAP6*) gene in schizophrenia

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Abstract

Accumulating evidence suggests that the pathologic lesions of schizophrenia may in part be due to the altered cytoskeletal architecture of neurons. Microtubule-associated proteins (MAPs) that bind to cytoskeletal microtubules to stabilize their assembly are prominently expressed in neurons. Of the MAPs, MAP6 (STOP) has a particular relevance to schizophrenia pathology, since mice deficient in the gene display neuroleptic-responsive behavioral defects. Here we examined the genetic contribution of *MAP6* to schizophrenia in a case ($n=570$) –control ($n=570$) study, using dense single nucleotide polymorphism (SNP) markers. We detected nominal allelic ($p=0.0291$) and haplotypic (global $p=0.0343$ for 2 SNP-window, global $p=0.0138$ for 3 SNP-window) associations between the 3′ genomic interval of the gene and schizophrenia. *MAP6* transcripts are expressed as two isoforms. A postmortem brain expression study showed up-regulation of mRNA isoform 2 in the prefrontal cortex (Brodmann's area 46) of patients with schizophrenia. These data suggest that the contribution of *MAP6* to the processes that lead to schizophrenia should be further investigated.

Keywords: Microtubule-associated protein; Cytoskeleton; Haplotype; Gene expression; Isoform; Postmortem brain

1. Introduction

Cumulative evidence suggests that schizophrenia is associated with cytoskeletal alterations in neuron architecture. Affected neurons lose synaptic connectivity and

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the ability to transmit incoming axonal information to the somatodenritic domain (for review see Benitez-King et al., 2004). The neuronal cytoskeleton plays a key role in synaptogenesis and neurotransmitter release. The cytoskeleton also participates in the development of the nervous system and functional plasticity of the mature brain. Microtubules, the main cytokeletal components, are long protein polymers assembled from subunits formed by alpha and beta tubulins. They maintain a dynamic equilibrium between polymers and dimers through polymerization–depolymerization cycles. Microtubule-associated proteins (MAPs) that bind along the sides of microtubules, regulate these processes. A number of MAPs have been identified; among these, MAP1B was one of the first to be expressed in vitro, from cultured neurons (Tucker et al., 1989). MPA1B is more abundant in neurons than in non-neuronal cells (Diaz-Nido and Avila, 1989) and its expression is down regulated during mammalian brain development (Binder et al., 1984; Tucker et al., 1989). MAP2 is specifically expressed in neurons (Papandrikopoulou et al., 1989). An increasing number of studies have reported altered expressions of MAPs, particularly of MAP2, in schizophrenic brains (Anderson et al., 1996; Cotter et al., 2000; Jones et al., 2002; Rioux et al., 2003).

Characterization of neuronal MAP6, or STOP (*s*table-*t*ubule-*o*nly *p*olypeptide) suggests that it is restricted to vertebrates and provides high resistance to extreme depolymerizing conditions such as low temperature (for review see Bosc et al., 2003). Interestingly, *Map6* deficient mice displayed synaptic defects with depleted synaptic vesicle pools and impaired synaptic plasticity associated with severe behavioral disorders but no catastrophic consequences for mouse organogenesis or viability. Importantly these behavioral defects were alleviated with long-term administration of antipsychotic drugs (Andrieux et al., 2002). Therefore, in this study we set out to test the genetic role of *MAP6* in schizophrenia and examine transcript levels in postmortem brains.

2. Materials and methods

2.1. Subjects

For the case-control genetic association study, 570 unrelated schizophrenics (285 males, 285 females, mean age; 48.7 ± 12.1 years) and 570 age- and sex-matched controls who showed no history of mental illness in a brief psychiatric interview (285 males, 285 females, mean age; 48.4 ± 11.8 years) were used. All subjects resided in central Japan. A consensual diagnosis was made according to DSM-IV by at least two experienced psychiatrists, on the basis of direct interviews, available medical records and information from hospital staff and relatives. None of the patients had additional Axis-I disorders as defined by DSM-IV.

The present study was approved by the ethics committees of RIKEN and Hamamatsu University School of Medicine. All controls, patients and family members gave informed written consent to participate in the study, after provision and explanation of study protocols and purposes.

2.2. Polymorphism screening and single nucleotide polymorphism (SNP) selection

We performed a polymorphism screen of *MAP6* using primer sets that covered exons 1, 2, 3 and 4 and their flanking introns (Fig. 1) (information on these primer sets and PCR conditions are available upon request), to examine 20 schizophrenic patients. We also consulted The International HapMap Project database (http://www.hapmap.org/index.html.ja) (Altshuler et al., 2005). Based on this information, we first chose 17 SNPs from the *MAP6* genomic interval with the following criteria: (1) minor allele frequencies $\geq 5\%$, (2) inclusion of missense polymorphisms, (3) successful TaqMan probe design and (4) an even as possible spacing between SNPs. For marker sparse regions, we added two SNPs (rs656064 and rs617449; SNP 13 and SNP 16, respectively in Table 1) taken from a selection of frequency-annotated SNPs in the Celera Discovery System (Applied Biosystems, Foster City, CA; https://www.appliedbiosystems.com/).

2.3. SNP genotyping

We genotyped a total of 19 polymorphisms distributed within the *MAP6* interval. For brevity, these polymorphisms were designated as SNP01–SNP19 (Fig. 1). We used the TaqMan assay (Applied Biosystems) to genotype SNPs: probes and primers