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A family-based association study of PLP1 and schizophrenia

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Abstract

Recently, proteolipid protein 1 (PLP1) has been identified as downregulated in schizophrenia by quantitative PCR and other technologies. In this work we attempted to investigate the role of PLP1 in the etiology of schizophrenia using a family based association study in 487 Chinese Han family trios. The TDT for allelic association demonstrated that, in male, a weak association was detected in SNP rs475827 with $p = 0.0294$, suggesting that the genetic polymorphisms within *PLP1* in male are likely to confer an increased susceptibility to schizophrenia in the Chinese population.

Keywords: Schizophrenia; Proteolipid protein 1 (PLP1); SNP; Polymorphism; Transmission disequilibrium test (TDT)

Schizophrenia is a severe psychiatric disorder with a lifetime prevalence of approximately 1% and is characterized by hallucinations, delusions, disorganized thought, and various cognitive impairments. Multiple studies have consistently demonstrated that it is highly heritable, but that the genetics are complex [4,13,14,16,18,24]. After several well-publicized false leads, many plausible candidate regions for schizophrenia are now under intense scrutiny; these include regions 1q21-22, 1q32-42, 5q21-34, 6p24-21, 6q13-26, 8p22-21, 10p15-11, 13q14-32, 15q13-15, and 22q11-13. Neuropathological and neuroimaging studies have reported a number of anatomical alterations associated with the disease [1,12]. Biochemical and RNA analyses have shown alterations in various neurotransmitter pathways and presynaptic components [2]. It is of great interest that some of the evidence for oligodendrocyte and myelin dysfunction in schizophrenia has been provided. Reduction of key oligodendrocyte-related and myelin-related genes in schizophrenic patients was identified [23]. Of these genes proteolipid protein 1 (PLP1) was the first one to be verified, by quantitative PCR and other technologies, as being down-regulated. PLP1 is an integral membrane protein belonging to the myelin proteolipid protein family. It is a major myelin protein from the central nervous system and plays an important role in the formation or maintenance of the multilamellar structure of myelin. The PLP1 gene encodes two proteins of the myelin sheath, PLP, the major isoform, and DM20. In human, mutations of this protein cause Pelizaeus-Merzbacher disease (PMD), an X-linked dysmyelinating neuropathy, and spastic paraplegia type II. Transgenic mice without *PLP1* expression show ultrastructural changes in myelin structure and deficits in myelin compaction and

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impairment in organisation of the periaxonal region [15,27]. These abnormalities might correlate with ultrastructural changes of oligodendroglial cells and myelin sheaths noted in schizophrenia disorder. Here we report the result of TDT analysis for *PLP1*, in the Han Chinese population.

The PLP1 gene, which locates in Xq22.2, spans approximately 16 kb of DNA. Approximately 80 SNPs have been identified within the locus (http://www.ncbi.nlm.nih.gov/SNP/), of which we randomly selected five that can be detected by the real-time quantitative PCR analysis: rs475827, rs532117, rs509691, rs2233697, and rs1126707, in which rs2233697 and rs1126707 are two synonymous coding SNPs, rs475827 is approximately 1.5 kb upstream of the 5′ UTR and the other two are intronic.

Four hundred and eighty-seven Han Chinese parent-offspring trio families, in which the offspring were affected with schizophrenia, were recruited for this study. They comprised 170 trio families from Jiangxi province, 191 trio families from Shenyang province, and 126 trio families from Sichuan province. Of these families, 740 (50.7%) were male and 721 (49.3%) were female with a mean age of 31.47 years, S.D. = 7.96. In affected offspring 253 (51.95%) were male and 234 (48.05%) were female with a mean age of 21.17 years, S.D. = 3.81. All subjects were Han Chinese in origin. The patients underwent a structural clinical interview for diagnosis of schizophrenia using Structured Clinical Interview for DSM-III-R: patient edition (SCID-P), version 1.0. Clinical diagnosis was made by two independent psychiatrists according to the DSM-III-R criteria for schizophrenia (American Psychiatric Asssociation, 1987). Subjects gave their standard informed consent for inclusion in the study, which was reviewed and approved by the Shanghai Ethical Committee of Human Genetics. Genomic DNA was extracted from the whole blood using the phenol–chloroform method [8].

The SNPs were genotyped by allele-specific PCR for specifically amplifying the reference allele or its variant in separate PCR reactions [11]. PCR reactions were performed and analyzed on an ABI7900 according to the manufacturer's protocol (User Bulletin No. 2, SYBR Green PCR master Mix and RT-PCR protocol, Applied Biosystems). The PCR primers used in this study were designed by a tetra-primer ARMS-PCR design program [26]. The sequences of the primers are listed in Table 1. The assay used in this study combines kinetic (real-time quantitative) PCR with allele-specific amplification [10]. PCR reactions were carried out in a total volume of 5 μl containing: 10 ng genomic DNA, 2.5 μl Taqman® Universal PCR Master Mix (Applied Biosystems), 0.2 μM allele-specific primer, 0.2 μM common primer and 0.2 × SYBR® Green I (Molecular Probe, Inc.) on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). To reduce well-to-well variability in PCR reaction conditions, an automated dispenser (Hydra® microdispenser, Robbins Scientific) and digital multi-channel pipettes (Thermo Labsystems) were used. After an initial 2 min at 50 °C to activate the AmpErase® uracil-*N*-glycosylase (UNG) and a step of 12 min at 95 °C to deactivate UNG and activate AmpliTaq Gold® enzyme, followed by 50 cycles consisting of 15 s at 95 °C and 30 s at annealing temperature, followed by a final stage of dissociation of checking PCR product, were carried out. Allele calling that is identical to the previous research in our laboratory was manually performed [22].

The transmission disequilibrium test (TDT) was applied to assess allelic association for individual SNPs [21]. In such a family-based analysis, the allele transmitted by heterozygous parents to affected offspring was used as "case" and that nontransmitted as "control". ETDT program version 2.4 was used for transmission/disequilibrium test analysis of individual SNPs [19]. The pairwise linkage disequilibrium (LD) values, as measured by D', were estimated with software 2LD [28]. All tests were two tailed and significance was accepted at $p = 0.05$. Hardy–Weinberg equilibrium analysis was conducted using the online calculator http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm. Other statistical results were performed by SPSS11.0 for WINDOWS.

SNP rs509691 and rs2233697 were non-polymorphic in our sample, therefore only rs532117, rs1126707, and rs475827 were used in the association study. All three markers were in Hardy–Weinberg equilibrium (p values >0.34).

Table 1
Primers used for allele-specific PCR

Marker	Primer sequence[a]	Fragment size (bp)	Annealing temperature (°C)
rs 475827	5′ TGCTCGCCAGCCACATAT/C 5′ ATTCCGACAGTGACCATCCA	88	58
rs 532117	5′ AGTCAAGAAAAGGAGCGTG/A 5′ AGAAAAGTCTAAGAGTTTGGGG	93	60
rs509691	5′ TACTAAAAATACAAAAATTACCC/T 5′ AGCCTCCTGAGTAGCTGGGAT	65	60
rs2233697	5′ GACCTATTTCTCCAAAAACTACAAG/A 5′ AAAAAAGATGGGTCTGTGTGG	85	56
rs 1126707	5′ CAGTATAGGCAGTCTCTGTGCTCAT/C 5′ ATATAGTGCTTCCATAGTGGGTAGG	98	60

[a] An additional mismatch was deliberately put at position-3 from the 3′-terminus of the allele-specific primer to confer the specificity of PCR amplification.