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Original Article

Polymorphism of the CLDN5 gene and Schizophrenia in an Iranian Population

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Abstract

Background: The gene coding claudin (*CLDN5*) is located on 22q11. Since the proteins of *CLDN5* family are a major component for barrier-forming tight junctions, it may be important to test whether or not the *CLDN5* locus could be associated with schizophrenia.

Methods: A total of 150 individuals affected with schizophrenia and 150 healthy persons were recruited. The relationship between the three single nucleotide polymorphism (SNPs) and schizophrenia disease was studied using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) technique. The PCR products were completely digested with restriction enzymes of *Dpn*II, *Pvu*II and *Bst*NI, and then separated on agarose gel. The statistical investigations and haplotype analysis were also performed.

Results: The transmission disequilibrium test (TDT) exhibited weak association between rs10314 [C/G] and schizophrenia ($v2 = 3.55$, $P = 0.022$), but the other two SNPs did not show such an association. The global chi-square test showed that the 3-SNP haplotype system was not associated with schizophrenia although the 1-df test for individual haplotypes showed that the rs1548359(C)-rs10314(G)-rs739371(C) haplotype was excessively non-transmitted ($v2 = 6.33$, $P = 0.025$). The $v2$ test for LD between SNPs indicated that these three SNPs were in strong LD.

Conclusion: Collectively, LD analysis showed that the *CLDN5* locus was associated with schizophrenia in an Iranian population.

Keywords: *CLDN5* gene, Schizophrenia, Polymorphism, Iran

Introduction

Schizophrenia is a form of common mental disease with incidence of nearly 1% worldwide. The disease generally occurs in late youth and early maturity with psychological symptoms which result in a hard, painful life for both the patient and the family (1, 2). According to report of World Health Organization (WHO), considering the losses inflicted by diseases on developed countries, schizophrenia is ranked 5th among all existing diseases in terms of the years in person's life devastated by diseases (3). The reason behind its stigma is its high mortality rate (about 1-14%).

The most important feature of schizophrenia is the change in pattern of thought and destruction of psychological functions which in turn leads to abnormalities in social life. The reports of brain

imaging show that this disease is the consequence of disorder in brain structure and function in the left temporal lobe. On the other hand, surveys on identical twins indicate that schizophrenia can have genetic origin (4, 5). Studies conducted on heredity of schizophrenia denote that it is a very complex genetic disorder that may be affected by several genes. Hereditability of schizophrenia has been estimated to be about 82-84% (6). According to meta analyses of scan genome and gene linkage analyses, there are highly susceptible genes on chromosomes 1q, 3p, 5q, 6p, 8p, 11q, 14p, 20q and 22q contributing to schizophrenia (7, 8).

One of the most important genes accounting for the disease is Claudin 5 (*CLDN5*) gene which is located on chromosome 22q11 (8). Claudin 5 is a member of the Claudin family. It is a trans-membrane protein reported to be a major cell adhesion molecule of tight junctions in brain endothelial cells. Tight junctions are well-developed between adjacent endothelial cells of blood vessels in the central nervous system, and playa central role in establishing the blood-brain barrier (BBB) (9). Claudin-5 is involved in the function of the blood-brain barrier (BBB) and plays a major role in tight junction-specific obliteration of the intercellular space. Mutations in this gene have been found in patients with velocardiofacial syndrome. It is expressed ubiquitously, even in organs lacking epithelial tissues such as the brain, skeletal muscle, and spleen (10). It is found that the CLDN5 gene was associated with schizophrenia in a Chinese population (11). It is also found that the combination of the CLDN5 gene with the DQB1 gene to be associated with celiac disease. These initial findings suggest that abnormalities of tight junctions may be involved in schizophrenia by changing the permeability in the gut and in the brain.

In this research, we investigated three SNPs present at the CLDN5 locus. The chromosomal order of these SNPs is as follow; rs10314 in the 3'-untranslated region of the CLDN5 locus, rs1548359 present in the CDC45L locus centromeric of rs10314 and rs739371 in the 5'-flanking region of the CLDN5 locus. This study is the first association study of CLDN5 gene polymorphisms with schizophrenia in an Iranian population.

Materials and Methods

Samples

A total of 150 unrelated patients with schizophrenia (mean age age +SD: 41.7+13.5 years, including 80 female and 70 male aged 18-60 years in 2010 were collected from psychiatry hospitals and clinics, Tehran, Iran. These patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), and their disease was confirmed by psychiatrists. Then, 150 unrelated healthy control volunteers collected from different regions of country (mean age +SD: 40.5+13.2, including: 72 female and 78 male, aged 20-58 years).They were anonymous and had not been evaluated by psychiatrists. Patients and controls almost resided in the same area of Iran. All participants signed written informed consent.

Genotyping of SNPs

Genomic DNA was extracted from the whole blood sample using the salting out procedure. After amplifying, PCR amplicons were digested with restriction enzymes of *Dpn*II, *Pvu*II and *Bst*NI, and then separated on agarose gel. The genotyping of SNPs was performed by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP). The primers specifically annealing to a target sequence and PCR programs are given in Table 1.

Statistical analysis

The chi-square ($\nu 2$) goodness-of-fit test was applied to test for Hardy–Weinberg equilibrium. The $\nu 2$ values and the degree of freedom (df) were summed over all SNPs genotyped to estimate the statistical significance. The transmission disequilibrium test (TDT) was applied to analyze the family-based genotyping data. The haplotype analysis was performed with the program Haploview software program (http://www.Broad. Mit. Edu/mpg/haploview/).

Table 1: The primers and conditions for PCR amplification of SNPs

SNPs	Primers	PCR conditions
rs1548359	Forward: TCGAGGATGAGAGTTTATGCCG Reverse: GTGTTCACCCATTTCACGGATG	95°C for 5 min 58°C for 1 min 72°C for 30 s 35 cycles
rs10314	Forward: AGGAGGTCTCCACAGGAGTC Reverse: CTGCCTTACTTCCCAGAGGC	95°C for 5 min 50°C for 1 min 72°C for 30 s 35 cycles
rs739371	Forward: CTTAAGACCCTCCATGGCTC Reverse: ACGGTGCAGCAAGGTGTTCC	95°C for 5 min 52°C for 1 min 72°C for 30 s 35 cycles

This program produces two $v2$ tests, the global test for association on H-1 degree of freedom (df), where H is the number of haplotypes for which transmission data are available, and the 1-df test for excess transmission of each haplotype. The E H program (version 1.11) was applied to estimate the degree of linkage disequilibrium (LD) between SNPs (12).

Results

The $v2$ goodness-of-fit test indicated that the genotypic distribution of three SNPs was not deviated from Hardy–Weinberg equilibrium either in the patient sample ($v2$ = 2.94, df = 3, P = 0.515) or in the healthy group ($v2$ = 7.24, df = 3, P = 0.086). The TDT analysis displayed a weak association between rs10314 and schizophrenia ($v2$ = 4.75, P = 0.022), but the other two SNPs did not show such an association (Table 2). For the haplotype analysis, the global $v2$ test did not show a disease association for the rs1548359-rs10314-rs739371 haplotype system ($v2$ = 6.43, df = 7, P = 0.30). As shown in Table 3, the 1-df test for individual haplotypes indicted that the rs1548359(C)-rs10314 (G)-rs739371(C) haplotype was excessively non-transmitted ($v2$ = 4.22, P = 0.026). The $v2$ test for LD between SNPs indicated that these three SNPs were in strong LD (Table 4).

Table 2: TDT analysis for genetic association between schizophrenia and SNPs

SNP	Restriction with	Transmitted	x^2	*P*-value
rs1548359	*Dpn*II	C = 55/G = 59	0.14	0.708
rs10314	*Pvu*II	C = 103/G = 74	4.75	0.022
rs739371	*Bst*NI	C = 77/G = 92	1.33	0.049

Table 3: Analysis of haplotype transmission with the program Haploview program

Haplotype	Observed	Expected	x^2	*P*
CCC	18.4	55.6	2.12	>0.04
CCG	150.6	103.1	1.81	>0.04
CGC	71.9	64.9	4.22	0.026
CGG	33.8	41.1	0.28	>0.047
GCG	1.3	1.9	1.66	0.046
GGC	66.3	55.1	1.45	>0.04
GGG	12.5	15.3	0.90	>0.055

Table 4: Estimation of LD between three SNPs

Group	x^2	Dr	*P*
Patient	70.8	7	<0.000011
Patient	91.7	7	<0.000011

Discussion

The CLDN5 gene belongs to a multigene family consisting of more than 20 members that serve mainly as structural and functional components of the tight junctions in paracellular transport (12, 13). The tight junctions occur in both endothelial cells and simple epithelial cells. The brain is an organ lacking epithelial tissues. Interestingly, CLDN5 is expressed only in endothelial junctions and it has been found to be present in the tight junctions formed from endothelial cells in rat and chicken brains (13). These findings suggest that CLDN5 may be involved in forming the blood-brain barrier (BBB). Moreover, CLDN5 also functions during myelination of Schwann cells in developing nerves. Because CLDN5 is an important component of the endothelial tight junctions, it could play a role in blocking exogenous pathogenic substances entering the body and the brain. If CLDN5 were defective, the brain would be exposed more frequently to harmful environmental factors (13).Association studies have revealed that at least three loci in the del22q11 region are likely to be associated with schizophrenia, including the COMT locus, the UFD1L locus and the PRODH2/DGCR6 locus (14). However, these data remain needed to be replicated further before a firm conclusion is drawn. The present study was conducted with the detection of three SNPs located at the CLDN5 locus and nearby. These SNPs are as follow; rs10314, rs1548359 and rs739371. Rs10314 is located in the 3'-untranslated region of the CLDN5 locus, the rs1548359 in the CDC45L locus centromeric of rs10314 and the rs739371 in the 5'-untranslated region of the CLDN5 locus (14). Our results demonstrated that only rs10314 at the CLDN5 locus was associated with schizophrenia. Of eight individual haploypes, the rs1548359(C)-rs10314 (G)-rs739371(C) haplotype was excessively non-transmitted. These three SNPs were in strong LD and they may then affect each other in LD with a disease underlying variant. Thus, the multilocus test is not independent and the nominal *P*-value does not need to be corrected by Bonferroni correction. This data exhibited the association between the CLDN5 locus and schizophrenia. CLDN5 has been reported as one of the most critical genes implicated in the schizophrenia disease and numerous polymorphisms of this gene has been investigated in a Chinese population (11). Collectively, LD analysis showed that the CLDN5 locus was associated with schizophrenia in an Iranian population. The rs1548359(C)-rs10314 (G)-rs739371(C) haplotype at the CLDN5 locus is very likely to carry a disease-resistant variant as it was excessively non-transmitted by parents of schizophrenic patients. Because the Claudin proteins are a major component for barrier-forming tight junctions that could play a crucial role in response to changing natural, physiological and pathological conditions, association between CLDN5 and schizophrenia may be an important clue leading to look into a meeting point of genetic and environmental factors.

Conclusion

Overall, LD analysis indicated that the *CLDN5* locus was associated with schizophrenia in an Iranian population.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Iran. The authors declare that there is no conflict of interest.

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