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Research Article

Association between the g.296596G > A genetic variant of *RELN* gene and susceptibility to autism in a Chinese Han population

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Abstract

Autism is a childhood neuro-developmental disorder, and Reelin (*RELN*) is an important candidate gene for influencing autism. This study aimed at investigating the influence of genetic variants of the *RELN* gene on autism susceptibility. In this study, 205 autism patients and 210 healthy controls were recruited and the genetic variants of the *RELN* gene were genotyped by the created restriction site-polymerase chain reaction (CRS-PCR) method. The influence of genetic variants on autism susceptibility was analyzed by association analysis, and the g.296596G > A genetic variant in exon10 of the *RELN* gene was detected. The frequencies of allele/genotype in autistic patients were significantly different from those in healthy controls, and a statistically significant association was detected between this genetic variant and autism susceptibility. Our data lead to the inference that the g.296596G > A genetic variant in the *RELN* gene has a potential influence on autism susceptibility in the Chinese Han population.

Keywords: autism, susceptibility, association analysis, *RELN* gene, genetic variants.

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Introduction

Autism is a severe childhood neuro-developmental disorder characterized by significant impairments, such as social deficits, delay and deviance in language development and communication skills, and displays of stereotypic behaviors, activities and interests (Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Holt *et al.*, 2010; Tian, 2012). Evidence from previous studies suggests that certain genetic variants can contribute to autism susceptibility (Zhang *et al.*, 2002; Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Ashley-Koch *et al.*, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; Tian, 2012). Several candidate genes, such as Reelin (*RELN*), Serotonin transporter (*5HTT*), Oxytocin receptor (*OXTR*), Metabotropic glutamate receptor 8 (*GRM8*), Engrailed 2 (*EN2*), Wingless-type MMTV integration site family member (*WNT2*), and Apolipoprotein E (*APOE*), have been investigated with respect to possible associations between genetic variants and autism susceptibility (Petit *et al.*, 1995; Cook *et al.*, 1997; Yirmiya *et al.*, 2001; Bonora *et al.*, 2003; Zhong *et al.*, 2003; Gharani *et al.*, 2004; Li *et al.*, 2004; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Wu *et al.*, 2005; Ashley-Koch *et al.*, 2007; Jacob *et al.*, 2007; Dutta *et al.*, 2008; Lerer *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; He *et al.*, 2011). It has been reported that the *RELN* gene is one of the most important candidate genes for autism susceptibility (Turner *et al.*, 2000; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Ashley-Koch *et al.*, 2007; Freitag, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; Tian, 2012). The *RELN* gene is located in the region of linkage for autism on chromosome 7 (Monaco *et al.*, 2001a,b). Recently, several studies observed the potential associations of *RELN* genetic variants with autism risks (Turner *et al.*, 2000; 2001; Persico *et al.*, 2001; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Serajee *et al.*, 2006; Ashley-Koch *et al.*, 2007; Freitag, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; Tian, 2012). Some of these reports confirmed that certain *RELN* genetic variants are statistically associated with autism risk (Persico *et al.*, 2001; Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Serajee *et al.*, 2006; Ashley-Koch *et al.*, 2007; Li *et al.*, 2008; Holt *et al.*, 2010; Tian, 2012). However, the results from these observations were conflicting rather than conclusive (Krebs *et al.*, 2002; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Devlin *et al.*, 2004; Li *et al.*, 2004; Skaar *et al.*, 2005; Serajee *et al.*, 2006; Dutta *et al.*, 2008; Li *et al.*, 2008; He *et al.*, 2011). Up to date, there are no similar studies on the association of g.296596G > A genetic variant in *RELN* gene with autism susceptibility. Therefore, the objective of this study was to detect this genetic variant and further evaluate its influence on autism susceptibility.

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Subject and Methods

Subjects

In this case-control study done from between January 2009 and December 2012 at the Tongji Hospital, a total of 415 subjects were enrolled, including 205 autism patients (mean age: 4.6 years, 148 males and 57 females) and 210 healthy subjects (mean age: 4.7 years, 151 males and 59 females). All subjects were genetically unrelated Chinese of Han ethnicity. The autism patients were diagnosed by the criteria defined by the American Psychiatric Association (2000) manual DSM-IV-TR. The controls were frequency-matched to autism patients in terms of age and gender. The protocol of this study was approved by Ethics Committee of the Tongji Hospital and informed consent was obtained from all subjects.

Genotyping

Venous blood samples were collected from each subject, and genomic DNA was isolated by using the DNA Blood Mini kit (QIAGEN, Valencia, CA), following the manufacturer's instructions. PCR primers were designed by Primer Premier 5.0 software and their sequences, annealing temperature, amplification region and sizes were shown in Table 1. The PCR amplifications were carried out in 20 μL reaction mixtures, containing 50 ng of mixed DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison, WI, USA). The PCR protocol was: 95 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 59.5 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 8 min. The genetic variants of *RELN* gene were genotyped by the created restriction site-polymerase chain reaction (CRS-PCR) method, where one of the primers contained a nucleotide mismatch, which enabled the use of restriction enzymes for discriminating sequence variations (Haliassos *et al.*, 1989; Zhao *et al.*, 2003; Yuan *et al.*, 2012; Yuan *et al.*, 2013a; Yuan *et al.*, 2013b). Aliquots of 5 μL PCR amplified products were digested with 2U *Mae*II restriction enzyme (MBI, Fermentas) at 37 °C for 10 h, following the manufacturer's instructions. The digested products were separated in 2.0% agarose gels stained with ethidium bromide and observed under UV light. To verify the accuracy of genotype determination by the CRS-PCR method, approximately 15% of the PCR amplified products were randomly picked and sequenced on an ABI 3730 sequencer by Bioasia Biotechnology Co., Ltd (Shanghai, China).

Statistical analyses

The chi-squared (χ^2) test was used to evaluate the Hardy Weinberg equilibrium (HWE) for genotype and allele frequencies. A p value < 0.05 was considered statistically significant. All statistical analyses were down using the Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA).

Results

In this case-control study, we detected the g.296596G > A genetic variant of *RELN* gene using the CRS-PCR method. The sequence analyses indicated that this genetic variant resulted from a non-synonymous mutation caused by a G to A mutation in exon 10 of the *RELN* gene, this leading to the replacement of a valine (Val) by an isoleucine (Ile) (p.Val359Ile). The PCR products was digested with *Mae*II restriction enzyme and divided into three genotypes: GG (191 and 23 bp), GA (214, 191 and 23 bp) and AA (214 bp, Table 1). Table 2 shows the respective allele and genotype frequencies in autism patients and healthy controls. The allele-G and genotype-GA were predominant in the studied subjects. The allele frequencies in autism patients (G, 53.66%; A, 46.34%) were significantly different from those of healthy controls (G, 62.38%; A, 37.62%; $\chi^2 = 6.4827$, $p = 0.0109$). The genotype frequencies were also statistically significant different between autism patients and healthy controls ($\chi^2 = 7.2176$, $p = 0.0271$, Table 2). The distributions of genotype in autism patients and healthy controls were fitted with HWE (all p-values > 0.05, Table 2).

Discussion

Recent studies indicate that autism is a disease of polygenic inheritance, wherein genetic variants play key functions in the development of autism. Candidate gene studies have proven a useful approach for identifying genetic variants associated with increasing autism susceptibility (Holt *et al.*, 2010; Tian, 2012). The human *RELN*

Table 1 - Primer pairs, PCR products and CRS-PCR analysis details used for g.296596G > A genetic variant detection of the *RELN* gene.

Primer sequences	Annealing temperature (°C)	Amplification fragment size (bp)	Amplification fragment region	Restriction enzyme	Genotype (bp)
5'-ATCAATTCAGCTCACAGACAAGAC-3'	59.5	214	Exon10	*Mae*II	GG:191, 23
5'-CTGGTCCTTTAATAGTGGTTTTGG-3'					GA:214, 191, 23
					AA:214

The underlined nucleotide marks the nucleotide mismatch enabling the use of the selected restriction enzyme for discriminating sequence variations in the CRS-PCR analysis.

Table 2 - Genotype and allele frequencies of the *RELN* gene g.296596G > A genetic variant in the studied populations.

Group	Genotype frequencies			Allele frequencies		χ^2	p
	GG (%)	GA (%)	AA (%)	G (%)	A (%)		
Autism (n = 205)	62(30.24)	96(46.83)	47(22.93)	220(53.66)	190(46.34)	0.6985	0.7052
Control (n = 210)	80(38.10)	102(48.57)	28(13.33)	262(62.38)	158(37.62)	0.2555	0.8801
Total (n = 415)	142(34.22)	198(47.71)	75(18.07)	482(58.07)	348(41.93)	0.1701	0.9185
	$\chi^2 = 7.2176$, p = 0.0271			$\chi^2 = 6.4827$, p = 0.0109			

gene is an important candidate gene for influencing autism (Turner *et al.*, 2000; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Ashley-Koch *et al.*, 2007; Freitag, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; Tian, 2012), and analysis of genetic variants in the *RELN* gene allows to effectively screen for autism risk (Bonora *et al.*, 2003; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Holt *et al.*, 2010; Tian, 2012). Several previous studies have been carried out using *RELN* as a candidate gene for autism susceptibility in different populations (Persico *et al.*, 2001; Krebs *et al.*, 2002; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Devlin *et al.*, 2004; Li *et al.*, 2004; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Serajee *et al.*, 2006; Ashley-Koch *et al.*, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; He *et al.*, 2011). In the present study, we found the g.296596G > A genetic variant in the *RELN* gene and evaluated the relationship of this genetic variant with respect to autism susceptibility in a Chinese Han population by an association analysis. The allele and genotype frequencies for this genetic variant in autism patients were significantly different from those in healthy subjects (Table 2). Our data indicate that the g.296596G > A genetic variant of the *RELN* gene has a statistically significant association with autism susceptibility and may affect the subjects susceptibility toward autism in the Chinese Han population. Several similar studies concerned the influence of other genetic variants in the *RELN* gene on autism susceptibility (Persico *et al.*, 2001; Krebs *et al.*, 2002; Zhang *et al.*, 2002; Bonora *et al.*, 2003; Devlin *et al.*, 2004; Li *et al.*, 2004; Bartlett *et al.*, 2005; Skaar *et al.*, 2005; Serajee *et al.*, 2006; Ashley-Koch *et al.*, 2007; Dutta *et al.*, 2008; Li *et al.*, 2008; Holt *et al.*, 2010; He *et al.*, 2011; Tian, 2012), which are in accordance with our results, including a significant association between the *RELN* genetic variant (rs2073559) and autism susceptibility (Ashley-Koch *et al.*, 2007). Evidence for the association of the *RELN* genetic variant (rs362780, p = 0.00165) with autism susceptibility was found by Holt *et al.* (Holt *et al.*, 2010). Tian (2012) reported that the g.504742G > A polymorphic variant in the *RELN* gene might affect subjects susceptibility toward autism in the Chinese Han population, but that the g.333509A > C variant was not significantly associated with autism. These observations all corroborate that *RELN* genetic variants are important contributors to the genetic risk in autism susceptibility.

In conclusion, the present study revealed that the g.296596G > A genetic variant in *RELN* gene is statistically associated with autism susceptibility in a Chinese Han population. Gene function studies and association studies on larger population are still necessary to confirm these findings and to investigate the biological mechanism underlying *RELN*-mediated autism susceptibility.

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