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Replication of an association of a common variant in the Reelin gene (*RELN*) with schizophrenia in Ashkenazi Jewish women

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Abstract

A single nucleotide polymorphism (rs7341475) in *RELN* has recently been shown to be associated with schizophrenia (SZ) in an Ashkenazi Jewish (AJ) case-control study specifically in women by Shifman *et al.* We have replicated this association in women in another large independent AJ collection (721 cases, 259 female; 1455 controls, 834 female) and confirmed that it applies to both SZ and schizoaffective disorder. Further, we explore the effects of this polymorphism through quantitative trait loci (QTL) analysis of 9 SZ related factors providing information on sex-specific genotype-phenotype correlations.

Keywords

Schizophrenia; Reelin; Association; Endophenotype; Sex-specific; Ashkenazi; Jewish

INTRODUCTION

Schizophrenia (SZ) is a common, disabling, neuropsychiatric disorder with onset typically in young adults and characterized by multiple sex-specific phenotypic differences: females make up only ~30% of the cases, have a later age of onset, higher familiarity and a somewhat different presentation (more affective symptoms). Consistent with these sex-specific phenotypic differences, a recent study by Shifman *et al.* (Shifman *et al.* 2008) reported that a common DNA variant (rs7341475) in *RELN* was associated with an increased risk for SZ in Ashkenazi Jewish (AJ) females but not males. *RELN* is expressed at peak levels in the fetal brain during the 2nd trimester and encodes a ~388 kDa protein whose function is important for corticogenesis. Consistent with the hypothesis that SZ is a neurodevelopmental disorder (Sawa, Snyder 2002), certain regions of brains from SZ patients show a significant reduction in *RELN* expression (~50%) (Impagnatiello *et al.* 1998).

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We have a large, independent collection of AJ SZ patients and controls and therefore sought to replicate the association results and observed sex effect of Shifman *et al.* We also explored the possible relationship between phenotype and rs7341475 genotype in this sample by performing quantitative trait loci (QTL) analysis with 9 phenotypic factors, derived from our recently published principal components factor analysis of 73 items from our consensus diagnostic ratings and direct assessment interviews (McGrath et al. 2009).

MATERIALS AND METHODS

Our analysis sample of 721 AJ SZ probands and 720 AJ controls was collected by the Johns Hopkins Epidemiology-Genetics Program in Psychiatry (<http://www.hopkinsmedicine.org/epigen/>), and has been described in detail elsewhere (Fallin et al. 2003). Of the 721 AJ SZ probands, 124 (~17%) are schizoaffective patients. We also included an additional 735 control individuals of AJ descent from the New York Cancer Project (NYCP) Biorepository (Foulkes et al. 2002).

We genotyped SNP rs7341475 using the Taqman 5'-exonuclease allelic discrimination assay according to the manufacturer's recommendations in 721 independent AJ SZ cases and 1455 AJ controls. We performed a one-tailed Chi-Square test, considering only results consistent with the original study of Shifman *et al.* as statistically significant, i.e., AJ female SZ cases would carry more G alleles than controls. In our sample 17% of the affected individuals have the diagnosis of schizoaffective disorder (SZA). Patients with this diagnosis were specifically eliminated from the analysis performed by Shifman *et al.* (Shifman et al. 2002), but explored in a later study and found to show the same association (Pisante et al. 2009). For this reason we also explored the effect of including or removing SZA individuals.

To further investigate the possibility of complexity in the genotype-phenotype relationship, we performed a QTL analysis (UNPHASED v3.0.9) using the 9 factor scores derived from 73 dichotomous signs and symptoms from each of the 721 AJ SZ cases (McGrath et al. 2009; *Dudbridge 2006). These factors were labeled: affective (AFF), child/adolescent sociability (CHA), disorganization (DIS), hallucination (HAL), disability/impairment (IMP), negative (NEG), delusion (DEL), prodromal (PRO), and scholastic (SCH).

RESULTS AND DISCUSSION

The SNP rs7341475 was successfully genotyped in totaling 2176 individuals and the genotype frequencies were in Hardy-Weinberg equilibrium. The distribution of genotypes and alleles of rs7341475 is shown in the Table 1. As predicted, we observed a consistent and significant (one tail $p=0.0475$) difference in case versus control allele frequencies only in AJ women with an Odds Ratio of 1.23.

When we subtracted the SZA individuals from our cases and repeated the analysis, we observed the same trend but with reduced statistical significance (one tail $p=0.065$, data not shown) again in AJ females only. We conclude that the SZA patients contribute to the association and excluding them reduces the sample size and, consequently, the statistical power. This result is consistent with the result published later by the same group (Pisante et al. 2009).

For our QTL analysis with the 9 factors from the McGrath et al factor analysis (McGrath et al. 2009), as shown in the Figure 1, two factors (CHA, HAL) show nominal association with rs7341475 with the best signal ($p=0.018$) in male-allelic analysis for factor CHA and the second best signal ($p=0.035$) in female-genotypic analysis for factor HAL. In both cases, the G allele is nominally associated with higher scores (i.e., more symptoms). However, these results do not withstand multiple test correction and we include them only to suggest approaches that

could improve the power of future replication attempts and provide information on the complex nature of the observed associations.

In summary, we have replicated the genetic association between a common variant in *RELN* and SZ in AJ women reported by Shifman *et al.* in a population of the same origin. Our results strengthen the evidence for *RELN*'s involvement in SZ and provide sex specific connections of the disease-associated variant with phenotypic features of SZ. It is of interest that *RELN* expression has also been shown to be sex-related in SZ patients (Eastwood, Harrison 2003). Our data add to the accumulating evidence that implicate *RELN* in SZ through sex specific mechanism(s).

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Figure 1. Quantitative trait loci (QTL) association analysis of rs7341475 with 9 factors in AJ SZ patients

The 9 factors shown on the horizontal axis are defined in the text. The vertical axis is the negative logarithm (base 10) of the p-value. Six analyses were performed: All_allelic: allelic test for all cases; All_genotypic: genotypic test for all case; Female_allelic: allelic test for female cases only; Female_genotypic: genotypic test for female cases only; Male_allelic: allelic test for male cases only; Male_genotypic: genotypic test for male cases only.

Table
Genotype and Allele Frequencies of the SNP rs7341475 of *RELN* Gene in Cases and Controls

Subjects (N)	Genotype(%)			chisq-value	p-value(one-tailed)	Allele (%)		chisq-value	p-value(one-tailed)
	AA	AG	GG			A	G		
Overall_cases(721)	32(4.4)	248(34.4)	441(61.2)	1.474	0.2393	312(21.6)	1130(78.4)	0.912	0.1698
Overall_controls(1455)	82(5.6)	503(34.6)	870(59.8)			667(22.9)	2243(77.1)		
Male_cases(462)	25(5.4)	162(35.1)	275(59.5)	0.026	0.4935	212(22.9)	712(77.1)	0.008	0.4644
Male_controls(621)	35(5.6)	217(34.9)	369(59.5)			287(23.1)	955(76.9)		
Female_cases(259)	7(2.7)	86(33.2)	166(64.1)	4.033	0.0666	100(19.3)	418(80.7)	2.788	0.0475
Female_controls(834)	47(5.6)	286(34.3)	501(60.1)			380(22.8)	1288(77.2)		