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Short communication

Genetic analysis of the *RELN* gene: Gender specific association with Alzheimer's disease

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ABSTRACT

Association between genetic variants of the reelin (*RELN*) gene and the risk for developing Alzheimer's disease (AD) was examined in a sample of 432 patients and 308 controls. Single marker and haplotype analyses revealed that the strongly linked rs528528 and rs607755 polymorphisms are associated with AD risk in a gender specific manner. Among men, but not in women the rs528528 T/T and rs607755 A/A genotypes were significantly associated with the susceptibility to AD.

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1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease, the leading cause of dementia among elderly people. Neuropathologically, AD is defined by extensive neuronal loss and the accumulation of intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein and extracellular deposits of amyloid- β ($A\beta$) peptide in the brain (Selkoe, 2001). The complex late-onset form of AD is most likely caused by multiple genetic and environmental susceptibility factors. Inheritance of the Apolipoprotein E (*APOE*; MIM# 107741) $\epsilon 4$ variant is a widely studied genetic predisposing factor for developing AD (Mahley et al., 2006).

Reelin, an extracellular matrix glycoprotein, is encoded by the *RELN* gene (MIM# 600514), which is a potentially relevant candidate gene for AD on account of its biological function and chromosomal localization. *RELN* gene maps to 7q22, which is located next to an AD linkage region on the long arm of chromosome 7 (Pericak-Vance et al., 2000; Hahs et al., 2006; Ertekin-Taner, 2007). Reelin plays a crucial role in the migration and positioning of neurons during brain development and has been linked to processes of synaptic plasticity, learning and memory formation (Herz and Chen, 2006; Lakatosova and Ostatnikova, 2012).

Despite its expected important role in neurodegeneration, according to our knowledge so far only three studies have examined genetic associations of *RELN* with AD, and one with AD-related neuropathology (Seripa et al., 2008; Antoniadou et al., 2011;

Kramer et al., 2011; Bufill et al., 2013). This study aimed to replicate the previous reports on associations between *RELN* single nucleotide polymorphisms (SNP) and AD in an independent sample. We determined the allelic variation of *RELN* by using 5 markers (rs2299356, rs528528, rs607755, rs6943822 and rs4298437) in a Hungarian sample of 432 patients and 308 controls, with potential linkage disequilibrium (LD) between genetic markers.

2. Methods

The study included 432 patients with late-onset AD (74.9 ± 6.7 years of age (mean \pm SD), 70.3 ± 5.1 years of age at onset (mean \pm SD), men 35.1%) and 308 elderly, cognitively healthy control individuals (74.2 ± 7.1 years of age (mean \pm SD), men 34.4%), both groups of Hungarian origin. A consensus clinical diagnosis of late-onset AD was established according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRD) criteria (McKhann et al., 1984). The minimum age at onset was 65 years. All recruitment and protocols were conducted with written informed consent and with the approval of the Ethics Committee of the Hungarian Council on Science and Health (ETT-TUKEB).

Genotyping of blood-derived DNA samples was performed by applying commercial TaqMan single-nucleotide polymorphism assays (Applied Biosystems, Foster City, CA). The polymerase chain reaction amplification was conducted in single-plex reactions in 96-well plates with a total volume of 20 μ l using the following amplification protocol: 95 °C for 10 min, and 40 cycles of 92 °C for 15 s and 60 °C for 1 min. Fluorescence measurements were

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performed using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA).

Categorical variables were studied applying Fisher exact and Pearson χ^2 -tests. The mean age of the AD and control groups was compared by using the *t*-test for independent samples. Multiple logistic regression model was used to test for interaction between the *RELN* and *APOE* polymorphisms (under both dominant and additive models), and between the *RELN* polymorphisms and gender. In the case of significant genotype by gender interaction effect (rs2299356, rs528528, rs607755), we also compared genotype distribution between AD patients and controls separately by gender using Pearson's χ^2 -test. To exclude Type I errors, we carried out Bonferroni's correction for multiple testing for 3 single-marker and 8 haplotype comparisons.

Hardy-Weinberg equilibrium testing, LD calculations, and haplotype analyses were conducted using Haploview 4.2 (Barrett et al., 2005). Power analysis was performed using G*Power 3.0 software (Faul et al., 2007), and the effect size was determined according to the method published by Cohen (1988). Based on the calculated effect sizes in men, our study sample has 82% power at the significance level of 0.05 to detect differences in rs528528, and 75% to find differences in rs607755 genotype frequencies between AD and control groups (effect sizes: $w=0.198$ for rs528528 and $w=0.184$ for rs607755).

3. Results

No statistically significant differences were found between AD and control groups in terms of gender distributions and mean age. All observed genotype frequencies in both controls and cases conform to Hardy-Weinberg equilibrium.

Genotype distributions of the different *RELN* polymorphisms did not differ significantly between AD and control groups (Table 1). The *APOE* $\epsilon 4$ allele was significantly over-represented in the AD group as compared to the control group (AD: 27.9%; control: 11.4%; $p < 0.0001$). None of the investigated *RELN* polymorphisms showed

Table 2

Genotype and haplotype frequencies of the *RELN* rs2299356, rs528528 and rs607755 polymorphisms in men.

Polymorphisms	AD patients	Controls	Chi-square	<i>P</i> value (corrected <i>p</i> value ^a)
rs2299356			7.159	0.028 (0.084)
A/A	41 (27.5%)	15 (14.0%)		
A/G	71 (47.7%)	56 (52.4%)		
G/G	37 (24.8%)	36 (33.6%)		
rs528528			10.084	0.006 (0.018)
T/T	44 (29.5%)	15 (14.0%)		
T/C	74 (49.7%)	57 (53.3%)		
C/C	31 (20.8%)	35 (32.7%)		
rs607755			8.617	0.013 (0.039)
A/A	43 (28.9%)	15 (14.0%)		
A/G	72 (48.3%)	57 (53.3%)		
G/G	34 (22.8%)	35 (32.7%)		
Haplotypes ^b				
A-T-A	44.3%	31.2%	8.998	0.003 (0.024)
G-C-G	39.4%	50.9%	6.631	0.010 (0.080)
G-T-A	8.0%	7.5%	0.037	0.847
A-C-G	5.5%	6.6%	0.244	0.621
A-T-G	1.4%	1.4%	0.0	0.997
G-C-A	0.7%	1.0%	0.144	0.704
G-T-G	0.7%	0.5%	0.043	0.835
A-C-A	n.d.	0.9%	2.425	0.119

AD: Alzheimer's disease, n.d.: not detected, *RELN*: reelin gene

^a To exclude Type I errors, we carried out Bonferroni's correction for multiple testing for 3 single-marker and 8 haplotype comparisons.

^b Haplotypes of the *RELN* rs2299356, rs528528 and rs607755 polymorphisms. Chi-squares and *p* values for comparisons of the haplotype frequencies were determined by using the Haploview 4.2 program.

epistasis with *APOE* $\epsilon 4$ allele on AD risk in the logistic regression model ($p > 0.05$). However, a marginally significant interaction effect was found between the rs2299356 polymorphism and gender

Table 1

Genotype frequencies of the investigated *RELN* polymorphisms in the overall population.

Chromosomal localization	Polymorphisms	AD patients	Controls	Chi-square	<i>P</i> value
7:103669375	rs2299356			1.242	0.537
	A/A	107 (24.8%)	68 (22.1%)		
	A/G	214 (49.5%)	151 (49.0%)		
	G/G	111 (25.7%)	89 (28.9%)		
7:103748638	rs528528			3.087	0.214
	T/T	117 (27.1%)	70 (22.7%)		
	T/C	213 (49.3%)	150 (48.7%)		
	C/C	102 (23.6%)	88 (28.6%)		
7:103749507	rs607755			1.206	0.547
	A/A	112 (25.9%)	72 (23.4%)		
	A/G	218 (48.2%)	146 (47.4%)		
	G/G	112 (25.9%)	90 (29.2%)		
7:103958224	rs6943822			0.434	0.805
	C/C	88 (20.4%)	57 (18.5%)		
	C/T	207 (47.9%)	153 (49.7%)		
	T/T	137 (31.7%)	98 (31.8%)		
7:103985430	rs4298437			0.623	0.732
	C/C	190 (44.0%)	127 (41.2%)		
	C/T	190 (44.0%)	144 (46.8%)		
	T/T	52 (12.0%)	37 (12.0%)		

AD: Alzheimer's disease, *RELN*: reelin gene

($p=0.058$) and a significant interaction effect was detected between the rs528528 polymorphism and gender ($p=0.048$) and between the rs607755 polymorphism and gender ($p=0.023$) in the logistic regression model.

Stratification of the data according to gender revealed significant differences of the rs2299356, rs528528 and rs607755 genotype distributions in men (Table 2), but not in women. The frequency of the rs2299356 A/A genotype was significantly higher in the AD than in the control group among men ($\chi^2=7.159$ (2) $p=0.028$, corrected: $p=0.084$). Compared to the controls, the rs528528 T/T genotype was significantly over-represented in the AD group in men ($\chi^2=10.084$ (2) $p=0.006$, corrected: $p=0.018$). Regarding the rs607755 polymorphism, the A/A genotype occurred with a significantly higher frequency in the AD than in the control population among men ($\chi^2=8.617$ (2) $p=0.013$, corrected: $p=0.039$).

Strong LD was observed between the rs528528 and rs607755 ($D'=0.952$, $r^2=0.873$) and between the rs6943822 and rs4298437 polymorphisms ($D'=0.926$, $r^2=0.355$); while moderate LD was found between the rs2299356 and rs528528 ($D'=0.724$, $r^2=0.494$) and between the rs2299356 and rs607755 polymorphisms ($D'=0.687$, $r^2=0.454$). According to our data two haplotype blocks can be detected: rs2299356-rs528528-rs607755 (block 1) and rs6943822-rs4298437 (block 2).

Haplotype frequencies of these polymorphisms did not differ significantly between cases and controls ($p > 0.05$). Stratification of the data on the basis of gender resulted in significant differences in block 1 haplotype distributions in men (Table 2), in contrast with the results in women. The A-T-A haplotype of the rs2299356-rs528528-rs607755 polymorphisms was more frequent in the AD than in the control group in men ($\chi^2=6.661$ (1) $p=0.003$; corrected $p=0.024$), while the G-C-G haplotype had a higher occurrence in the control group ($\chi^2=7.768$ (1) $p=0.010$, corrected: $p=0.080$).

4. Discussion

In single marker case-control analysis no significant correlation between *RELN* polymorphisms and AD risk was found in the overall sample, whereas significant association of the rs2299356 A/A, rs528528 T/T and rs607755 A/A genotypes with AD was observed in men, but not in women. After correction for multiple testing, only the effect of the SNPs rs528528 and rs607755 remained significant. Since reelin competitively binds to the same receptors as APOE (D'Arcangelo et al., 1999), a possible interaction between *RELN* and *APOE* genes on AD risk was also hypothesized, but no epistasis was found.

The *RELN* genotype distribution observed in our control sample is similar to earlier reports on other Caucasian control populations (Bufill et al., 2013; Seripa et al., 2008; Kramer et al., 2011). In a Spanish case-control study rs2299356 polymorphism was found to be associated with AD and rs528528 with mild cognitive impairment (Bufill et al., 2013). Our study did not confirm these results in the Hungarian population. Similar to our findings no effect of the rs607755 polymorphism was detected in an Italian sample in the entire cohort; however, an opposite pattern was found after stratification according to gender: G/G genotype was associated with AD risk among females (Seripa et al., 2008).

A genome wide association study aimed to identify genetic variants that distinguish non-demented elderly with a heavy neurofibrillary tangle burden from those with a low neurofibrillary tangle burden. In the *RELN* gene rs6943822 and rs4298437 were found to be associated with this AD-related neuropathology (Kramer et al., 2011). Based on these results, we assumed that these polymorphisms can confer susceptibility to AD, but our findings did not support this hypothesis. A possible reason is that

our control group can be heterogeneous regarding the absence or presence and magnitude of neurofibrillary tangles in spite of the fact that they are cognitively healthy individuals.

In haplotype analysis no significant association was found between *RELN* haplotypes and AD risk in the overall sample, but the A-T-A haplotype frequency of block 1 (rs2299356-rs528528-rs607755) was significantly higher in the AD compared to the control group among men. These results are consistent with the single marker findings. The rs2299356 polymorphism did not have a significant effect in itself, but it is linked to rs528528 and rs607755 which were significantly correlated with AD.

In the present study single marker and haplotype assessments led to the conclusion that the strongly linked *RELN* rs528528 and rs607755 polymorphisms may be associated with AD risk in a gender specific manner. The medium sample size of our study is an important limitation, therefore our findings should be interpreted with caution until further replication.

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