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The Q7R Saitohin gene polymorphism is not associated with Alzheimer disease

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Abstract

Previous studies have reported conflicting results regarding the association of the Q7R polymorphism in the Saitohin gene with late-onset Alzheimer disease (AD). Given that AD is a tauopathy but no mutations or polymorphisms in Tau have been consistently associated with AD, and that Saitohin is nested in intron 9 of Tau and shares a similar expression pattern, we tested this association in 690 multiplex AD families and in a case-control sample (903 patients and 320 controls). We found no evidence of significant association of this polymorphism with risk of AD using family-based and case-control tests of association.

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Apolipoprotein E-4 (APOE-4) accounts for about 50% of the genetic susceptibility to late-onset Alzheimer disease (AD), suggesting the existence of additional genetic risk factors. The microtubule-associated protein Tau is the main component of the neurofibrillary tangles, a major AD neuropathological hallmark. Tau thus constitutes a good candidate gene but no mutations have been identified in AD patients and association studies have reported conflicting results [11]. Tau is mutated in frontotemporal dementia and other tauopathies, and the Tau H1 haplotype is associated with progressive supranuclear palsy and Parkinson disease. It is therefore possible that other polymorphisms in that genomic region demonstrate an association with AD. Interestingly, the Saitohin gene (STH) localizes to intron 9 of Tau and shares its expression pattern in most human tissues and brain areas [2]. Conrad et al. [2] investigated the association of the Q7R single nucleotide polymorphism (SNP) in STH with AD and found that distributions of

alleles and genotypes were significantly different between 51 AD patients versus 30 controls, with the arginine (R) allele and the RR genotype significantly over-represented in AD patients (odds ratio [OR] = 3.109 for allele and OR = 11.92 for genotype) [2]. If this initial report was confirmed by other studies, this polymorphism would represent the second strongest genetic susceptibility factor in AD. To test these results in an additional case-control group and, for the first time, in a family-based setting, we investigated the association of this polymorphism in 690 multiplex AD families and in a case-control sample consisting of 903 patients and 320 controls.

Our sample ($N = 690$ families) for family-based association studies consists of three sets of families: the Collaborative Alzheimer Project families (ascertained through The Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC), the Department of Psychiatry and the Center for Human Genetics at Duke University, the Program in Human Genetics at Vanderbilt University, and the UCLA Neuropsychiatric Institute), families ascertained by the NIMH AD Genetics Initiative,

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Table 1
Genotype and allele frequencies of the Saitohin Q7R polymorphism in the AD patients and controls

Genotype	Genotype frequency		Allele	Allele frequency	
	Cases count (%)	Control count (%)		Cases count (%)	Control count (%)
QQ	570 (63.0)	189 (59.1)	Q	1427 (79.0)	488 (76.2)
QR	287 (31.8)	110 (34.4)	R	379 (21.0)	152 (23.8)
RR	46 (5.1)	21 (6.5)			

and families from the Indiana Alzheimer's Disease Center's National Cell Repository. AD neuropathology examinations were done according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and National Institute on Aging and the Reagan Institute (NIA-Reagan) criteria [5,9]. Both age-at-onset (AAO) and age-at-examination (AAE) were recorded, with AAO based on caregiver or spouse recollection or medical record review of the age at the onset of the first symptom of AD. These 690 multiplex families comprise 1499 AD patients and 909 unaffected relatives. Our case-control sample is composed of 903 patients and 320 controls. The patients consist of unrelated AD patients ascertained through the Joseph and Kathleen Bryan ADRC, and patients selected at random, one from each family in the familial data set. Controls were ascertained through the Joseph and Kathleen Bryan ADRC and the Center for Human Genetics at Duke University, and were most often the unrelated and unaffected spouse of an AD patient. All individuals are Caucasian.

We genotyped the Q7R Saitohin SNP using a TaqMan allelic discrimination assay with the following primers and probes: 5'-TCCCTAGTCTGGGCCATGAG-3', 5'-GGCCACCTGCACAGTCTTGT-3', 56FAM-AGGGTG-GAGGCCAAGTCTCATGC-3BHQ-1, 5TET-AGGGTG-GAGGCCGAGTCTCATGC-3BHQ-1. The polymerase chain reaction (PCR) amplification was performed in 5 µl reactions (30 ng dried DNA, 1 × TaqMan universal PCR master mix from Applied Biosystems, 900 nM of each primer, 200 nM of each probe) using the GeneAmp PCR system 9700 thermocyclers (Applied Biosystems) for a 40-cycle program (50°C/2 min; 95°C/10 min; 40 × [95°C/15 s, 60°C/1 min]). The fluorescence generated during the PCR amplification was read using the ABI Prism 7900HT sequence detection system and analyzed with the SDS software (Applied Biosystems). To identify new polymorphisms in the STH gene, its open reading frame was PCR-amplified (primers: 5'-TCCACCCAGCATGGTGAC-3', 5'-TCCAAGTTCAGTTGCCATCTC-3') in 30 pools of five individuals each and sequenced. APOE genotyping was performed as previously described [4].

The test for Hardy–Weinberg disequilibrium was conducted in the group of controls using the Genetic Data Analysis program [6]. *P*-values were generated using a permutation test with 3200 replicates.

Family-based association analysis was conducted using

the Pedigree Disequilibrium Test (PDT) [8] and geno-PDT [7] to assess association between alleles and genotypes, respectively, with AD risk. To investigate the association of STH with AAO of AD in families, we used the variance components approach, considering both the Fulker model and the orthogonal model implemented in the Quantitative Trait Disequilibrium Test (QTDT) program [1]. Because the variance components framework accounts for familial correlations it is more appropriate to use than linear regression for multiplex families. Both models employ a likelihood ratio test (LRT) to evaluate the association between AAO and alleles of the STH SNP.

Case-control analyzes for association with risk of AD were conducted using unconditional multiple logistic regression implemented in SAS (SAS Institute, Cary, NC). All analyzes were adjusted for AAE and gender. For single-allele tests, the Wald chi-square value was used to assess significant association between alleles and risk of AD. For genotypic tests, the LRT comparing nested models (the baseline model containing gender and AAE versus the full model with AAE, gender, and the terms for two of the three genotypes) was used to assess significant improvement of fit between the two models.

Case-control analyzes for association with AAO were conducted using multiple linear regression controlling for gender. The *F*-test associated with the Type III (simultaneous) sums of squares was used to assess relationships between alleles or genotypes and AAO of AD.

No evidence for deviation from Hardy–Weinberg equilibrium was detected in the control sample for the Q7R SNP (*P* = 0.53). The genotype and allele frequencies in the patient and control populations are similar to those

Table 2
Results of family-based Saitohin Q7R polymorphism association tests

	Number of families	<i>P</i> -value	
		PDT	Geno-PDT
Overall	690	0.861	0.974
Autopsy-confirmed ^a	339	0.961	0.406
AD with LB ^b	39	0.706	0.697

^a Families with at least one family member who had an autopsy that met CERAD or NIA-Reagan criteria for definite AD.

^b Families with at least one individual having AD and Lewy bodies (LB) at autopsy [10].

Table 3
Results of case-control Saitohin Q7R polymorphism association tests

	Number of cases	Number of controls	QR (95% CI)		
			Genotype model		Allele model
			QR	RR	R
Overall	903	320	0.90 (0.66–1.22)	0.75 (0.41–1.37)	0.87 (0.65–1.17)
AD autopsy-confirmed cases versus controls	266	320	0.83 (0.53–1.30)	0.58 (0.25–1.35)	0.78 (0.51–1.19)
APOE-4 positive	553	80	1.35 (0.77–2.36)	1.54 (0.43–5.52)	1.37 (0.80–2.35)
APOE-4 negative	350	240	0.70 (0.47–1.04)	0.59 (0.28–1.27)	0.68 (0.46–0.99)
Males	301	146	1.00 (0.64–1.56)	0.56 (0.24–1.33)	0.92 (0.60–1.39)
Females	602	174	0.83 (0.54–1.27)	1.01 (0.42–2.41)	0.85 (0.57–1.28)
AAO/AAE < 70	372	172	0.98 (0.63–1.52)	1.53 (0.58–4.03)	1.04 (0.68–1.58)
AAO/AAE \leq 70	531	148	0.91 (0.57–1.44)	0.47 (0.20–1.10)	0.81 (0.53–1.25)

previously reported [3,12,13], but no significant differences were observed between patients and controls (Table 1).

The family-based tests of association PDT and geno-PDT did not detect any association between alleles or genotypes of the Q7R SNP and risk of AD (all $P > 0.05$) in the overall, autopsy-confirmed, and AD with Lewy bodies (families with at least one individual with both AD and Lewy bodies at autopsy) groups (Table 2). In addition, the Q7R SNP was not found to influence AAO ($P = 0.48$ with the Fulker and orthogonal models).

Case-control analyzes also showed no association between alleles or genotypes and risk of AD in the overall, autopsy-confirmed, Lewy body, APOE-4 allele positive, male, female, AAO/AAE < 70, or the AAO/AAE \geq 70 groups (Table 3 and data not shown). We found marginal evidence of negative association (OR = 0.68; 95% CI: 0.46–0.99; $P = 0.045$) between the R allele and risk of AD in the APOE-4 negative subgroup (Table 3). There was no evidence of association of Q7R with AAO ($P > 0.05$ for all tests).

The Saitohin gene coding region and 50 bp of flanking sequence on each side were screened in 150 individuals for additional polymorphisms but none were found, so no other coding SNPs in this gene could be evaluated for association with risk for developing AD.

Although the R allele and RR genotype at the Q7R polymorphism in the STH gene were previously found to be significantly over-represented in AD patients when compared to controls [2], our data in a much larger sample did not replicate these findings. In the present report, no significant association was found in any statistical test performed, with the exception of a P -value of 0.045 in the case-control study in individuals lacking the APOE-4 allele. This finding, that the R allele was significantly negatively associated with risk, is counter to the previous report [2] where the opposite allele, the Q allele, was found to be negatively associated irrespective of APOE-4 status. In light of previous results and considering multiple tests, it is likely that this single significant result is a false positive.

The discrepancy between the study of Conrad et al. [2]

and the present report may derive from a type I error (false-positive result) in their data, different patient ascertainment methods, or from population-specific effects. The present study also differs from the previous reports [2,3,12,13] in the very large number of individuals involved and in the family-based tests of association performed in addition to a case-control study. Although our results do not point to any association of Saitohin with risk for AD, it may be involved in other neurological diseases such as PD and further investigation in large cohorts is required to answer this question. It would be very interesting to determine if this protein localizes to the neuropathological hallmarks of several neurological diseases, such as amyloid plaques, neurofibrillary tangles or Lewy bodies.

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