SHORT REPORT

Saitohin gene is not associated with Alzheimer's disease

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J Neural Neurosurg Psychiatry 2003;**74**:362–363

Background: The deposition of tau protein in neurofibrillary tangles constitutes an important feature of many neurodegenerative disorders, including Alzheimer's disease. A polymorphic gene, saithin (STH), nested within the tau gene (microtubule associated protein tau, MAPT), was recently identified and an association of a non-synonymous polymorphism in STH with increased risk for Alzheimer's disease was suggested.

Objective and methods: To test the above hypothesis in a case-control association study of two independent white populations within Switzerland and Greece, comparing genotype and allele frequencies from 225 Alzheimer's disease patients and 144 healthy control subjects.

Results: No differences in allelic or genotypic distributions between Alzheimer's disease patients and controls was found in the individual samples (Swiss/Greek) or in the combined sample. Stratification for the presence of apolipoprotein E (APOE) ϵ 4 allele, sex, or age did not show significant effects in the populations studied, nor was there an effect on the age of onset.

Conclusions: No evidence was found for an association of the non-synonymous polymorphism (Q7R) in STH and Alzheimer's disease. This finding is in line with earlier studies showing no association between MAPT and Alzheimer's disease.

Recently, Conrad *et al* identified a gene nested within an intron of the tau gene (microtubule associated protein tau, MAPT), termed saithin (STH).¹ STH encodes for a 128 amino acid protein and is located in intron 9 of MAPT (2.5 kb downstream of exon 9), a critical region for alternative splicing of exon 10. Splicing in of exon 10 results in a protein with four repeated microtubule binding domains (4R), in contrast to tau without the exon 10, which displays only three microtubule binding domains (3R). Distribution of STH mRNA in human tissue is similar to MAPT, and high in muscle, placenta, and fetal and adult brain. To date, no specific function is known for STH. However, putative phosphorylation sites for protein kinase C and casein kinase II have been identified, which may be in line with a combined function of STH and MAPT¹; a non-synonymous polymorphism (Q7R; CAA to CGA) was used to carry out a case-control association study, which suggested overrepresentation of the GG genotype, as well as the G allele, in Alzheimer's disease patients compared with controls.

Deposition of hyperphosphorylated tau in neurofibrillary tangles is a central feature of Alzheimer's disease,² the most common form of dementia in the elderly,³ and other neurodegenerative diseases, termed tauopathies, including frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), Pick's disease, progressive supranuclear palsy, and corticobasal degeneration.⁴ An imbalance of the different tau isoforms is associated with the pathological changes in these diseases. Familial forms of these tauopathies have been shown to be caused by intronic and exonic mutations in the MAPT gene^{5,6} affecting mainly the region around the alternatively spliced exon 10. So far, studies examining potential associations

between Alzheimer's disease and polymorphisms in MAPT remain controversial.^{7–13} In this study we tested whether the STH polymorphism was associated with risk for Alzheimer's disease, because first, STH and tau are in close vicinity, and second, the allelic variance is positioned in a region involved in the alternate splicing of MAPT, and thus also involving the regulation of the ratio between 3R and 4R tau isoforms.

METHODS

We examined 225 patients with Alzheimer's disease (according to NINCDS-ADRDA criteria¹⁴) and 144 controls (tested with CERAD battery and MMS¹⁵) in two white populations (Swiss: 91 Alzheimer patients and 92 healthy controls; Greece: 134 Alzheimer patients and 52 controls) and undertook a case-control association study for the non-synonymous polymorphism in STH (Q7R; CAA to CGA).

DNA was isolated from whole blood with a DNA extraction kit (Qiagen, Hilden, Germany). Genotyping for STH was done with the PSQ™ 96 system (Pyrosequencing AB, Uppsala, Sweden) (primer forward, GCC CCT GTA AAC TCT GAC CA; primer reverse, GCT GAG GGT TCT GTC TCT GG, pyrosequencing primer, GTG AGG GTG GAG GC; for details see www.pyrosequencing.com). APOE genotypes were assessed using the LightCycler™ instrument (Roche Diagnostics Corporation, Mannheim, Germany).¹⁶ Pipetting was done with a Genesis Workstation 200/8 using Gemini v3.5 software (TECAN).

Statistics

For statistical analysis, we used Fisher's exact test, Pearson's χ^2 (testing association), and the Kolmogorov–Smirnov test (testing distributions between two groups). A probability (p) value of < 0.05 was regarded as significant. Non-significant p values are not shown. The study was approved by the local ethics committee and informed consent was obtained before the investigation.

RESULTS

The allelic distribution for STH in the combined sample was 78.59% (A) and 21.41% (G), and the genotypic distribution was 61.8% (A/A), 33.6% (A/G), and 4.6% (G/G) (table 1, which also includes single population data), following Hardy–Weinberg equilibrium in both populations. Based on the genotype frequencies reported by Conrad *et al*,¹ our study had a statistical power of more than 95% for $\alpha = 0.05$. In contrast to Conrad *et al*, we did not detect any significant difference in the distribution of genotypes or alleles between the patients with Alzheimer's disease and the controls.

In previous studies associations of single nucleotide polymorphisms assigned to MAPT with Alzheimer's disease were found in APOE ϵ 4 carriers.^{7–11} We therefore stratified our sample for APOE ϵ 4 carriers. As previously described, the APOE ϵ 4 allele was significantly associated with the risk of Alzheimer's disease in our samples (Pearson's $\chi^2 = 20.44$; $p = 2 \times 10^{-4}$). Stratification in APOE ϵ 4 carriers and non-carriers did not reveal any significant differences between the two groups, nor did stratification for age (≥ 70 years or < 70 years at the age of onset) or sex. Finally, to test whether STH may act as a progression marker, we determined the age of onset by Kaplan–Meier analysis, as well

Table 1 Analysis of 225 patients with Alzheimer's disease and 144 healthy control subjects

			Diagnosis		
Sample			HCS (n (%))	AD (n (%))	Total
Swiss	Genotype:	A/A	55 (59.8)	53 (58.2)	108 (59.0)
		A/G	35 (38.0)	31 (34.1)	66 (36.1)
		G/G	2 (2.2)	7 (7.7)	9 (4.9)
	Allele:	A	145 (78.80)	137 (75.27)	282 (77.05)
		G	39 (21.20)	45 (24.73)	84 (22.95)
Greek	Genotype:	A/A	29 (55.8)	91 (67.9)	120 (64.5)
		A/G	21 (40.4)	37 (27.6)	58 (31.2)
		G/G	2 (3.80)	6 (4.50)	8 (4.30)
	Allele:	A	79 (75.96)	219 (81.72)	298 (80.11)
		G	25 (24.04)	49 (18.28)	74 (19.89)
Combined	Genotype:	A/A	84 (58.3)	144 (64.0)	228 (61.8)
		A/G	56 (38.9)	68 (30.2)	124 (33.6)
		G/G	4 (2.8)	13 (5.8)	17 (4.6)
	Allele:	A	224 (77.78)	356 (79.11)	580 (78.59)
		G	64 (22.22)	94 (20.89)	158 (21.41)

The samples were age and sex matched. Mean (SD) age at diagnosis was 71.61 (6.9) years in the Alzheimer's disease group; age at examination in the control group was 70.0 (6.3) years. The samples came from two white populations (Swiss/Greek).

There were no statistically significant associations between to STH genotypes or alleles and Alzheimer's disease in single or combined samples.

AD, Alzheimer's disease; HCS, healthy control sample.

as the mean age of onset, and found no differences between the genotypes (AA: mean age 72 years; 95% confidence interval (CI), 71 to 73; AG: mean age 71 years; 95% CI, 69 to 73; GG: mean age 73 years; 95% CI, 70 to 76).

DISCUSSION

Our findings are in line with two recent reports,^{17,18} both showing no association between STH polymorphism and Alzheimer's disease. In addition, Verpillat and colleagues¹⁸ found a complete linkage of the Q genotype (A allele) with the H1 tau haplotype in 91 patients with frontotemporal dementia. They even concluded that the STH Q genotype could be part of the extended H1 tau haplotype. In the controversial discussion of the association between Alzheimer's disease and polymorphisms in MAPT,⁷⁻¹³ these three studies (Cook *et al*, 2002,¹⁷ Verpillat *et al*, 2002,¹⁸ and our own data) argue strongly in favour of the absence of an association between variations in MAPT and the risk of developing Alzheimer's disease.

Conclusions

This study, like others, fails to support a significant association between STH and Alzheimer's disease. Stratification did not reveal effects in subpopulations or special risk groups. The promising gene saitoihin (STH) (by its proposed function and location) is not associated with the risk of Alzheimer's disease. Further analysis should be directed towards sporadic tauopathies.

ACKNOWLEDGEMENT

We thank Mr Jay Tracy for careful reading and preparation of the manuscript, and Ms Esmeralda Garcia, Ms Christin Wilde, Ms Andrea Hauer, and Ms Estelle Obrist for patient care and sampling. This work was supported by grants of the National Center for Competence in Research (NCCR) "Neuronal Plasticity and Repair", the Swiss Science National Foundation (32-65869.01), and the Roche Research Foundation (22-2001).

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Received 15 October 2002

Accepted 2 December 2002

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