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ORIGINAL COMMUNICATION

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Somatostatin genetic variants modify the risk for Alzheimer's disease among Finnish patients

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Abstract The levels of somatostatin are consistently decreased in the brain and cerebrospinal fluid of Alzheimer's disease (AD) patients. The somatostatin gene is located on chromosome 3q27.3 close to an association region identified in late-onset AD patients originating from Finland. Since somatostatin is a good candidate on both positional and functional grounds, we studied whether single nucleotide polymorphisms (SNPs) in the somatostatin gene were associated with AD in the Finnish population. We genotyped three SNPs within this gene in Finnish AD patients (n = 424) and non-demented controls (n = 466). AD patients were compared with non-demented control subjects using single-locus and haplotype approaches. In the whole study group, the age, sex and APOE adjusted OR for the risk of AD in C-allele carriers of the SNP rs4988514 was 1.42 ($p < 0.05$). Interestingly, in APOE ε4-allele carriers, the age and sex adjusted OR for the risk of AD in C-allele carriers of the rs4988514 increased to 2.05 ($p < 0.01$). Additionally, SNP rs4988514 may interact with the APOE ε4-allele to increase the risk of AD. Assessment of individual haplotype distributions revealed a 2-fold overrepresentation of the TCG haplotype of SNPs rs3864101, rs4988514 and rs7624906 in the AD APOE ε4-allele group ($p < 0.01$). Conversely, a major haplotype TTG was significantly underrepresented among all the AD patients as well as APOE ε4-allele carrying AD patients. Thus the major haplotype TTG of somatostatin may have a protective effect against AD. This first genetic association study between somatostatin and AD indicates that genetic variations in the somatostatin gene may modify the risk for AD among Finnish AD subjects.

Key words somatostatin · Alzheimer's disease · association · SNP

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease leading to dementia. AD is characterized by typical neuropathological changes including extracellular amyloid plaques with amyloid-beta protein (Aβ) and intracellular neurofibrillary tangles. According to the amyloid cascade hypothesis, accumulation and deposition of Aβ in the brain are the major events in the pathology of AD [10].

Several lines of evidence indicate that abnormalities in numerous neurotransmitters and neuropeptides are involved in AD. Somatostatin is an important neuropeptide in the mammalian central nervous system. The concentration of somatostatin consistently declines in the brain and cerebrospinal fluid of AD patients suggesting that some genetic determinant(s) may be the cause of

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the altered somatostatin expression or stability [1, 4, 16, 20]. Somatostatin protein levels are also known to be significantly lower in AD patients carrying the Apolipoprotein E (APOE) ε4-allele [8]. Additionally, the somatostatin gene is located in close proximity (~ 500 kb) to the microsatellite marker D3S2436 on chromosome 3q28 which we have previously shown to be associated with late-onset AD [11]. Two other family-based studies of individuals from different ethnic backgrounds have also identified the same locus as possibly harboring a genetic variant that increases late-onset AD susceptibility [9, 12]. Moreover, Saito et al. recently demonstrated that somatostatin may be associated with AD via modulation of membrane metallo-endopeptidase (MME), a major Aβ degrading enzyme [17]. A genetic deficiency of somatostatin may induce a long-term decline in the MME activity for over a decade or more and this may lead to an elevation in the steady-state levels of amyloid-beta. Accumulation of Aβ would in turn trigger the AD pathological cascade. Thus previous biochemical and genetic findings indicate that somatostatin can be considered as a plausible candidate gene for contributing to AD.

In the present study, single nucleotide marker analysis in a large number of AD and control subjects was carried out to evaluate whether genetic variations across the somatostatin gene modify the risk for AD in a Finnish population. The data indicate that somatostatin is a possible risk gene for AD especially among the APOE ε4-allele carrying patients.

Materials and methods

The study population was examined in the Department of Neurology, Kuopio University Hospital. The ethical committee of the University hospital approved the study. The subjects consisted of 424 AD patients (mean onset-age was 71 ± 7 years; 67 % women) and 466 controls (mean age at examination or death was 70 ± 5; 60 % women), who exhibited no signs of cognitive decline on interview or during neuropsychological testing. All AD subjects underwent a comprehensive clinical evaluation during which the clinical diagnosis of probable AD was made according to NINCDS-ADRDA criteria [14].

Somatostatin has been mapped to 3q27.3 (spans ~2300 bp) and has two exons. Three single nucleotide polymorphisms (SNPs), rs3864101 (intergenic, 3'), rs4988514 (3'UTR) and rs7624906 (intergenic, 5'), at ~3 and 7 kb intervals, were selected for screening using SNPbrowser™ software 3.0 and genotyping was done using TaqMan® Pre-Developed Assay Reagents for Allelic Discrimination (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was amplified on a MJ Research PTC-200 Cycler (50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min), and fluorescence was detected on an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA). APOE genotyping in the study cohort has previously been determined by using a standard method [13].

Statistical analyses for comparing allele and genotype frequencies were performed using the SPSS/Win statistical program (version 11.5.1 for windows; SPSS, Chicago, IL) and the GenePop program on the Web (http://wbiomed. curtin.edu.au/genepop/). The Bonferroni correction was applied by multiplying P values with the number of tests. Hardy-Weinberg distribution of genotypes among the AD and control groups was assessed using GenePop option 1. Pair-wise linkage disequilibrium (LD) of SNPs was determined using the Haploview software (http://www.broad.mit.edu/mpg/haploview/). The Expectation-maximization (EM) algorithm was used to obtain maximum-likelihood estimates of haplotype frequencies of somatostatin gene SNPs with Arlequin version 2 software (http://anthro.unige.ch/arlequin). Haplotype frequencies were compared for case and control samples using the RxC program employing the metropolis algorithm to obtain unbiased estimates for exact p-values with standard errors (http://www.marksgeneticsoftware.net/rxc.htm). Two different approaches namely standard Pearson χ^2-test and logistic regression analysis were performed to identify any interactions between APOE ε4-allele and somatostatin C-allele carriers. The level of statistical significance was set at $p < 0.05$.

Results

APOE ε2/3/4 allelic frequencies differed as expected in the case (n = 424; 0.02/0.51/0.47) and control (n = 466; 0.04/0.80/0.16) groups ($p < 0.001$) and were consistent with those previously described in the same population [13]. The APOE ε4-allele was five times more common for cases (odds ratio (OR) = 4.8 (95 % confidence interval (CI) 3.8 to 5.9). All SNPs were in Hardy-Weinberg equilibrium in cases and controls ($p > 0.05$) in the whole group and in APOE stratified groups.

The allele and genotype frequencies of the three SNPs did not differ significantly between the whole AD and control groups (Table 1). However, an increased frequency of C-allele carriers of SNP rs4988514 can be seen in AD cases (0.31) compared to controls (0.26). Regression analysis revealed a significant age, sex and APOE adjusted OR for the risk of AD in C-allele carriers of the rs4988514 (OR 1.42; 95 % CI 1.02–2.00, p = 0.04, Bonferroni corrected p = 0.12). When AD patients and controls were analyzed together in a pair-wise LD analysis (1780 alleles), a strong LD was observed between all three SNPs in the somatostatin gene (D' = 1.0 in all pairs, $p < 0.01$) and these SNPs originated from the same haplotype block. The frequencies of all observed haplotypes were above 0.05 and they were included into the statistical tests (Table 1). The association testing of individual haplotype distributions in the whole study group revealed that the major haplotype TTG was underrepresented in the cases compared to controls.

It is known that the somatostatin protein levels are significantly lower in AD patients carrying the APOE ε4-allele [8]. To study the genetic background of the somatostatin gene in this particular AD group, we stratified the data according to APOE. In the APOE ε4-allele group, allele and genotype frequencies of rs4988514 differed significantly between AD cases (n = 312) and controls (n = 129) and the age and sex adjusted OR for the risk of AD in C-allele carriers of the rs4988514 was 2.05 (95 % CI 1.23–3.41; p = 0.006, Bonferroni corrected p = 0.018, Table 2). As in the whole group, the major haplotype TTG in the APOE ε4-allele group was underrep-

Table 1 Allele, genotype and haplotype frequencies of somatostatin (SST) polymorphisms among the whole study group

SST SNP	Alleles	Allele frequency		P-value[a]	Genotypes	Genotype frequency		P-value[a]	OR (95% CI), adjusted for *APOE*, age and gender[b]
		AD (n = 848)	CO (n = 932)			AD (n = 424)	CO (n = 466)		
rs7624906	C	0.14	0.12	0.127	TT	0.73	0.78	0.134	1.08 (0.76–1.54)
	T	0.86	0.88		CT	0.25	0.21		(TT vs TC + CC)
					CC	0.02	0.02		
rs4988514	C	0.16	0.14	0.078	TT	0.69	0.74	0.079	**1.42 (1.02–2.00)***
	T	0.84	0.86		CT	0.29	0.25		(TT vs TC + CC)
					CC	0.02	0.01		
rs3864101	G	0.79	0.81	0.160	GG	0.62	0.66	0.166	1.14 (0.83–1.57)
	T	0.21	0.19		GT	0.34	0.30		(GG vs GT + TT)
					TT	0.04	0.04		

Haplotypes	AD (n = 848 alleles)	CO (n = 932 alleles)	P-value[c]	OR (95% CI)
H1 (T-T-G)	0.622 (528)	0.678 (632)	**0.012 ± 0.004**	0.78 (0.64–0.95)
H2 (T-T-T)	0.069 (59)	0.067 (62)	0.854 ± 0.006	1.05 (0.73–1.52)
H3 (T-C-G)	0.164 (139)	0.135 (126)	0.110 ± 0.009	1.25 (0.97–1.63)
H4 (C-T-T)	0.143 (122)	0.120 (112)	0.136 ± 0.008	1.23 (0.93–1.62)
Overall			0.073 ± 0.011	

[a] P-values calculated by using unbiased estimate of exact p-value; [b] OR calculated by using binary logistic regression model with SPSS, * $p < 0.05$; [c] P-values calculated by using RxC program (± SE)

Table 2 Allele, genotype and haplotype frequencies of somatostatin (SST) polymorphisms for AD patients (n = 312) and controls (n = 129) among the *APOE* ε4-allele group

SST SNP	Alleles	Allele frequency		P-value[a]	Genotypes	Genotype frequency		P-value[a]	OR (95 % CI), adjusted for *APOE*, age and gender[c]
		AD (n = 624)	CO (n = 258)			AD (n = 312)	CO (n = 129)		
rs7624906	C	0.15	0.15	0.839	TT	0.71	0.71	0.828	1.04 (0.66–1.65)
	T	0.85	0.82		CT	0.27	0.28		(TT vs TC + CC)
					CC	0.02	0.01		
rs4988514	C	0.17	0.09	**0.004**	TT	0.69	0.81	**0.002**	**2.05 (1.23–3.41)****
	T	0.83	0.90	**(0.024)**	CT	0.29	0.19	**(0.012)**	(TT vs TC + CC)
					CC	0.03	0		
rs3864101	G	0.78	0.79	0.661	GG	0.05	0.04	0.656	1.12 (0.73–1.72)
	T	0.22	0.21		GT	0.35	0.33		(GG vs GT + TT)
					TT	0.60	0.63		

Haplotypes	AD (n = 624 alleles)	CO (n = 258 alleles)	P-value[b]	OR (95 % CI)
H1 (T-T-G)	0.609 (380)	0.702 (181)	**0.012 ± 0.004**	0.66 (0.49–0.91)
H2 (T-T-T)	0.067 (42)	0.058 (15)	0.652 ± 0.007	1.17 (0.64–2.15)
H3 (T-C-G)	0.169 (106)	0.093 (24)	**0.004 ± 0.001**	2.00 (1.25–3.19)
H4 (C-T-T)	0.154 (96)	0.147 (38)	0.839 ± 0.005	1.05 (0.70–1.58)
Overall			**0.012 ± 0.002**	

[a] P-values calculated by using unbiased estimate of exact p-value; [b] P-values calculated by using RxC program (± standard error); [c] OR calculated by using binary logistic regression model with SPSS, * $p < 0.05$
The Bonferroni corrected P values are given in parenthesis

resented for cases compared to controls. Additionally, the TCG haplotype was significantly overrepresented among AD cases carrying the APOE ε4-alleles. We did not detect any significant differences between the study groups with respect to the APOE ε4-negative carriers.

To investigate the possible interaction between somatostatin and APOE genes, ORs were calculated for somatostatin and APOE genes separately and in combination, using cases and controls who had neither APOE ε4 nor somatostatin C-alleles of SNP rs4988 514 as the ref-

Table 3 OR of AD cases when taking the subjects with neither APOE ε4 nor somatostatin (SST) C alleles as reference

[a]APOE ε4+	[a]SST rs4988514 C+	AD	Control	OR (95 % CI)	[b]P-value	
−	−	80	240	reference		
−	+	32	97	0.99 (0.62–1.59)	1.0	
+	−	214	105	6.11 (4.33–8.63)	<0.001	[b] P<0.01; OR 2.0
+	+	98	24	12.25 (7.33–20.46)	<0.001	(95 % CI 1.21–3.32)

[a] The minus sign indicates cases/controls who lack these genotypes; plus indicates number of cases with these genotypes
[b] Pearson χ^2 test

erence (Table 3). The results indicated that the somatostatin C-allele carriers with APOE ε4-allele were associated with an increased risk of AD.The interaction option of logistic regression analysis also supported the conclusion that APOE ε4 and somatostatin C-alleles interacted with AD (p = 0.045; OR 2.0 with 95 % CI 1.01–4.04). Both analyses indicated that the somatostatin C-allele was not independently associated with the AD risk, whereas the APOE ε4-allele was an independent risk factor.

Discussion

At least three independent studies have observed support for significant linkage with late-onset AD at chromosome 3q28 [9, 11, 12] including our paper with the microsatellite marker D3S2436 [11]. The somatostatin gene is located ~500 kb away from D3S2436 marker and thus it can be considered as a positional candidate gene for AD. The expression levels of somatostatin are also known to decrease in the brain and cerebrospinal fluid of AD patients suggesting that some genetic determinant(s) may underlie the altered somatostatin expression or stability [1, 4, 8, 16, 20]. Moreover, somatostatin is believed to be involved in the regulation of the metabolism of Aβ in the brain by modulating the protein turnover and cellular localization of Aβ degrading enzyme MME [17]. Based on previous biochemical and genetic findings, somatostatin could be considered as a good candidate on both positional and functional grounds. To assess the possible genetic association of somatostatin gene with AD, we genotyped three SNPs among Finnish AD patients and controls. As far as we are aware, this is the first genetic association study between somatostatin and AD, which indicates that the somatostatin gene is a possible risk gene for AD, especially among the APOE ε4-allele patients.

In the whole study group only nominally significant age, sex and APOE adjusted OR for the risk of AD in C-allele carriers of the rs4988 514 was found (OR 1.42; 95 % CI 1.02–2.00, p = 0.04). In subjects with the APOE ε4-allele, the risk haplotype TCG encompassed the C-allele of SNP rs4988514, which was also significantly overrepresented among the AD APOE ε4-allele group in the single locus analyses even if we used a conservative Bonferroni correction to compensate for multiple testing and corrected for 6 tests (three SNPs and APOE 4 status). Additionally, SNP rs4988 514 may interact with the APOE ε4-allele to increase the risk of AD. The APOE ε4-allele is the major genetic risk factor for both early-onset AD and late-onset AD [2, 5, 18]. It has also been shown to be associated with higher amyloid-plaque burden in the brain and decreased levels of CSF Aβ42 [3, 7, 15, 19, 21]. Interestingly, in somatostatin knockout mice there is known to be decreased Aβ degrading enzyme MME activity and disrupted localization as well as an increase in the amounts of Aβ42 [17]. One possible hypothesis is that the down-regulation of somatostatin expression particularly in the APOE ε4-allele group [8] in the human brain initiates a gradual decline in MME activity. This could be due to the possibly erroneous genetic information in somatostatin gene and may lead to a corresponding elevation in the steady-state levels of Aβ, over a decade or more, and this may cause the Aβ accumulation that triggers the AD pathological cascade in AD patients carrying the APOE ε4-allele. Due to the smaller sample sizes in the APOE stratified study groups, independent replication studies will be needed to verify the observed association findings in other populations.

The haplotype TTG was significantly underrepresented among the whole group of AD patients as well as in the APOE ε4-allele AD patients. This major haplotype TTG may provide a protective effect against AD. Consistently, the protective haplotype TTG does not include the risk allele C of SNP rs4988514.

The three genotyped markers for somatostatin covers ~10 kb and were located ~1.4 kb upstream, in the 3'UTR region and ~7 kb downstream of the gene and found to be in complete LD with each other. According to the HapMap database, they constitute a 40 kb haplotype block defined by confidence intervals of LD [6] from Utah residents with ancestry from northern and western Europe populations (CEU) encompassing the whole genomic region of somatostatin. No other genes are known to exist within this haplotype block. We conclude that according to previous data and the results observed here, genetic variations in the somatostatin gene may modify the risk for AD among Finnish AD subjects.

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