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The frequency of the TRPC4AP haplotype in Alzheimer's patients

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ABSTRACT

A haplotype in the gene for transient receptor potential cation channel, subfamily C, member 4 associated protein (TRPC4AP), has been identified in two extended pedigrees with late-onset Alzheimer's disease. Nine of the SNPs in the haplotype were analyzed in our unrelated Alzheimer's patients and controls. The H1 haplotype was found in 36% of the patients (199 patients) and in 26% of the controls (85 controls) ($P = 0.0282$; OR = 1.56; 95%CI = 1.05–2.32). The latent classification method of analysis showed that the H1 haplotype was characteristic of Alzheimer's patients, with ages-of-onset between 66 and 80 years. When clinical phenotypes were analyzed, there was a suggestion that the patients with this haplotype may have more behavioral changes and hallucinations. Moreover, both the latent classification analysis and logistic regression analysis indicated that there was no association of the haplotype with either APOE status or gender. The gene is part of a superfamily of cation channels that are involved with calcium entry into cells.

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Late-onset Alzheimer's disease is a complex neurodegenerative disorder manifested by severe cognitive impairment. Extensive research has identified many putative susceptibility genetic variants associated with the disease, using case/control paradigms. Replication has been equivocal. Mutations in the presenilins and the amyloid precursor protein have been identified for early-onset disease, while the apolipoprotein E4 polymorphism (APOE) has been identified as a risk factor that increases susceptibility for the disease [2,5,8,13–15]. Another important risk factor for the disease is family history.

Large pedigrees with late-onset Alzheimer's disease in which those affected are still alive are rare. We identified two of these large families that we used for a genome-wide screen, using the Affymetrix GeneChip® Human Mapping 500K SNP microarrays. We identified a set of SNPs in the gene, transient receptor potential cation channel, subfamily C, member 4 associated protein (TRPC4AP), which were significant after Bonferroni correction. After analyzing an additional panel of SNPs in this gene in each family, we identified a haplotype of 10 SNPs which was significant for the disease in these families [11]. We analyzed the haplotype of nine of these SNPs in our Alzheimer's patients vs. controls to determine its prevalence.

There were 199 patients with Alzheimer's disease (primarily Caucasian; 135 female and 64 male) and 85 control spouse subjects (Caucasian; 54 female and 31 males) used for the haplotype analysis. The age-of-onset for the patients was 71 ± 8 years, with a range of 50–92 years. The reference age for the spouses was 60 ± 17 years, with a range of 50–88 years. The clinical diagnosis of senile dementia of the Alzheimer's type was made according to NINCDS-ADRDA criteria [10]. The medical records were carefully reviewed to verify the progressive cognitive decline and to document appropriate blood work to eliminate other medical conditions, including thyroid and B12 deficiencies. We also included a computed tomography and/or magnetic resonance imaging scan of the brain, which showed cortical atrophy with no evidence of strokes or tumors. The spouses were of similar ages, ethnic background, and environment, which controlled for unmeasured risk factors, as well as age and race. Participants or authorized representatives for the patients gave informed consent for the study, in accordance with the institutional review board guidelines.

Genomic DNA was extracted from blood samples using either proteinase K digestion and chloroform extraction or the Qiagen QIAamp DNA blood midi kits (Qiagen, Inc., Valencia, CA). SNPs were genotyped using fluorescent-detected single base extension with the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA), as described [7]. Nine of the 10 SNPs were genotyped in all of the samples. The SNP (rs6087664) was not easily multiplexed and not used. The nine SNPs in physical order were rs1058003, rs3746430, rs3736802, rs6088677, rs6087660, rs4911460, rs13042358, rs6120816, rs1885119.

Haplotypes were determined for each individual by use of the expectation maximization algorithm (EM), implemented in HelixTree, of which we had the trial version. Haplotype data with EM

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Table 1
Haplotype associations.

	H1*	H2	H3	H4	H5	Others	Total
Patient	143(35.93%)	105(26.38%)	55(13.82%)	33(8.29%)	18(4.52%)	44(11.06%)	398
Control	45(26.47%)	57(33.53%)	32(18.82%)	15(8.82%)	10(5.88%)	11(6.47%)	170

* $P = 0.0282$ and the OR = 1.56 (95%CI: 1.05–2.32).

probabilities greater than 0.88 were exported to SAS for logistic regression analysis to determine the risk associated with each haplotype. Each haplotype was further compared with the combination of the other haplotypes.

The latent classification statistical model, the Grade of Membership (GoM, developed at the Center for Demographic Studies at Duke University) was used to investigate the various clinical phenotypes simultaneously without multiple comparisons [3,6,9,12,16]. The data are represented by model-based groups which are defined by the frequencies of the responses for the variables. Individuals are not assigned to a group. They are assigned a membership score for each group. The internal variable used to define the pure types was the presence of the multilocus genotype. External variables were the clinical phenotypes, which may be encountered during the Alzheimer's disease process: behavior changes, hallucinations, problems with calculations or language, or depression. Age-of-onset was also included as an external variable. The clinical phenotypes were determined either from the initial form completed by the families upon entry into the study or upon examination of the medical records. The data for the multilocus genotypes and clinical phenotypes were analyzed simultaneously. Missing or limited information and small samples sizes can be used without specifying a particular model.

Five major haplotypes with frequencies higher than 5% were estimated for the data. The five haplotypes as read from the forward strand, starting from rs1058003 as listed were:

H1: GTTCTGGGT
H2: ACCTCAACC
H3: ACTCCAGCC
H4: ACCCCAACC
H5: GCCTTGGGT

There were 143 patients (36%) and 45 controls (26%) with the H1 haplotype (Table 1). The H2–H5 haplotypes had lower frequencies in the samples.

When the H1 haplotype was compared with the combination of the other haplotypes by chi-square analysis, the results were significant: $P = 0.0282$ and the OR = 1.56 (95%CI: 1.05–2.32). The standard power for the association analysis was 0.88 [1]. The data obtained from using logistic regression to account for age-of-onset, gender, and APOE4 status for each haplotype were not significant. For example, the significance for APOE4 carriers with the H1 haplotype was $P = 0.3520$; OR = 1.36 (95%CI: 0.71–2.62). The results for APOE4 non-carriers were $P = 0.1980$; OR = 1.45 (95%CI: 0.82–2.55). Thus risk associated with the H1 haplotype appears to be independent of APOE status as well as age-of-onset and gender.

Using the latent classification analysis with the diplotype H1H1 as the internal variable and the data from the Alzheimer's patients, three groups were identified, as expected. (Table 2). There was a distribution of 80 in Group I, 104 in Group II, and 148 in Group III.

Group III had the H1H1 diplotype. Group I was heterozygous, while Group II did not have the H1 haplotype. There is an indication that the Group III patients may have more behavioral changes, as well as psychiatric issues, such as hallucinations. Interestingly, the Group III patients were late-onset, with age-of-onset ranging from 66 to 80 years. Groups I and II had ages-of-onset that were more widespread. When either APOE4 or gender was used as external variables, there was a wide distribution among all three groups, indicating again that the risk associated with this haplotype was independent of gender and APOE status.

Our previous study with two extended pedigrees, each having five siblings affected with late-onset Alzheimer's disease, identified a haplotype in the gene, TRPC4AP, as causative for the disease [11]. Moreover, the genotype was homozygous for these SNPs in the affected siblings. Genotypes for the controls were generally heterozygous or were homozygous for the opposite genotype. We have extended our study to include 199 unrelated patients and 85 control unaffected spouses to determine the prevalence of the disease. We have found that 36% of the patients' haplotypes were H1, while only 26% of the spouse controls have this haplotype. There was no association of the H1 haplotype with either APOE status or gender. This was confirmed by using both logistic regression analysis as well as the latent classification analysis. The latter analysis also indicated that those patients having the H1 haplotype may have more behavioral changes, as well as psychiatric issues. Thus the H1H1 diplotype may indeed be associated with late-onset Alzheimer's disease.

The H1 haplotype is found in one linkage disequilibrium block which contains all of the 19 exons in the TRPC4AP gene. The gene on chromosome 20q11.22 is large, with a length of 90,411 bases, 19 exons, and two alternative transcripts. According to GenBank, there may be 17 spliced variants while AceView lists 20 different mRNAs, indicating the complexity of the gene.

The transient receptor potential cation channels are part of a superfamily with 28 channels. The channels provide calcium ion entry and are involved in the regulation of calcium-dependent cell functions. Recently another study described a second gene,

Table 2
Alzheimer's disease clinical phenotype correlated with TRPC4AP haplotype.

Attributes	Frequency	I	II	III
Diplotype				
H1H1	15.06	8.14	0	100
H1 other	43.98	84.88	0	0
Other	40.96	6.98	100	0
Behavior changes				
Yes	75.30	72.09	75.81	88.89
No	24.70	27.91	24.19	11.11
Hallucinations				
Yes	41.77	38.55	40.68	62.50
No	58.23	61.45	59.32	37.50
Calculation difficulty				
Yes	70.19	70.24	68.33	76.47
No	29.81	29.76	31.67	23.53
Language difficulty				
Yes	69.33	69.88	67.74	72.22
No	30.67	30.12	32.26	27.78
Depression				
Yes	54.09	51.81	55.00	62.50
No	45.91	48.19	45.00	37.50
Age-of-onset				
50–60	11.45	22.09	0.00	0.00
61–65	14.46	18.6	12.90	0.00
66-70	18.07	11.63	20.97	38.89
71–75	22.89	19.77	27.42	22.22
76–80	22.89	19.77	22.58	38.89
>80	10.24	8.14	16.13	0.00

CALHM1, which encodes a transmembrane glycoprotein controlling cytoplasmic calcium levels as well as Aβ levels [4]. CALHM1 is on a different chromosome, 10q24.33. In their large study, it was found that the SNP rs2986017 was significantly associated with the disease. Thus calcium homeostasis may play a major role in the mechanism of Alzheimer's disease and warrants further investigation.

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