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Association studies between common variants in prolyl isomerase *Pin1* and the risk for late-onset Alzheimer's disease

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Abstract

Alzheimer's disease (AD) pathology is associated with two proteins, the microtubule-binding protein tau and the β-amyloid-precursor protein (APP). When tau becomes hyperphosphorylated, it forms neuritic aggregates, called neurofibrillary tangles. APP is cleaved by several enzymes to generate Aβ peptides, which are – depending on their length – more or less amyloidogenic and form senile plaques. Pin1, a peptidyl-propyl *cis/trans*-isomerase, seems to be involved in both pathologies. Pin1 may facilitate dephosphorylation of tau by PP2A phosphatase, while cellular overexpression of Pin1 causes a reduction in the amyloidogenic processing of APP, making this enzyme an interesting target for pharmaceutical intervention. The gene encoding Pin1 maps to 19p13.2, a region previously linked to late-onset Alzheimer's disease (LOAD). Therefore, Pin1 is an excellent positional and functional candidate for LOAD. In this study, we investigated whether common single nucleotide polymorphisms (SNPs) in *Pin1* can influence the risk for developing late-onset Alzheimer's disease. No association was observed with any of six polymorphisms or their resulting haplotypes. A meta-analysis of two promoter SNPs, which combined the data from this study with two previous ones, did not show any association either suggesting that common SNPs in *Pin1* do not increase the risk for LOAD.

Keywords: Pin1; Late-onset Alzheimer's disease; Genotyping

Pin1 is a prototypic member of the family of peptidyl-prolyl isomerases. These enzymes catalyze the *cis–trans* isomerization of peptide bonds N-terminal to specific phospho-Ser/Thr–Pro motifs. Alzheimer's disease (AD) pathology is defined by the presence of two deposits: senile plaques, formed by β-amyloid peptide (Aβ) and neurofibrillary tangles, which are aggregates of hyperphosphorylated tau protein.

Pin 1 binds to tau phosphorylated on pThr231–Pro and induces conformational changes, which dephosphorylate tau and restore its biological function [4]. Pin1 also regulates β-amyloid-precursor protein (APP) processing. Pastorino et al. [7] showed that Pin1 controls APP isomerization at the phosphorylated Thr668–Pro motif, which affects the processing of APP into neurotrophic or toxic cleavage products. Pin1 overexpression reduces Aβ secretion from cell cultures, while knockout of *Pin1* increases Aß secretion. In Pin1$^{-/-}$ mice, there is an age-dependent increase in insoluble Aß42 in the brain [7]. Pin1 also regulates cell-cycle control, cell proliferation and apoptosis and has been linked to risk for certain types of cancer [3].

A region on chromosome 19 has recently been associated with late-onset Alzheimer's disease (LOAD) independently of the apolipoprotein E gene [10]. The gene encoding Pin1 maps to 19p13.2, which makes Pin1 an excellent positional and functional candidate for AD.

To test whether common variants in *Pin1* are a risk factor for LOAD, we genotyped six single nucleotide polymorphisms (SNPs). These SNPs were tagging SNPs chosen from Hapmap (http://www.hapmap.org) or – as in the case of rs2233678 (-842G/C) and rs2233679 (-667C/T) – were used in two previous studies testing *Pin1* promoter polymorphisms for their

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association with AD [1,9]. Segat et al. [9] found an association with –842G/C and with a haplotype of the two SNPs. In contrast, Lambert et al. [1] failed to detect association between these polymorphisms or haplotypes in a large French case-control series. In another Italian study, in which AD cases and controls were screened for sequence variations in *Pin1*, only rare variations were found, which had no effect on the genetic risk for AD [8].

We used Sequenom™ technology to genotype the *Pin1* SNPs in a Caucasian LOAD case-control series from the WashU-ADRC and a case-control series generated by selecting one case per family from our genetic linkage sample [6] and matched controls, also recruited from the WashU-ADRC. The combined sample consists of 750 cases and 658 healthy controls. For the cases, a clinical diagnosis of probable or definite dementia of the Alzheimer's type was made using the NINCDS-ADRDA or similar criteria with minimum age of onset of 60 years [5]. The cases have an average age-of-onset of 75 ± 6.8 years (range 58–99 years) while the average age at last assessment for the controls is 77.1 ± 8.9 years (range 60–104 years). The controls are 60% women and the cases are 69% women. All studies were approved by the Washington University School of Medicine Human Studies Committee and informed consent was obtained from all the subjects.

The primers for the Sequenom genotyping are summarized in Table 1.

APOE genotyping was performed following the protocol on the Pyrosequencing™ website (http://www.pyrosequencing.com/pages/assay_register_clin_gen.html). The combined case-control series had an expected *APOE ε4* allele distribution. Using the program STRUCTURE, we found no evidence of population stratification among the WashU-ADRC case-control data set [2]. Chi square tests were used to test for deviation from Hardy–Weinberg equilibrium and to test for allelic association. A Fisher's exact test was used for the genotypic tests and to stratify for presence or absence of the *APOE4 ε4* allele. Odds ratios and 95% confidence intervals were calculated for allelic associations and haplotypes. Linkage disequilibrium (LD) analysis was carried out on a pair-wise basis for all the SNPs in the control sample using the Haploview program (http://www.broad.mit.edu/mpg/haploview/index.php).

Table 1
Sequenom primers for the six Pin1 SNPs

SNP ID	SEQUENCE
rs1077220m-F	ACGTTGGATGCCAAACAGTTCACCAGAACC
rs1077220m-R	ACGTTGGATGCTCTAGAGCCCATAGCAATC
rs1077220m-snp	CTCAACCAAGGAGAATTATTA
rs2010457-F	ACGTTGGATGTGGGAGCCCAATGCCCAGA
rs2010457-R	ACGTTGGATGAGCAGAGGTGCGCAAGGAAT
rs2010457-snp	CAATGCCCAGACCCTCC
rs2233678-F	ACGTTGGATGCATATAAGAACACGGAGGAG
rs2233678-R	ACGTTGGATGGGGCTCTGCAGACTCTATTT
rs2233678-snp	GTGGGAGGAGATGGGCT
rs2233679-F	ACGTTGGATGTTCCCACAGATGTCCAAAGC
rs2233679-R	ACGTTGGATGGTCCAGAGCCTAGGGAAAAG
rs2233679-snp	CCAGCCTCTTTTATTTTTTGCAG
rs2287838-F	ACGTTGGATGGTAGAGATGATGCCAGGAAG
rs2287838-R	ACGTTGGATGTTCAGAGGCTGCTCTGCTGG
rs2287838-snp	GATGCCAGGAAGAAAGTG
rs2287839-F	ACGTTGGATGTGTTGCTTTGTTGTTGTCTC
rs2287839-R	ACGTTGGATGGTGCAGTGATGACCAAACAG
rs2287839-snp	TTGTTGTTGTCTCTGTATGA

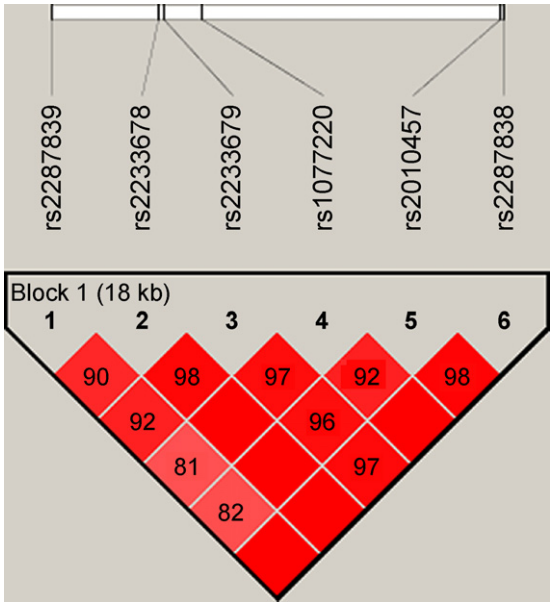

Fig. 1. LD map of the six SNPs genotyped in Pin1 was computed using Haploview. The pairwise *D*'-values were 1.0 except when noted in the cell.

The distribution of allele and genotype frequencies of the six SNPs is shown in Table 2. The frequencies of all SNPs were in Hardy–Weinberg equilibrium in cases and controls. We found no differences in the allelic or genotypic frequencies between cases and controls for any of the six SNPs (Table 2) even after stratifying for presence or absence of the *APOE ε4* allele (data not shown). The SNPs cover the region of the *Pin1* gene, which is 14 kb in size, and around 4 kb 5′ of the first exon of *Pin1*. All the SNPs were in strong LD (Fig. 1). Haplotypes formed by the tagging SNPs from Haploview (rs2287839, rs1077220, rs2010457 and rs2287838) did not show any significant association (global *p*-value 0.4251). The three-SNP haplotype including rs1077220, rs2010457 and rs2287838 resulted in a *p*-value of 0.7555. One SNP (rs2287839) was dropped because inclusion of this SNP resulted in a rare haplotype.

With this study, we confirmed the results from [1]. They studied the two promoter SNPs, rs2233678 and rs2233679, for their potential to influence the risk of developing LOAD in a large French case-control population. Their data suggested that these two Pin1 SNPs didn't make a significant contribution to AD risk. This finding was in contrast to an earlier study from [9]. We combined the data from our study and the two previous studies in a meta-analysis, which resulted in a *p*-value of 0.55 for the genotypic and a *p*-value of 0.60 for the allelic test with rs223678 and in a *p*-value of 0.98 for the genotypic test and a *p*-value of 0.84 for the allelic test with rs223679.

We also sequenced the four exons and approximately 100 bp in the adjacent introns of *Pin1* in 45 individuals. We confirmed the known SNP in exon 2 (rs2233682). We found two SNPs in intron 2 (rs2233683 and a rare polymorphism at chromosome

Table 2
Distribution of allele and genotype frequencies of the six SNPs in *Pin1*

SNP	*n*	Genotype	*n* (%)		Allele *n* (%)		*p*-value genotype (allele)
rs2287839		CC	CG	GG	C	G	
Controls	635	4 (0.6)	73 (11.5)	558 (87.9)	81 (6.4)	1189 (93.6)	0.85 (0.75)
Cases	741	7 (0.9)	85 (11.5)	649 (87.6)	99 (6.7)	1383 (93.3)	
rs2233678		CC	CG	GG	C	G	
Controls	632	6 (0.9)	114 (18)	512 (81)	126 (10)	1138 (90)	0.31 (0.26)
Cases	725	14 (1.9)	136 (18.8)	575 (79.3)	164 (11.3)	1286 (88.7)	
rs2233679		CC	CT	TT	C	T	
Controls	631	73 (11.6)	270 (42.8)	288 (45.6)	416 (33)	845 (67)	0.86 (0.78)
Cases	732	83 (11.3)	324 (44.3)	325 (44.4)	490 (33.5)	966 (66.5)	
rs1077220		CC	CT	TT	C	T	
Controls	621	360 (58.0)	228 (36.7)	33 (5.3)	948 (76.3)	294 (23.7)	0.64 (0.51)
Cases	728	435 (60.3)	249 (34.2)	40 (5.5)	1127 (77.4)	329 (22.6)	
rs2010457		CC	CT	TT	C	T	
Controls	624	71 (11.4)	264 (42.3)	289 (46.3)	406 (32.5)	842 (67.5)	0.94 (0.90)
Cases	712	77 (10.8)	306 (43.0)	329 (46.2)	460 (32.3)	964 (67.7)	
rs2287838		CC	CT	TT	C	T	
Controls	624	146 (23.4)	283 (45.4)	195 (31.3)	575 (46.1)	673 (53.9)	0.38 (0.64)
Cases	726	163 (22.5)	356 (49)	207 (28.5)	682 (47)	770 (53)	

position 9810393 (*C*/*T*, freq = 2%)) and rs2010457, which we had already genotyped.

In our study, none of the six common SNPs in *Pin1*, including the two promoter SNPs, rs223678 and rs223679, was associated with increased LOAD risk. Our study was designed to test whether common variation in Pin1 explains any risk for AD. Therefore, we cannot rule out the possibility that rare variants in *Pin1*, that increase Pin1 expression, might influence the risk for LOAD since this type of association would not be detected by a method that relies on linkage disequilibrium.

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