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Two rare *AKAP9* variants are associated with Alzheimer disease in African Americans

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The authors of this paper have no conflicts of interest to report.

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Abstract

Background—Less is known about the genetic basis of Alzheimer disease (AD) in African Americans (AAs) than in non-Hispanic whites.

Methods—Whole exome sequencing (WES) was performed on seven AA AD cases. Disease association with potentially AD-related variants from WES was assessed in an AA discovery cohort of 422 cases and 394 controls. Replication was sought in an AA sample of 1,037 cases and 1,869 controls from the Alzheimer Disease Genetics Consortium (ADGC).

Results—Forty-four SNPs from WES passed filtering criteria and were successfully genotyped. Nominally significant ($p < 0.05$) association to AD was observed with two African-descent specific *AKAP9* SNPs in tight linkage disequilibrium: rs144662445 ($p = 0.014$) and rs149979685 ($p = 0.037$). These associations were replicated in the ADGC sample (rs144662445: $p = 0.0022$, odds ratio [OR] = 2.75; rs149979685: $p = 0.0022$, OR = 3.61).

Conclusions—Because *AKAP9* was not previously linked to AD risk, this study indicates a potential new disease mechanism.

Keywords

whole-exome sequencing; late-onset Alzheimer disease; rare variant; genetic association; African American; *AKAP9*

1. Background

Late onset Alzheimer disease (AD) is the most common form of dementia in the elderly. It has a substantial genetic component, with heritability as high as 75%[1]. The apolipoprotein E (*APOE*) gene $\epsilon 4$ variant has been shown to be a major component of this risk[2]; among non-Hispanics whites (NHW), $\epsilon 4/\epsilon 4$ homozygotes are estimated to have up to 15-fold increased odds of AD compared to those with the most common $\epsilon 3/\epsilon 3$ genotype[3].

Genome-wide association studies (GWAS) including very large NHW samples have identified genes harboring additional modest-effect AD risk susceptibility loci (odds ratios between 1.1 and 1.3) [4–8].

Interestingly, even though the proportion of risk due to genetic influences is similar in African Americans (AA) and NHWs[9], there is evidence that the genetic architecture of the disease differs between these two populations. For example, the effect of *APOE* $\epsilon 4$ on AD risk is considerably weaker in AAs than NHWs, [3, 10, 11] although this difference may be accounted for in part by the age-dependent effect of $\epsilon 4$ [11]. Several genes associated with AD and AD-related hippocampal atrophy measured by magnetic resonance imaging in NHWs also show evidence of association in AAs, however most SNPs associated with NHWs have not been replicated in AAs[12–14]. While it may be that the AA studies are underpowered compared to studies with NHWs, it also may be due to differences in the linkage disequilibrium (LD) structure between AAs and NHWs or population-specific causal variants underlying the associations. Interestingly, two GWAS replicated the association with *ABCA7* in AAs[12, 14] and the risk associated with *ABCA7* (odds ratio [OR]=1.79) approaches that of *APOE* $\epsilon 4$ in AAs (OR=2.31)[14]. Nevertheless, a large proportion of heritability in AAs remains unexplained. Much of the heritability may be ascribed to uncommon (frequency < 5%) or rare (frequency < 1%) variants which would be undetectable in GWAS except in very large samples[15]. Causal high-penetrant rare variants in *APP*, *PSEN1* and *PSEN2* have been identified primarily in the early-onset familial form of the disease [16]. However, to date no causal variants in these gene have been reported in AAs.

Next-generation sequencing methodologies have the potential to identify rare or private risk variants many of which would be expected to exert moderate to high effects on disease risk compared to most common disease-associated variants[15, 17]. One efficient study design to detect highly penetrant disease variants entails sequencing individuals from families with multiple affected members who are more likely than sporadic cases to possess such variants. Potentially disease-causing variants identified in familial cases can then be genotyped in other family members or in a larger sample of unrelated individuals to evaluate association with disease. This study design was recently used in Caucasian samples to identify rare variants in the phospholipase D3 (*PLD3*) gene[18]. Resequencing *PLD3* exons in a larger number of cases and controls identified additional AD-associated *PLD3* variants including Ala442Ala, which was subsequently shown to be nominally-significantly associated ($p=0.03$) with AD in a modest AA sample (130 cases, 172 controls). In this study, we performed whole-exome sequencing (WES) of 7 familial AA late-onset AD cases and subsequently tested association of AD risk with 44 rare variants selected on the basis of frequency, sequencing quality, and relationship to previously implicated AD genes in 422 AA cases and 394 cognitively normal AA controls. We identified two African-descent specific genetic variants in the A kinase anchor protein 9 (*AKAP9*) gene which are significantly enriched in AA AD cases versus controls, and subsequently replicated this association in a large independent AA cohort. The study design is summarized in Figure 1.

2. Methods

2.1 Study samples

The discovery sample included AA participants of the Multi-Institutional Research on Alzheimer Genetic Epidemiology (MIRAGE) Study and the Genetic and Environmental Risk Factors for Alzheimer Disease among African Americans Study (GenerAAtions). Ascertainment methods, inclusion criteria, and genome-wide genotyping in the MIRAGE and GenerAAtions cohorts are described in detail elsewhere[9, 12]. The MIRAGE cohort consists of nuclear families most of which are discordant sib pairs. There are also several affected sib pairs and larger sibships. Seven MIRAGE-Study AD cases with multiple affected family members, and thus more likely to be enriched with rare AD-related variants, were selected for whole-exome sequencing. Descriptive statistics for these subjects are summarized in Supplementary Table 1. Four of these subjects were affected sib-pairs. The genotypes of single nucleotide polymorphisms (SNPs) identified as potentially AD related in WES were genotyped in 218 cases (including the seven sequenced subjects) and 237 controls from the MIRAGE cohort and 223 cases and 190 controls from the GenerAAtions cohort. Analyses were performed on a discovery cohort made up of the genotyped subjects excluding the seven sequenced AD cases and subjects less than 60 years at the time of assessment. The discovery cohort includes 199 cases and 209 controls from the MIRAGE cohort and 223 cases and 185 controls from the GenerAAtions cohort. Characteristics of the discovery cohort are summarized in Supplementary Table 1. The most significant variants were genotyped in replication cohort comprising a subset of the Alzheimer Disease Genetics Consortium (ADGC) AA sample, excluding the MIRAGE and GenerAAtions cohorts, which contains 1,037 cases and 1,869 controls (Supplementary Table 2). The ADGC AA cohort is described in detail elsewhere[14]. Studies were performed with appropriate institutional review board approval and informed consent was obtained from all study participants.

2.2. Sequencing and Genotyping

A detailed description of whole-exome sequencing, SNP genotyping methods and quality control, and Sanger-sequencing used to confirm genotypes of the most significant SNPs from the analysis of the discovery data is provided in Supplementary Methods.

2.3. Variant Filtering

Variants identified by whole exome sequencing and passing quality control (QC) were filtered for novelty (absence in the dbSNP database v. 132, using time since discovery as a proxy for low frequency because rare SNPs are more likely to be included in later dbSNP revisions) and potential for deleterious effect (must be a non-synonymous base pair change). To filter out possible sequencing artifacts, we additionally required that variants be observed in more than one case, whether or not the variants occurred in siblings or unrelated subjects. The novel non-synonymous variants were selected for genotyping if they were in genes which had been either previously-identified as associated with AD—that is, genes that are listed in the AlzGene database—or in genes belonging to networks with established AD genes. We constructed gene networks using the INGENUITY Pathway Analysis (IPA) software. IPA analyses were seeded with genes emerging from WES and established risk

genes for early or late onset AD (*APOE*, *BINI*, *CLU*, *CRI*, *PICALM*, *MS4A6E*, *CD2AP*, *CD33*, *ABCA7*, *EPHA1*, *SORL1*, *ACE*, *PSEN1*, *PSEN2*, and *APP*) [2, 4–8, 19–25] in order to identify genes and the variants contained therein that might be related physiologically to AD-related processes or are related to genes with a known connection to AD. These networks do not exclusively consist of genes involved in a single AD-related biological function, but instead include genes that interact, either directly or indirectly, with known AD genes and hence may implicate new disease-related pathways.

2.4. Statistical Analysis

Tests of association between genotyped SNPs and AD were performed using R[26]. Significance was determined using a one-sided Fisher's exact test because rare SNPs can cause numerical stability issues in logistic models, and because we specified *a priori* that our search was focused on deleterious rare variants. The test of association within the discovery cohort may underestimate the strength of a true association in the MIRAGE dataset because many of the controls in this sample are unaffected siblings of AD cases who would presumably be carriers of risk alleles at a higher rate than unrelated controls. Although the MIRAGE study ascertained primarily discordant sib-pairs, the sample contained three affected sib-pairs including the two affected sib-pairs in the WES sample. Both members of the sib-pair not included in the WES sample were included in the discovery cohort. The possibility of inflated significance due to non-independence of the samples is unlikely (see the generalized estimating equation [GEE] analysis in the Supplementary Results). Principal components (PC) analysis with EIGENSTRAT[27] based on all minor allele frequency (MAF) >5% markers from a whole-genome genotyping panel (described in [12]) found no evidence of AD-associated population substructure within the discovery sample (all $p > 0.05$ on tests of association between AD and each of the first 10 PCs). In the replication sample, SNPs were analyzed for association with AD using a logistic regression model adjusting for sex and study site. Methods for the haplotype, phylogenetic dendrogram, and bioinformatic analyses (using PolyPhen-2[28], SIFT[29], and MutPred[30] software) are described in Supplementary Methods. Haplotype analyses were performed with PLINK[31] using both whole-exome sequence variant data and tag SNPs from whole-genome genotyping.

3. Results

A total of 88,867 variants were identified by WES. No coding *APP*, *PSEN1*, *PSEN2* or *PLD3* variants were identified in the 7 AA AD cases. After filtering, 63 SNPs from 58 genes were selected for further study and 44 of these SNPs were successfully genotyped in the entire sample. Detailed results of sequencing and variant filtering are presented in the supplementary materials, including a complete list of variants that were genotyped (Supplementary Table 4). The majority of genotyped SNPs were rare (29 SNPs had a $MAF < 1\%$). Nominally significant results were obtained with *AKAP9* SNPs rs144662445 ($p = 0.014$, $OR = 8.4$) and rs149979685 ($p = 0.037$; the OR for rs149979685 could not be estimated because the rare allele was not observed in any controls) (Table 1, Supplementary Table 5). The genotypes for these *AKAP9* SNPs were confirmed by Sanger sequencing. Five AD cases, in addition to the two cases included in WES, were observed with rare alleles for

both rs144662445 and rs149979685 (Supplementary Table 6). Five other subjects (four AD cases and one control) had the minor allele for rs144662445 but not rs149979685. Subjects having at least one of these two rare variants are designated *AKAP9+*. No subjects were observed having the minor allele for rs149979685 but not rs144662445. No rare-allele homozygotes were observed for either SNP. One control subject who was genotyped but not included in the discovery sample due to age—a 54 year-old unaffected sibling of an AD proband (whose age at onset was 64)—had rare alleles for both rs144662445 and rs149979685. The *AKAP9+* subjects are all unrelated according to their self-reported data and identity by descent (IBD) estimates calculated by PLINK[31] using genome-wide SNP data. A PC analysis in EIGENSTRAT[27] indicated that these subjects were not members of a clearly defined population subgroup within AAs (Supplementary Figure 1). *AKAP9* genotypes were available for relatives of four *AKAP9+* AD cases. Two of these cases each had an unaffected sibling who was *AKAP9-*. Each of the other two *AKAP9+* cases who were included in the WES discovery sample had an *AKAP9-* affected sibling.

Next, we sought replication of the association only with the two *AKAP9* SNPs in the ADGC AA sample. Valid genotypes for both SNPs were obtained in 1,037 AD cases and 1,869 controls. In this sample, the average age at onset among AD cases was 80.1 and the average exam age for controls was 74.5. Average ages were similar among *AKAP9+* (79.7) and *AKAP-*(80.1) cases ($p=0.83$) and among *AKAP9+* (75.9) and *AKAP-* (74.5) controls ($p=0.47$). The *APOE* $\epsilon 4$ allele frequency was 33.7% in cases and 18.5% in controls in the replication sample. This does not differ between *AKAP9+* (38.8%) and *AKAP9-* (33.6%) cases ($p=0.59$) or between *AKAP9+* (12.5%) and *AKAP9-* (18.6%) controls ($p=0.50$). A logistic regression model adjusting for age and study site demonstrated significant association of AD risk with the minor alleles of both rs144662445 ($p=0.0022$, $OR=2.75$) and rs149979685 ($p=0.0022$, $OR=3.61$) (Table 2). Hence, the association observed in the discovery data was replicated in a larger independent sample. A logistic regression analysis of the subset of ADGC AA replication subjects who had both *AKAP9* SNP genotypes and genome-wide genotype data (877 cases and 1641 controls) yielded nearly identical results for both SNPs when PCs were included (rs144662445 $p=0.0034$, $OR=2.72$ versus $p=0.0035$, $OR=2.72$ with PCs; 149979685 $p=0.0064$, $OR=3.17$ versus $p=0.0063$, $OR=3.19$ with PCs), indicating that population substructure did not contribute to the confirmation of the *AKAP9* association in the replication sample.

To examine the possibility that an untested AD causal variant in *AKAP9* or surrounding region could account for the observed association, we compared haplotypes of *AKAP9+* and *AKAP9-* individuals in the discovery sample. The 5' end of *AKAP9* overlaps a large LD block which includes the neighboring genes *CYP51A1*, *KRIT1*, *ANKIB1*, and *LRRD1* (see supplementary Figure 2). Haplotype analysis of the 10 common ($MAF>0.05$) *AKAP9* SNPs in the GWAS-genotyping array revealed that all of the *AKAP9+* subjects harbored the same haplotype (designated HAP0). That is, the minor alleles (either as a singleton or together) are present on the haplotype GGAAGGAAGC as defined by rs1859037, rs2299233, rs2282973, rs6465347, rs2158138, rs733957, rs2079082, rs13239875, rs4265, and rs1063243 (eTable 7). The frequency of this haplotype is greater in AD cases (6.1%) compared to unrelated controls (4.1%), but this difference is not quite significant ($p=0.071$).

No other coding variants in this region were observed in the two *AKAP9*+ subjects with WES data through whom this association was ascertained.

Next, we performed phylogenetic dendrogram analysis of the SNP information in the haplotype region among 11 of the 246 African-descent subjects in 1000 Genomes for whom genotypes are available (including 88 Yorubans from Nigeria, 97 Luhans from Kenya, and 61 AAs from the American Southwest) and who had the HAP0 haplotype as defined by the common SNPs. Three of these 11 individuals (NA18499, NA19448, and NA20126) had minor alleles at both rs144662445 and rs149979685, and one individual (NA20298) had only the rs144662445 variant. These *AKAP9* variants were observed in each of the three African-descent groups suggesting that the *AKAP9* variants are present in multiple African ancestral populations. Cluster analysis of the phased alleles for the 22 haplotypes further discriminated the HAP0+ haplotypes from the HAP0- haplotypes (Figure 2A). There is also a branch point separating the HAP0+ haplotype containing one *AKAP9* variant (designated HAP0+1) from the haplotype containing both *AKAP9* variants (HAP0+2).

Based on these results, we examined the 1000 Genomes phased haplotypes for plausible exonic candidate AD-risk SNPs on the same haplotypes as rs144662445 and rs149979685 by comparing the high-risk haplotypes (HAP0+2 and HAP0+1) to the low-risk haplotype (HAP0-). Within the region from 91.3 Mb to 92.1 Mb—which extends well beyond the regions shared across those harboring *AKAP9* mutations (Figure 2B)—the three subjects with HAP0+2 haplotypes shared 69 variants with MAF < 10% that were not observed in subjects with the HAP0- haplotype. Rs144662445 and rs149979685 are the only coding variants in this group of SNPs. Next, we compared the single copy of HAP0+1 from 1000 Genomes (NA20298A) to HAP0-. In the same region, NA20298A contains 49 variants with MAF < 10%, which were not observed on the (low-risk) HAP0- haplotypes, including three coding variants: rs144662445 and two additional non-synonymous coding *AKAP9* SNPs rs186619641 and rs201858518. Rs186619641 and rs201858518 are unique to individual NA20298 in the 1000 Genomes database. Using only 1000 Genomes data we cannot determine whether or not these two additional non-synonymous variants are shared by all subjects in our study with HAP0+1, present in only a portion of subjects with HAP0+1 haplotypes, or unique to 1000 Genomes individual NA20298 who is the only AA subject in 1000 Genomes with one HAP0+1 haplotype. We sought clarification in the ESP database which, based on allele counts of the AD-associated SNPs rs144662445 and rs149979685, contains 3 copies of HAP0+1. These two exonic SNPs (rs186619641 and rs201858518) were absent from the ESP database suggesting that the two AD-associated variants defining HAP0+1 and HAP0+2 are not surrogate markers for rs186619641 and rs201858518. Therefore, based on our examination of HAP0+1 and HAP0+2 in public databases, we conclude that rs144662445 and rs149979685 are the only plausible AD-related coding variants on these background haplotypes within *AKAP9* or the surrounding region.

Bioinformatic analysis using several methods suggests that rs149979685 (S3771L transcript Q99996-1) is likely to be functional whereas rs144662445 (I2558M transcript Q99996-1) is not (Table 3). The MutPred analysis also indicated that the most likely impact of the rs149979685 variant is loss of a helix ($p=0.0033$), but gain of a loop ($p=0.024$) or loss of phosphorylation ($p=0.029$) are also possible. However, since MutPred makes these

predictions based on homology to known functional variants and the 3D structure of *AKAP9* is yet unknown, it is unclear whether these structures are present near rs149979685 or critical to the function of *AKAP9*. The two NA20298-specific non-synonymous SNPs (rs186619641 and rs201858518) were also predicted to be benign according to Polyphen-2.

4. Discussion

In a small WES study of seven familial AD cases, we identified two *AKAP9* SNPs which co-occur on a single uncommon background haplotype (HAP0) spanning *AKAP9* and the surrounding area. These SNPs were significantly enriched in AD cases compared to cognitively normal controls in a large African-American sample, and the associations were replicated in a larger independent AA cohort. These missense variants are rare in African-descent populations (ESP AA sample: $MAF_{rs144662445}=0.43\%$ and $MAF_{rs149979685}=0.36\%$; 1000 Genomes AFR sample: $MAF_{rs144662445}=0.81\%$ and $MAF_{rs149979685}=0.61\%$). They are not present in approximately 4,000 Caucasians in the ESP database or in currently available data for 379 European and 286 East-Asian subjects in the 1000 Genomes database.

Although the estimated OR's are lower in the replication sample compared to the discovery sample (as expected due to 'winner's curse'), the magnitude of effect of these variants on AD risk in the replication sample (estimated ORs of 2.75 and 3.61) is slightly larger than that reported for *APOE* $\epsilon 4$ in AAs (OR=2.31)[14]. Hence, as expected given their frequency, the identified *AKAP9* risk variants not likely to explain a great proportion of AD risk in AAs. However, those inheriting at least one risk allele have a substantially increased risk of AD. Bioinformatic analysis indicated that one of these SNPs (rs149979685) likely has an adverse impact on the structure or post-translational modification of the AKAP protein.

We observed many differences in the 1000 Genomes database between the HAP0 haplotype containing the AD-associated *AKAP9* SNPs and HAP0 haplotypes lacking both mutations, suggesting that the instances of the rare rs149979685 and rs144662445 variants are likely identical by descent, although derived from a very old mutational event. This conclusion is supported by the observation of these variants in subjects of diverse African ancestry and by evidence in our study that subjects sharing these alleles do not appear to be related or from a particular population subgroup based on genome-wide measures of IBD sharing and PC analysis.

AKAP9, located on chromosome 7q21–22, encodes a member of the A kinase anchoring protein (AKAP) family. AKAPs bind or tether protein kinase A (PKA) and other signaling molecules to relevant targets[32]. *AKAP9* is differentially spliced into isoforms which localize to different cellular compartments[32] and are involved in distinct biological processes. *AKAP9* gene products include a short isoform called Yotiao and long isoforms (AKAP350, AKAP450, and CG-NAP) often referred to collectively as AKAP450 based on their molecular weight.

AKAP9 was identified in a cDNA library of human brain where the Yotiao isoform is expressed in the hippocampus, cerebellum, and cerebral cortex[33]. A SNP in Yotiao has been identified as a cause of long-QT syndrome[34], a congenital heart abnormality characterized by long-QT intervals and arrhythmias. Disruption of the binding of PKA and

AKAPs (including AKAP5 and Yotiao) at nerve terminals in the hippocampus has been shown to interfere with cellular mechanisms associated with spatial memory[35]. The locations of rs144662445 and rs149979685 are approximately 38 kb 3' and 61 kb 3', respectively, from the boundaries of the Yotiao coding region. Therefore, these variants probably do not affect the Yotiao isoform, unless they are regulatory or simply in LD with unidentified non-coding risk variants. Hence it is more likely that the AD-associated *AKAP9* variants affect the structure or function of AKAP450. AKAP450 localizes to centrosomes of cells[36]. It is expressed in most tissues including brain. Disrupted binding of AKAP450 to the centrosome interferes with centriole duplication and cell cycle progression[37]. Studies have also shown that AKAP450 is involved in microtubule anchoring and organization at the centrosome[38] and necessary for the initiation of new microtubule formation on the cis-side of the golgi[39]. The non-synonymous change induced by rs144662445 (I2558M transcript Q99996-1) is 5 amino acids from the R2 binding site of AKAP450 (Figure 3). Rs149979685 (S3771L transcript Q99996-1) is located within the pericentrin/AKAP450 centrosomal targeting (PACT) domain which is a highly conserved region near the 3' end of AKAP450 and is the region responsible for the localization of AKAP450 to the centrosome[40] (Figure 3). The role of AKAP450 in AD is unclear, however, it has functional similarity with tau protein which is involved in microtubule stability and assembly and is a key constituent of neurofibrillary tangles which accumulate in AD brain[41–44]. Reports also indicate that phosphorylated tau does not account for all of the microtubule loss and shortening observed in neurons of those with AD[45]. Thus, it is possible that functional defects in AKAP450 may contribute to AD pathogenesis.

These results should be interpreted cautiously. The bioinformatics programs have predicted that rs149979685 has a possible function while rs144662445 does not. However, without functional data and supporting molecular work, we cannot definitively demonstrate that rs149979685 is the causal locus or whether or not rs144662445 is potentially causal due to some other mechanism not accounted for in the software used, or even whether another non-coding regulatory variant in LD with both rs144662445 and rs149979685 is responsible for the observed association with AD. However, examination of the region in 1000 Genomes and the ESP database indicates that such a variant is not likely to be a non-synonymous coding change. Resolution of this question will require functional experimentation. Finally, we acknowledge that our findings do not represent the breadth of possible AD-related rare variation in African Americans because the majority of such variants would not be present in the exons of seven individuals. Thus, for example, it is not surprising that we did not observe previously reported rare AD-risk variants in *PLD3* [18]. It is also possible that several of the non-*AKAP9* variants identified by WES and selected for genotyping are actually true risk loci which the discovery sample was underpowered to confirm by association. Targeted resequencing in both African- and European-descent populations will be necessary to identify the full complement of genetic variants which will provide greater understanding of the role of *AKAP9* in AD pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	African Americans
AD	Alzheimer disease
ADGC	Alzheimer Disease Genetics Consortium
GEE	generalized estimating equation analysis
GenerAAtions	Genetic and Environmental Risk Factors for Alzheimer Disease among African Americans Study
GWAS	Genome-wide association study
IBD	identity by descent
IPA	INGENUITY Pathway Analysis software
LD	linkage disequilibrium
MAF	minor allele frequency
MIRAGE	Multi-Institutional Research on Alzheimer Genetic Epidemiology Study
NHW	non-Hispanics whites
OR	odds ratio
PC	principal components
SNP	single nucleotide polymorphism
WES	whole-exome sequencing

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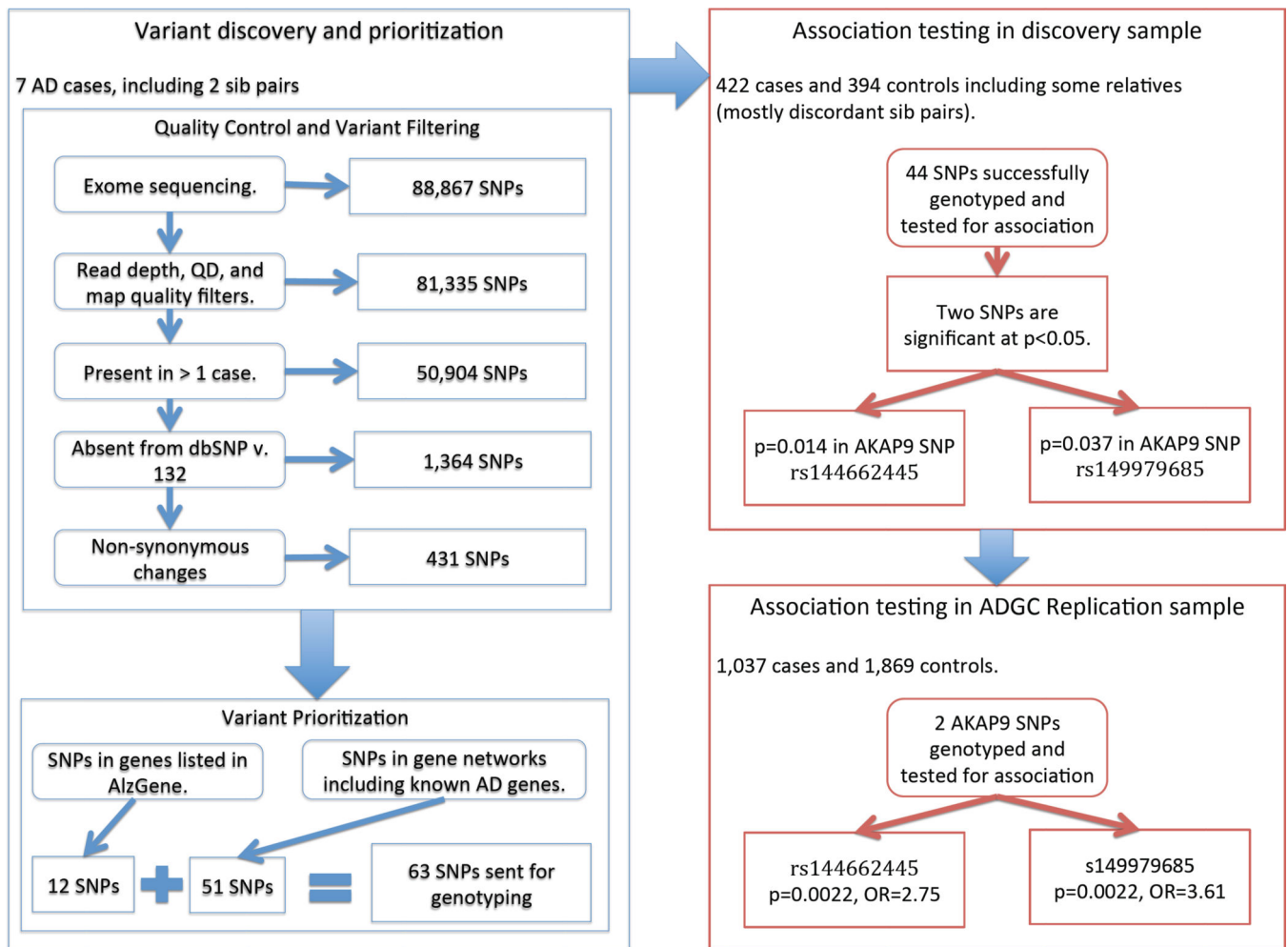


Figure 1. The overall study design including a whole-exome sequencing (WES) of 7 AA late-onset AD cases, followed by variant prioritization and filtering, genotyping in a larger AA discovery sample, and replication in a large AA ADGC cohort.

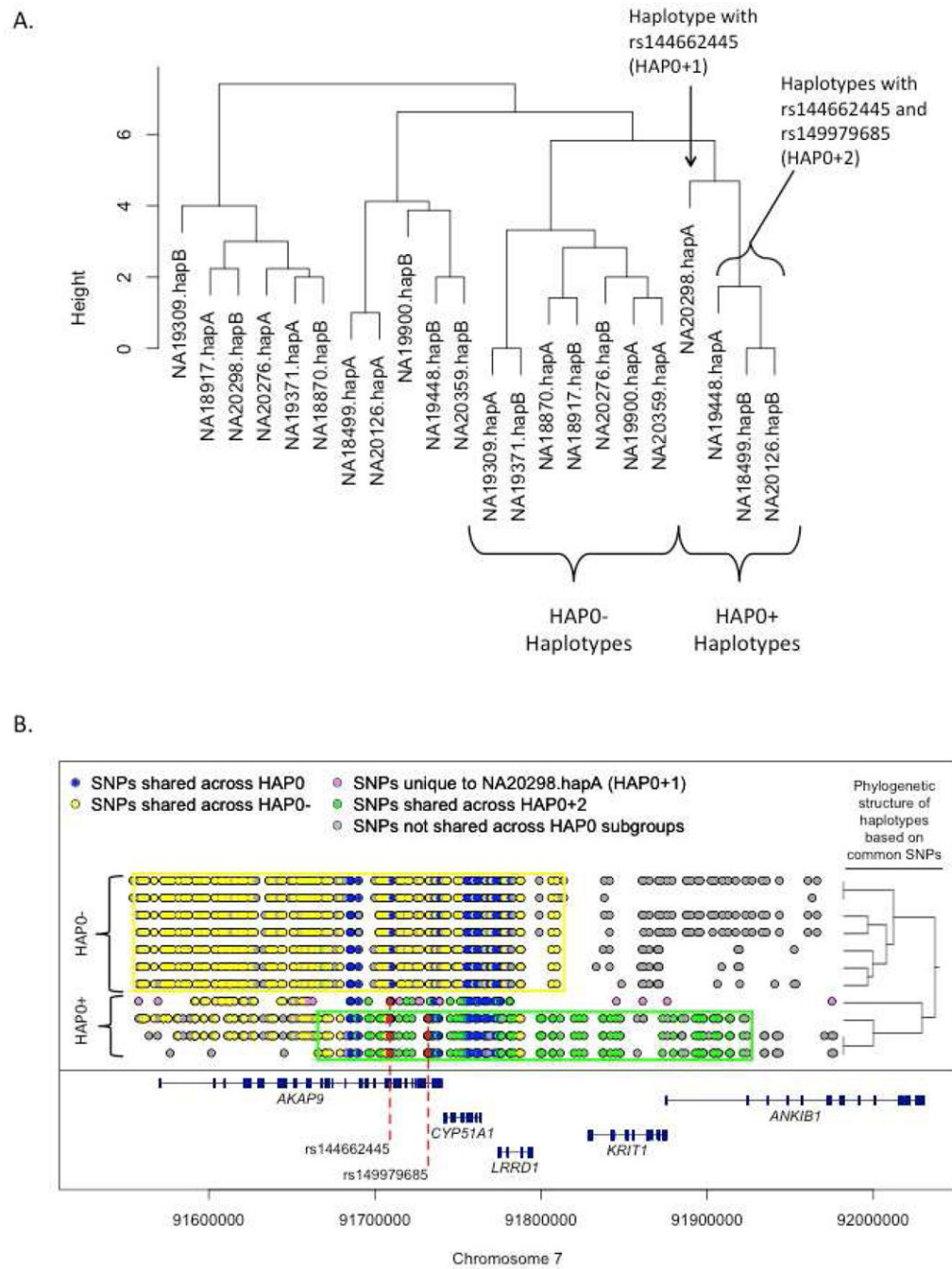


Figure 2. Examination of the 1000 Genomes subjects with the haplotype containing the two identified *AKAP9* variants including (A) a phylogenetic dendrogram of haplotypes and (B) positions of infrequent SNPs (<5% MAF) shared by haplotypes with 0, 1, and 2 variants. Key: HAP0: The background haplotype which contains the two identified *AKAP9* variants. HAP0+ the background variant with one or more of the two putatively AD-associated variants; HAP0- the background haplotype without either AD-associated variants; HAP0+1 the background

haplotype with one of the AD-associated *AKAP9* variants; HAP0+2 the background haplotype with both AD-associated *AKAP9* variants.

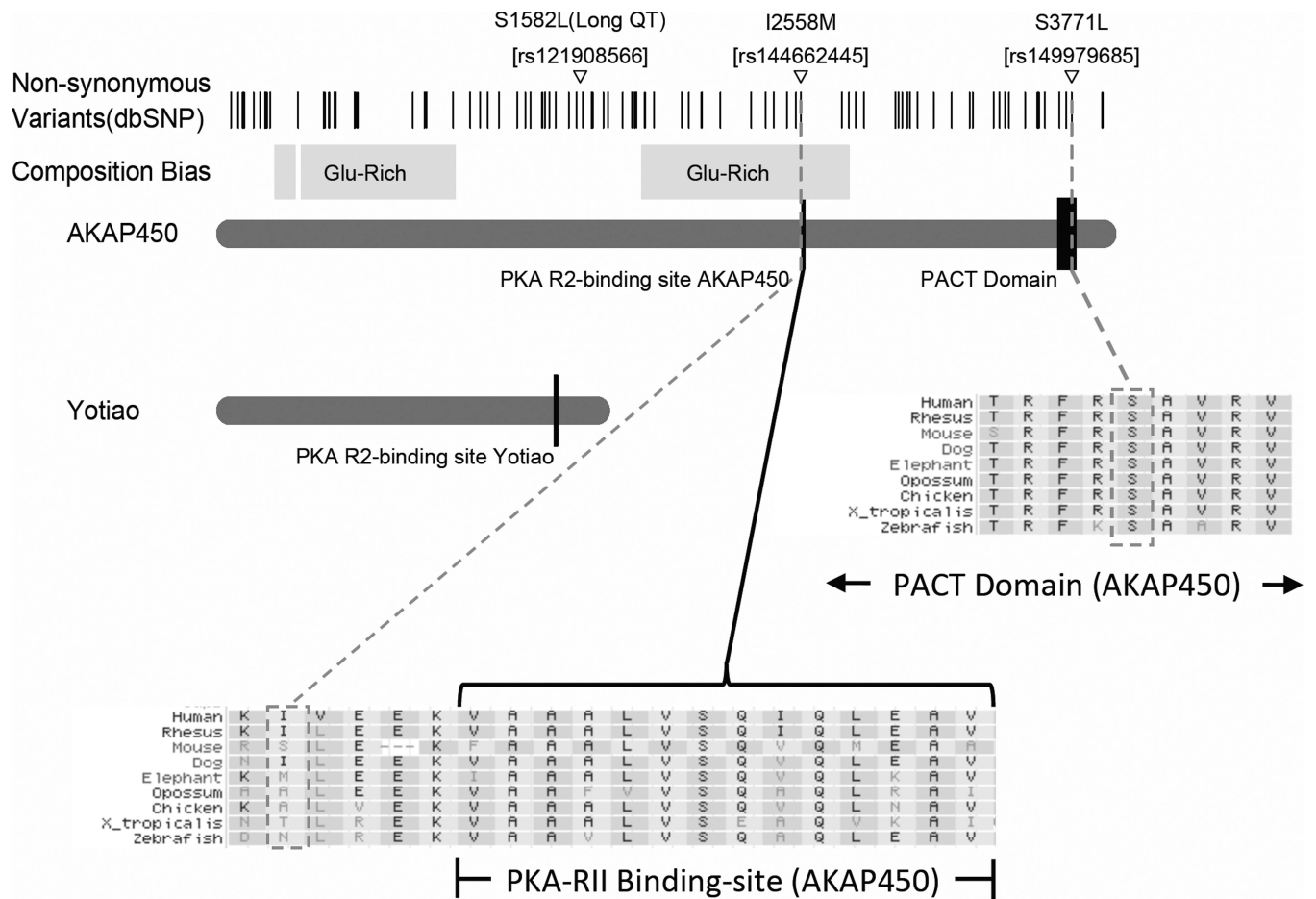


Figure 3. Annotated protein domain of *AKAP9* canonical transcript Q99996-1 including Yotiao; AKAP450; the PKA binding sites; the PACT domain; Long QT syndrome amino-acid change; and the conservation of the two AD-associated variants rs144662445 (I2558M) and rs149979685 (S3771L).

Table 1

The most significant associations between AD and genotyped SNPs in the discovery cohort.

Gene	Position	SNP ID	Freq Cases	Freq Controls	P
<i>AKAP9</i> *	7:91709085	rs1444662445	0.011	0.0013	0.014*
<i>AKAP9</i> *	7:91732110	rs149979685	0.0060	0.000	0.037*
<i>ATP2B4</i>	1:203672867	rs145963279	0.023	0.013	0.092
<i>LRP4</i>	11:46911956	rs138878258	0.0072	0.0026	0.17
<i>ITGA9</i>	3: 37774225	rs142726080	0.032	0.024	0.20

Abbreviations: AD, Alzheimer disease; Freq, frequency; SNP, single-nucleotide polymorphism.

* Genotypes for *AKAP9* SNPs confirmed via Sanger sequencing.

Table 2

The association between *AKAP9* variants and AD using a logistic regression adjusted for sex and study site in the replication cohort.

SNP ID	Frequency (Proportion) of Minor Alleles		Odds Ratio *	95% CI	P
	Cases	Controls			
rs144662445	0.011 (22 [†] /2052)	0.0043 (16/3722)	2.75	1.38–5.61	0.0022
rs149979685	0.0072 (15/1059)	0.0027 (10/3728)	3.61	1.51–9.00	0.0022

Abbreviations: AD, Alzheimer disease; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

* Effect size estimates, CI, and P values were derived from a logistic regression model adjusted for sex and study site.

[†] Includes one AD case who was homozygous for the minor allele

Table 3

Bioinformatic Examination of the Alzheimer disease-associated non-synonymous *AKAP9* variants.

SNP	Polyphen-2		Sift		MutPred	
	Score*	Prediction	Score [†]	Prediction	Probability Deleterious [‡]	Actionable Hypotheses [‡]
rs144662445	0.008	Benign	0.11	Tolerated	21%	NA
rs149979685	0.99	Probably Damaging	0.01	Damaging	57%	Loss of helix (P = 0.0033) Gain of loop (P = 0.024) Loss of phosphorylation (P = 0.029)

Abbreviations: NA, not applicable.

* In Polyphen-2 higher scores indicate that a SNP is more likely to be damaging.

† In SIFT, lower scores indicate that a SNP is more likely to be deleterious.

‡ In MutPred, a higher probability indicates that a is more likely to be damaging.

Actionable hypotheses are predicted functional changes for which the probability deleterious is <50% and for which the structural change P<.05.