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Candidate gene analysis for Alzheimer's disease in adults with Down syndrome

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Abstract

Individuals with Down syndrome (DS) overexpress many genes on chromosome 21 due to trisomy and have high risk of dementia due to the Alzheimer's disease (AD) neuropathology. However, there is a wide range of phenotypic differences (e.g., age at onset of AD, amyloid β levels) among adults with DS, suggesting the importance of factors that modify risk within this particularly vulnerable population, including genotypic variability. Previous genetic studies in the general population have identified multiple genes that are associated with AD. This study examined the contribution of polymorphisms in these genes to the risk of AD in adults with DS ranging from 30 to 78 years of age at study entry (N = 320). We used multiple logistic regressions to estimate the likelihood of AD using single-nucleotide polymorphisms (SNPs) in candidate genes, adjusting for age, sex, race/ethnicity, level of intellectual disability and *APOE* genotype. This study identified multiple SNPs in *APP* and *CST3* that were associated with AD at a gene-wise level empirical *p*-value of 0.05, with odds ratios in the range of 1.5–2. SNPs in *MARK4* were marginally associated with AD. *CST3* and *MARK4* may contribute to our understanding of potential mechanisms where *CST3* may contribute to the amyloid pathway by inhibiting plaque formation, and *MARK4* may contribute to the regulation of the transition between stable and dynamic microtubules.

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Keywords

Down syndrome; Alzheimer disease; Dementia; Gene mapping; Candidate genes; *APP*; *CST3*; *MARK4*

1. Introduction

Adults with Down syndrome (DS) are at high risk of developing Alzheimer's disease (AD) (Schupf, 2002; Zigman, 2013; Zigman and Lott, 2007), and many, but not all, will develop dementia by the end of their seventh decade of life (Lai and Williams, 1989; Zigman, 2013). The neuropathological manifestations of AD in DS have been attributed, at least in part, to triplication and overexpression of the gene for amyloid precursor protein (APP) located on chromosome 21 (Rumble et al., 1989), leading to an increased substrate for production of amyloid β (Aβ) peptides (Mehta et al., 1998; Schupf et al., 2001; Tokuda et al., 1997). Of the two major species of Aβ peptides—Aβ40 and Aβ42—generated by sequential proteolytic cleavage by β and γ secretases of the APP (Selkoe, 2001), lower levels of Aβ42 or the Aβ42/Aβ40 ratio in cerebrospinal fluid along with high levels of tau are associated with high risk of AD (Blennow and Hampel, 2003; Jack et al., 2013). However, even among individuals with full trisomy 21, age at onset of AD varies widely, and levels of Aβ40 and Aβ42 and Aβ42/Aβ40 ratio also vary widely even among individuals who are of comparable age (Coppus et al., 2008; Head et al., 2012; Holland et al., 2000; Lai and Williams, 1989; Schupf, 2002; Zigman et al., 2007).

Genetic as well as environmental factors may contribute to the observed variation in age at onset. Multiple genome-wide association studies (GWAS) and meta-analyses have identified at least 20 genes that are significantly associated with AD in the general population (Bertram et al., 2007; Hollingworth et al., 2011; Lambert et al., 2009, 2013; Lee et al., 2011; Naj et al., 2011; Wijsman et al., 2011). To date, however, only 1 genome-wide study of age at the onset of AD in DS based 67 autopsy samples has been reported (Jones et al., 2013). Several studies have examined the relation between single nucleotide polymorphisms (SNPs) and dementia in adults with DS using a candidate gene approach (Jones et al., 2013; Lee et al., 2007b; Liu et al., 2008; Margallo-Lana et al., 2004; Mok et al., 2014; Patel et al., 2011). In addition, mouse models of DS have identified genes that are differentially expressed between AD and controls (Chrast et al., 2000; Cook et al., 2005; Lyle et al., 2004; Prandini et al., 2007). Compared with individuals without DS, triplication and overexpression of genes that are located on chromosome 21, including *APP* and others, may contribute to AD risk or more general atypical aging in adults with DS. Some of these genes have also been implicated in AD pathogenesis. These include beta amyloid converting enzyme-2 (*BACE2*), superoxide dismutase (*SOD1*), and the astrocyte-derived neurotrophic factor S100 beta (*S100β*). In the present study, we examined SNPs in candidate genes on chromosome 21 as well as a subset of autosomes and chromosome X to determine their contribution to variation in risk for dementia due to AD in a large longitudinal cohort of adults with DS (refer to Supplement Table 1 for a complete list of candidate genes).

2. Materials and methods

2.1. Study participants

We examined 93 individuals with dementia and 227 without dementia for a total of 320 community-residing adults with confirmed DS (Table 1). All individuals were 30 years of age and older at the time of their study enrollment (range 31–78) and resided in New York, Connecticut, New Jersey, or eastern Pennsylvania. Participants were recruited with the help of state and voluntary service provider agencies and were eligible for inclusion in the present study if: (1) a family member or correspondent provided informed consent; (2) he or she either provided consent or assent indicating willingness to participate; and (3) he or she was willing and able to provide blood samples. Recruitment, informed consent, and study procedures were approved by the Institutional Review Boards of the New York State Institute for Basic Research in Developmental Disabilities, Columbia University Medical Center, and the Johns Hopkins University School of Medicine.

2.2. Clinical assessment

Assessments were conducted at the time of study entry and were repeated at intervals of approximately 18 months for up to 5 cycles of follow-up (mean duration of follow-up of 4.5 years; SD = 1.89). Assessments included evaluations of cognition and functional abilities, behavioral/psychiatric conditions, and an examination of medical records for information on health status and medication usage. Cognitive function was evaluated with a test battery designed for use with individuals varying widely in their initial levels of intellectual functioning, as previously described (Silverman et al., 2004). Structured interviews were conducted with caregivers to collect information on adaptive behavior and neuropsychiatric conditions. Past and current medical records were reviewed for all participants.

For diagnostic classification of dementia, recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability were followed (Aylward et al., 1997; Burt and Aylward, 2000). After each assessment cycle, dementia classification was made based on consensus case conferences relying on empirical evidence of stability or decline in performance profiles over time (Silverman et al., 2004). Each individual was classified as: (1) *no dementia*, indicating with reasonable certainty that significant impairment was absent; (2) *MCI-DS,* indicating that there was evidence of mild cognitive or functional decline, but importantly, the observed change did not meet dementia criteria; (3) *possible dementia*, indicating that some signs and symptoms of dementia were present but declines over time was not entirely convincing; and (4) *definite dementia*, indicating with reasonable confidence that dementia was present based on substantial decline over time.

2.3. Selection of candidate genes

Candidate genes (see Supplement Table 1) were selected based on previous reports of positive associations with AD or dementia, either in adults with DS or the general population. These genes included: (1) SNPs that were found to be significant in other genetic studies of DS; (2) the top candidate genes from the ALZGENE database when the customized SNP chips were being developed for this study between 2012 and 2013; and (3)

additional positional candidate genes from published genome-wide linkage and association studies. Due to the limited capacity of the Illumina's GoldenGate platform, only a subset of candidate genes was examined. For candidate regions from genome-wide linkage or association studies where precise genes have not been identified, we used SNAP (http://www.broadinstitute.org/mpg/snap/ldsearch.php) to identify genes within the candidate regions. This process generated 6 candidates on chromosome 21 and 41 genes on other chromosomes. Candidate genes on chromosome 21 included the genes for amyloid precursor protein (*APP*), β amyloid converting enzyme-2 (*BACE2*), the DS critical region-1 (*DSCR1;* also known as *RCAN1*), runt-related transcription factor 1 (*RUNX1*), the astrocyte-derived neurotrophic factor *S100β*, and CU/Zn superoxide dismutase (*SOD-1*). Additional candidate genes were on chromosomes 1, 2, 6–11, 15, 17, 19, 20, and X (see Supplement Table 1 for the full list of genes). Fig. 1 provides an overview of SNP selection and SNP analysis performed in this 2-stage candidate gene study.

2.4. SNP selection

We genotyped each gene with a sufficient number of SNPs to provide relatively dense coverage ($r^2 \sim 0.8$), and selected SNPs that had a relatively high minor allele frequency (>0.15) to increase the information content of each SNP, thereby enhancing statistical power. From these SNPs, we used the TAGGER program (de Bakker, 2009) to identify tag SNPs using the Caucasian samples from the HapMap data set (http://hapmap.ncbi.nlm.nih.gov). To ensure that coverage of the gene was relatively complete, we used SNAP (http://www.broadinstitute.org/mpg/snap/ldsearch.php) to check LD patterns across the genic region. For chromosome 21, 231 SNPS from the 6 genes had a median inter-marker distance of 2185 base pairs. For chromosomes other than 21, we identified 1114 SNPs from 41 genes with a median inter-marker distance of 2552 base pairs. In this article, we present top strands from the Illumina-customized platform.

2.5. SNP genotyping: customized SNP array in trisomic samples

Genomic DNA was genotyped using an Illumina GoldenGate custom array. Clustering and genotype calling of Chromosome 21 SNPs and non-Chr21 SNPs was performed using GenomeStudio genotyping module v1.8 which supports polyploidy loci. For SNPs on chromosome 21, the custom cluster option in GenomeStudio genotyping module v1.8 was used to specify 4 clusters and the custom GType was used to display genotype calls for polyploidy loci (AAA, AAB, ABB, or BBB). All genotype calls were then inspected manually by viewing SNP graph cluster plots (Schupf et al., 2015).

2.6. Quality control (QC) assessment

We included SNPs in the allelic association analysis when the Gencall value, a quality score, exceeded 0.25. This quality score was determined from allele cluster definitions for each SNP as determined by the Illumina GenomeStudio Genotyping Module version 3.0. For chromosome 21, we studied SNPs that produced call rate ≥90% (average call rate 98%) and dropped SNPs with call rate <90% (n = 23) or produced no genotypes (n = 9). For chromosomes other than 21, we dropped SNPs with call rates <98% (n = 11; average call rate: 99%). In addition, we randomly selected 15 samples and genotyped in duplicate. The concordance rates for genotyped SNPs in these samples ranged from 91.8% to 100% for

chromosome 21 SNPs and from 95.2 to 99.6 for nonchromosome 21 SNPs. Further QC assessments using PLINK (Purcell et al., 2007) excluded 15 additional SNPs with the following characteristics: missing genotyping rate >5%; minimum allele frequency <1%; Hardy-Weinberg Equilibrium test at a *p*-value <1E-6. Eventually, we analyzed 231 SNPs on chromosome 21 and 1099 SNPs from chromosomes other than 21.

2.7. Population stratification and covariates

We applied the multidimensional scaling method as implemented in PLINK to adjust for population stratification. Using all available SNPs that survived the QC process, genetic similarity across individuals was estimated by computing identity by state. In addition to our own samples, we included Whites (n = 165), Africans (n = 165), and Asians (170) from the Hapmap database (www.hapmap.org) to ensure proper classification of ethnicity. This analysis generated 3 ethno-racial clusters. These clusters, akin to principal components, were included in the multivariate model as covariates. For multivariable models, we adjusted for the following potential confounders: age, sex, level of intellectual disability (mild to moderate [IQ 35–70] vs. severe to profound [IQ <35]), ethno-racial clusters from population stratification analysis, and the presence or absence of an *APOE ε*4 allele.

2.8. Statistical analyses

To minimize type-1 error rate from multiple testing, we conducted a 2-stage analysis (Fig. 1). In stage 1, we selected tag SNPs to achieve an r^2 of 0.3 or below (variance inflation factor of 1.43) using the PLINK algorithm. We then applied a multivariable logistic regression model to examine the association between an SNP and AD, adjusting for confounders. An additive dosage model was used where we compared the risk associated with having none versus 1 versus 2 copies of the SNP. SNP-wise empirical *p*-value was estimated based on 10,000 replicates. In stage 2, to fine map the genes that had at least one SNP with SNP-wise empirical *p*-value <0.05, we then used all genotyped SNPs within the gene for further evaluation using the same logistic regression model. To take into account multiple SNP testing within any given gene, we applied the false discovery rate approach proposed by Benjamini and Yekutieli (Benjamini and Yekutieli, 2001) and computed gene-wise empirical *p*-value. The rationale for the false discovery rate approach was that: (1) the main goal of the present study is to confirm candidate genes for AD or dementia in adults with DS; thus, multiple testing correction at the level of each gene is reasonable and (2) even though this is the largest fine mapping study in DS to date, sample size is still relatively small. For the two most promising non-chromosome 21 genes (the loci that had more than one SNP with marginal significance [$p < 0.05$]), we performed sliding window haplotype analysis using 3 SNPs at a time to identify haplotypes that may contain functional variants within a gene. R statistical package (http://www.r-project.org/) was used for analysis.

3. Results

3.1. Demographic and clinical characteristics

The average age of the 320 study participants at the time of baseline was 49.9 year old (SD = 7.6), and the mean age of adults at the baseline for adults with dementia was 7 years older than those without (Table 1). The majority of the individuals had mild to moderate

intellectual disability. Ethnicity for over 90% of the study participants was reported in the medical charts as non-Hispanic White, and the allele frequency of the *APOE ε4* (11.6%) was comparable to other populations of Caucasian ancestry (Table 1).

3.2. Stage 1 screening analysis

In stage 1, we screened genes on chromosome 21 using a set of tag SNPs. Because these tag SNPs represent a group of SNPs in the chromosomal region with high linkage disequilibrium, they reduced the burden of multiple testing. Only *APP* and *RUNX1* had SNPs that had empirical *p*-values <0.05 (Table 2). rs17588612 in *APP* had an SNP-wise empirical *p*-value of 0.0126, and rs4816501 and rs13046934 in *RUNX1* had SNP-wise empirical *p*-values of 0.0308 and 0.0081, respectively. In stage 1, screening of genes on chromosomes other than 21, the following genes had one or more SNPs with a significant association at empirical $p < 0.05$: *MSRA* (empirical $p = 0.0230$), *DAPK1* (empirical $p = 0.0474$), *PITRM1* (empirical $p = 0.0498$), *SORCS1* ($0.0230 <$ empirical $p < 0.0469$), *SORL1* (empirical $p = 0.0387$), *TNK1* (empirical $p = 0.0477$), *LDLR* (empirical $p = 0.0442$), *ZNF224* (empirical $p = 0.0275$), *MARK4* (empirical $p < 0.0298$), and *CST3* (empirical $p = 0.0094$) (Table 3).

3.3. Stage 2 fine mapping analysis

For the genes that were significant in stage 1, we fine mapped the genes using all genotyped SNPs to better localize SNPs that are associated with AD (Tables 2 and 3). In the stage 2 analysis of chromosome 21, we computed a gene-wise empirical *p*-value to correct for multiple SNPs within the gene (Table 2). When this more rigorous correction was applied in stage 2, 18 SNPs in the *APP* gene, located within 100kb, had gene-wise empirical *p*-values that reached a threshold of $p \leq 0.05$, and odds ratios (ORs) for minor allele for these SNPs ranged from 2.04 (rs2070654) to 1.49 (rs2830066). Three minor alleles for the associated SNPs, namely rs2830036, rs1041420, rs2830048, were protective (OR <1). For genes on chromosomes other than 21, we identified 5 SNPs in the *CST3* gene that had gene-wise empirical *p*-value <0.05 (Table 3), and ORs for those SNPs ranged from 1.97 to 1.75. On the other hand, for the *MARK4* gene, 3 SNPs barely missed gene-wise empirical *p*-value of 0.05 (rs12976518, $p = 0.0534$; rs2377324, $p = 0.0534$; rs2306660, $p = 0.0715$). The minor allele for rs12976518 was putative (OR = 1.64), whereas the minor alleles for rs2377324 (OR = 0.55) and rs2306660 (OR = 0.60) were protective.

3.4. Haplotype analysis

To better localize the SNP signals and to potentially guide our future sequencing efforts for the 2 candidate genes that are located on chromosomes other than 21, we performed a 3-mer sliding window haplotype analysis for the two most promising candidate genes: *CST3* and *MARK4* (Tables 4 and 5; linkage disequilibrium patterns for SNPs in *CST3* and *MARK4* are shown in Supplement Fig. 1). For *CST3*, we examined the region containing rs2424577 to rs2405367 and found the strongest evidence in a contiguous 3-mer haplotype G-G-G for rs3787498-rs3827142-rs5030707 ($p = 0.00281$) to 3-mer haplotype G-A-A for rs2424582-rs2254635-rs2405367 ($p = 0.00884$). All associated haplotypes in this region were risk haplotypes. For *MARK4*, the strongest evidence was observed for haplotype A-A-G for rs12976518-rs10445572-rs2377324 ($p = 0.00796$) and haplotype A-G-A for rs10445572-

rs2377324-rs2240672 (p = 0.00924). For APP on chromosome 21, we are currently working to develop an algorithm to generate robust haplotypes for trisomy.

3.4.1. Comparison with the general population—When we compared allelic association of the significant candidate SNPs from our study of DS against those in the general population, we observed 3 SNPs—rs2830066 (p = 0.003) and rs2830088 (p = 0.033) in the *APP* gene, and rs2377324 in the *MARK4* gene (p = 0.023)—that had p-values <0.05 in a large GWAS study of adults without DS (Lambert et al., 2013). In addition, rs9423705 (p = 0.074) in the *PITRM1* gene and rs2306660 (p = 0.096) in the *MARK4* were weakly associated with AD. For this purpose, we used the first stage meta-GWAS data (Lambert et al., 2013) (n = 54,167).

4. Discussion

The present study confirmed that SNPs in *APP* and *CST3* were significantly associated with AD risk for adults with DS, whereas those in *MARK4* were suggestively associated; further, 2 SNPs in *APP* and 1 SNP in *MARK4* were also found to be associated with AD in a large-scale GWAS of adults without DS. Our results extend previous findings of a relationship between SNPs in candidate genes located on chromosome 21 and chromosomes other than 21 and risk of AD in adults with DS (Jones et al., 2013; Lee et al., 2007a; Liu et al., 2008; Margallo-Lana et al., 2004; Mok et al., 2014; Patel et al., 2011; Wegiel et al., 2008, 2011). Our analysis revealed that multiple SNPs in *APP* within a 100kb region on 21q21.3 were associated with AD in adults with DS at gene-wise level. Some of the minor alleles were risk alleles, whereas others were protective alleles. Beyond *APP*, this study reports significant allelic association for SNPs in *CST3*, suggesting potential contributions through vascular factors in adults with DS; and also reports suggestive allelic association for SNPs in *MARK4*, suggesting interaction between excess levels of Aβ peptides and tau.

To date, several studies have examined the role of genes on AD or age at onset of AD in adults with DS. Among genes on chromosome 21, a tetratnucleotide repeat in intron 7 on *APP* (Jones et al., 2010), SNPs on *BACE2* (Mok et al., 2014), and an SNP on *RUNX1* (Patel et al., 2011) have been associated with earlier age at the onset of AD, whereas among nonchromosome 21 candidate genes, SNPs in *APOE, SORL1, BACE1, ALDH18A1* (Lai et al., 1999; Lee et al., 2007b; Patel et al., 2011; Prasher et al., 2008; Schupf et al., 1998) and *PIC-ALM* (Jones et al., 2013) have also been associated with earlier age at onset of AD. Subsequently, Patel et al. (2014) examined the relation of 9 GWAS-derived SNPs with risk of AD in DS, including SNPs in *CR1*, *BIN1, CD2AP, EPHA1, CLU MS4A6A/4A, PICALM, ABCA7,* and *CD33* but found no significant relationship to dementia in adults with DS. Below we discuss 3 genes (*APP, CST3,* and *MARK4*) that had the most promising signals for genetic association in adults with DS, plus *PICALM,* which was previously reported to be associated with age at the onset in the only genome-wide study in autopsy samples from individuals with DS.

4.1. APP

Even though mutations in *APP* are among mutations in 3 genes that are known to cause AD, along with *PSEN1* and *PSEN2*, only a few variants have been implicated in population-

based studies of late onset AD (LOAD; Hindorff et al., 2014; Welter et al., 2014). Guyant-Marechal et al. (2007) found and replicated 1 variant rs463946 located at 27,546,187 bp that are somewhat proximal to the SNP signals in the present study, whereas Margallo-Lana et al. (2004) reported that individuals with 3 tetranucleotide repeats on intron 7 of the *APP* gene had significantly earlier age at the onset than those who did not, independent of *APOE.* Subsequently, Jones et al. (2010) confirmed the finding by Margallo-Lana et al. in a larger cohort of adults with DS. In 2002, Athan et al. (2002) reported +37G/C polymorphisms in the promotor region of the *APP* gene were associated with increased risk of AD. Jonsson et al. (2012) reported that rs63750847, a rare Icelandic mutation with allele frequency <1%, protects against age-related cognitive losses, but this finding has not yet been confirmed in other independent studies. To evaluate the relevance of these 2 previously reported loci, we will need additional genotyping of the 2 loci; however, it is unlikely that the present study of primarily US Caucasians will have sufficient number of carriers, since 2 large publicly available data sets (i.e., ADGC and IGAP) did not observe any individual with this coding mutation.

4.2. CST3

This gene codes for the cystatin c protein and colocalizes with Aβ in vascular walls and in senile plaque cores in the brains of individuals with AD as well as in adults with DS. Even though cystatin c protein binds with Aβ, it is reported to prevent oligomerization, and formation of fibrils in vitro (Kaur and Levy, 2012). Cystatin c protein plays the role of an inhibitor by suppressing the production of cathepsins B and D, where low levels of cathepsins were associated with reduced neuronal damages. Thus, cystatic c may rescue degenerating neurons (Kaur and Levy, 2012; Kaur et al., 2010). This idea was supported by an in vivo knockout mice experiment that showed deletion of cystatin c in knockout mice resulted in an elevated cathepsin B activity, leading to greater neuronal damages (Sun et al., 2008). Some have suggested that low levels of cystatin c protein may be a risk factor for AD (Gauthier et al., 2011).

The findings from genetic studies have been inconsistent in that allelic association between candidate SNPs in *CST3* and AD differs by ethnicity. A meta-analysis of SNPs in *CST3* using German, US, and European cohorts showed that the minor allele in rs5030707 was associated with an increased risk for AD (OR = 1.28) (Dodel et al., 2002; Finckh et al., 2000). In a separate study, a homozygous missense varint (G allele) in *CST3* was associated with both AD and ALS. Among Asian cohorts, however, the relationship between AD and SNPs in *CST3* was equivocal (Chuo et al., 2007; Maruyama et al., 2001; Wang et al., 2008). Further studies are needed to better understand the differences in allelic association for SNPs in *CST3* among different ethnic groups as these ethnic groups will differ in their genetic background as well as the distribution of environmental risk factors.

4.3. MARK4

Studies have reported that *MARK4* may be involved in early tau phosphorylation and/or may play a critical role for the *PAR1/MARK*-tau axis in mediating the toxic effects of Aβ on synapses and dendritic spines (Lund et al., 2014; Yu et al., 2012). The observed association between SNPs on *MARK4* and AD risk in adults with DS supports the possibility that a

variant in *MARK4* may influence this process. However, the potential mechanism underlying this 2-hit model is not well studied. Interestingly, the GWAS by Naj et al. (2011) observed association between AD and SNPs located in the *EXOC2L3,* and *MARK4* region, but they dismissed the association because it no longer was observed when adjustment was made for *APOE ε4.* However, it is possible that this gene is a significant modifier only in the presence of elevated levels of Aβ, as in the case of *APOE ε4* or in adults with DS with trisomy, consistent with the finding of the association between SNPs in *MARK4* and AD for adults with DS after adjusting for *APOE ε4*.

4.4. PICALM

We examined *PICALM,* implicated in a GWAS employing autopsy samples of adults with DS (Jones et al., 2013) and in large-scale genome studies (Harold et al., 2009; Lambert et al., 2013; Naj et al., 2011), using 40 SNPs. Based on our more conservative gene-wise empirical *p*-values, the SNPs (rs2888903, rs7941541, rs10751134 and rs561655) that were associated with AD in the general population, and an earlier age at onset in the DS population (Jones et al., 2013) were not significantly associated with AD in the present study of adults with DS.

4.5. Adults with versus without DS

Of the genes identified from the large-scale international genome-wide association studies in the general population (Lambert et al., 2013), we examined 9, but did not observe significant association.

Our study has several limitations. First, even though this is the largest gene mapping study of AD in adults with DS to date, this study is limited by a relatively small sample size, and lacked sufficient power for SNPs with low allele frequencies and those with small effect sizes. Despite this limitation, this study identified multiple SNPs with modest risk ratios at the gene-wise level, and most likely, the positive findings may be owed to studying a high-risk group that is known for early onset of AD and high levels of Aβ peptides. Second, this study examined the relation between AD and intronic SNPs, which serve as genetic markers to better localize the chromosomal location. Thus, further sequencing of these loci is needed to identify genetic variants that contribute to physiological alterations. Third, we interrogated known AD genes in adults with DS. Therefore, this candidate gene study was not designed to identify novel genes/variants, but rather, to better characterize the role of the SNPs in genes that are already shown to be associated with increased AD risk in adults with DS or in individuals with high levels of Aβ. Finally, study participants without AD were, on average, 7.7 years younger than those who had AD. It is possible that the effect sizes associated with the significant SNPs might be reduced if unaffected individuals were followed for a longer period of time. However, given that our multivariate model had adjusted for age and other potential confounders, the effect of age difference on allelic association would be limited.

In short, the present study reports SNPs in *APP, CST3*, and *MARK4* that are associated with elevated risk of AD in adults with DS. This study illustrates that genetic factors that contribute to AD in the general population are likely to play a similar role in adults with DS,

and a genetic study of AD in adults with DS can provide additional insight into the mechanisms for AD as these 2 genes may provide a new understanding for the role of cardiovascular factors and tau in AD. For these genes, further studies of adults with DS are needed to identify functionally relevant variants through extensive sequencing of exons and introns, and to evaluate how these variants may influence levels of gene expression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2017.04.018.

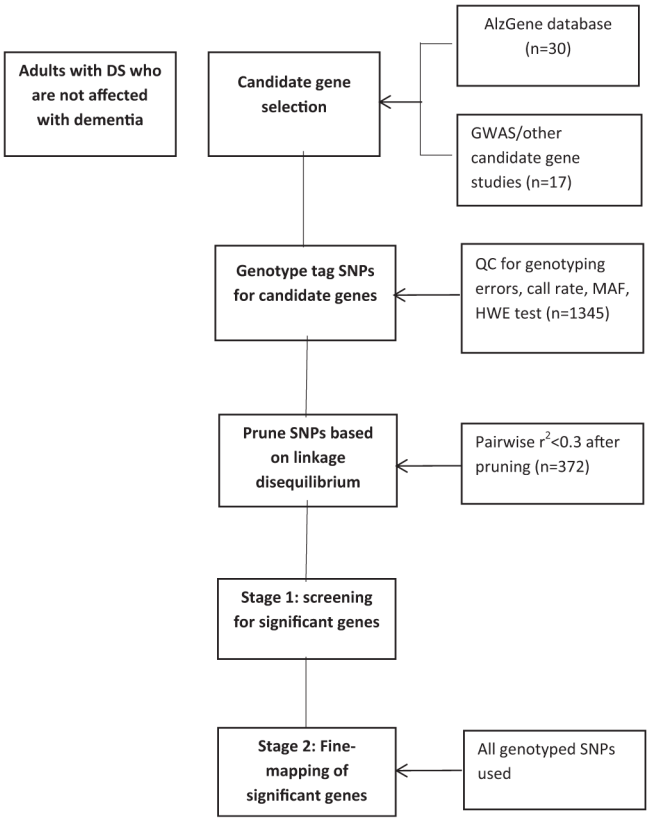

Fig. 1.
Flow chart for a 2-stage candidate gene study of Alzheimer's disease.

Table 1

Characteristics of the study participants

Characteristics	Combined	Dementia	No dementia
Number of individuals	320	93	227
Mean age at baseline (SD)	49.9 (7.58)	55.4 (7.28)	47.7 (6.48)
Level of intellectual disability (n, %)			
Mild/moderate	186 (58.1)	47 (50.5)	139 (61.2)
Severe/profound	134 (42.9)	46 (49.5)	88 (38.8)
Ethnicity (n, %)			
White	294 (91.9)	87 (93.5)	207 (91.2)
Non-White	26 (8.1)	6 (6.5)	20 (8.8)
APOE allele frequency[a]			
ε2	0.077	0.065	0.082
ε3	0.807	0.801	0.809
ε4	0.116	0.134	0.109
Sex (n, %)			
Female	235 (73.4)	65 (69.9)	170 (74.9)
Male	85 (26.6)	28 (30.1)	57 (25.1)

[a]Two subjects missing APOE status.

Table 2

Chromosome 21 SNPs that are associated with Alzheimer's disease at empirical $p < 0.05$

Chr	Gene	SNP[a]	BP (Hg19)	Risk allele	MAF	OR	OR_L95	OR_U95	Empirical *p* (pointwise)[b]	BH adjusted empirical *p* (genewise)
21	**APP**	**rs3991**	**27,428,256**	**T**	**0.236**	**1.893**	**1.384**	**2.618**	**0.0001**	**0.0031**
21	**APP**	**rs2830031**	**27,429,317**	**C**	**0.314**	**1.702**	**1.244**	**2.356**	**0.0010**	**0.0122**
21	**APP**	**rs2830033**	**27,430,925**	**A**	**0.314**	**1.763**	**1.280**	**2.458**	**0.0004**	**0.0081**
21	**APP**	**rs2830036**	**27,435,525**	**A**	**0.182**	**0.558**	**0.365**	**0.829**	**0.0050**	**0.0235**
21	**APP**	**rs1041420**	**27,443,650**	**A**	**0.221**	**0.554**	**0.371**	**0.804**	**0.0028**	**0.0203**
21	**APP**	**rs2830048**	**27,459,674**	**C**	**0.339**	**0.673**	**0.490**	**0.910**	**0.0135**	**0.0484**
21	**APP**	**rs2070654**	**27,462,727**	**T**	**0.298**	**2.044**	**1.439**	**2.948**	**0.0000**	**0.0000**
21	**APP**	**rs2830050**	**27,464,270**	**T**	**0.252**	**1.590**	**1.157**	**2.198**	**0.0046**	**0.0234**
21	**APP**	**rs2830054**	**27,476,104**	**G**	**0.407**	**1.564**	**1.156**	**2.137**	**0.0030**	**0.0203**
21	**APP**	**rs2830066**	**27,494,202**	**C**	**0.496**	**1.488**	**1.122**	**1.993**	**0.0063**	**0.0275**
21	**APP**	**rs2830076**	**27,502,468**	**A**	**0.357**	**1.541**	**1.143**	**2.093**	**0.0044**	**0.0234**
21	**APP**	**rs2830086**	**27,512,956**	**T**	**0.325**	**1.612**	**1.194**	**2.193**	**0.0018**	**0.0183**
21	**APP**	**rs2830088**	**27,514,740**	**C**	**0.487**	**1.433**	**1.085**	**1.909**	**0.0118**	**0.0480**
21	**APP**[c]	**rs17588612**	**27,517,203**	**C**	**0.082**	**1.766**	**1.124**	**2.794**	**0.0126**	**0.0480**
21	**APP**	**rs17588612**	**27,517,203**	**C**	**0.082**	**1.766**	**1.124**	**2.794**	**0.0126**	**0.0480**
21	**APP**	**rs13049230**	**27,521,417**	**G**	**0.318**	**1.550**	**1.149**	**2.104**	**0.0039**	**0.0234**
21	**APP**	**rs2830099**	**27,530,610**	**C**	**0.319**	**1.586**	**1.174**	**2.159**	**0.0023**	**0.0200**
21	**APP**	**rs2830100**	**27,533,329**	**T**	**0.334**	**1.658**	**1.228**	**2.261**	**0.0010**	**0.0122**
21	RUNX1	rs4816501	36,294,539	A	0.251	1.369	1.028	1.825	0.0308	0.4980
21	RUNX1	rs13046934	36,371,207	T	0.215	1.592	1.134	2.245	0.0081	0.4980

Key: MAF, minor allele frequency; OR, odds ratio; SNPs, single nucleotide polymorphisms.

[a] SNPs with gene-wise FDR-adjusted empirical *p*-value <0.05 are in boldface.

[b] For empirical *p*-value, 10,000 replicates were generated.

[c] Positive SNPs from stage 1 are highlighted in gray. SNPs without the gray highlight were added in stage 2 for fine mapping.

Table 3

Nonchromosome 21 SNPs that are associated with Alzheimer's disease at empirical $p < 0.05$

Chr	Gene	SNP[a]	BP (Hg19)	Risk allele	Allele freq	OR	(L95-U95)		Empirical *p* (pointwise)[b]	BH adjusted empirical *p* (genewise)
8	MSRA[c]	rs17692624	10,070,368	C	0.233	0.589	0.374	0.927	0.0230	0.8470
9	DAPK1	rs2058882	90,114,746	G	0.2	1.579	1.003	2.486	0.0474	0.9415
10	PITRM1	rs9423705	3,200,155	G	0.339	1.440	1.002	2.070	0.0498	0.4050
10	SORCS1	rs10748921	108,390,100	C	0.333	0.648	0.424	0.990	0.0469	0.5532
10	SORCS1	rs1538417	108,583,599	A	0.283	1.536	1.025	2.301	0.0361	0.5532
10	SORCS1	rs10787011	108,863,228	G	0.359	1.482	1.022	2.150	0.0371	0.5532
10	SORCS1	rs7897974	108,893,522	A	0.458	0.685	0.475	0.988	0.0426	0.5532
10	SORCS1	rs7099998	108,928,558	G	0.2	0.566	0.345	0.929	0.0230	0.5532
11	SORL1	rs11605969	121,430,872	A	0.153	1.683	1.032	2.747	0.0387	0.8816
17	TNK1	rs7219773	7,283,144	A	0.42	1.549	1.065	2.251	0.0257	0.0596
17	TNK1	rs12948090	7,285,104	A	0.416	1.458	1.013	2.098	0.0442	0.0596
17	TNK1	rs1554948	7,286,326	A	0.413	1.604	1.106	2.327	0.0135	0.0596
17	TNK1	rs3744549	7,293,715	G	0.251	0.655	0.429	0.998	0.0477	0.0596
19	LDLR	rs2738466	11,242,765	G	0.423	1.537	1.010	2.341	0.0442	0.2063
19	ZNF224	rs4803675	44,589,716	G	0.388	1.522	1.054	2.198	0.0275	0.1925
19	MARK4	rs12976518	45,759,344	G	0.475	1.636	1.141	2.347	0.0089	0.0534
19	MARK4	rs2377324	45,768,946	G	0.282	0.551	0.355	0.854	0.0089	0.0534
19	MARK4	rs2306660	45,802,863	G	0.245	0.599	0.378	0.948	0.0298	0.0715
20	**CST3**	**rs35610040**	**23,616,469**	**G**	**0.179**	**1.774**	**1.106**	**2.844**	**0.0165**	**0.0297**
20	**CST3**	**rs3787498**	**23,616,781**	**A**	**0.172**	**1.909**	**1.180**	**3.086**	**0.0085**	**0.0211**
20	**CST3**	**rs3827142**	**23,617,007**	**A**	**0.179**	**1.907**	**1.182**	**3.078**	**0.0079**	**0.0211**
20	**CST3**	**rs5030707**	**23,618,656**	**C**	**0.167**	**1.974**	**1.230**	**3.167**	**0.0049**	**0.0211**
20	**CST3**	**rs3827143**	**23,619,617**	**G**	**0.226**	**1.748**	**1.141**	**2.677**	**0.0094**	**0.0211**
20	**CST3**	**rs2254635**	**23,622,758**	**A**	**0.203**	**1.655**	**1.043**	**2.626**	**0.0316**	**0.0406**
20	**CST3**	**rs2405367**	**23,622,880**	**A**	**0.189**	**1.703**	**1.080**	**2.686**	**0.0200**	**0.0300**

[a] SNPs with gene-wise FDR adjusted empirical *p*-value <0.05 are in boldface.

[b] For empirical *p*-value, 10,000 replicates were generated.

[c]Positive SNPs from stage 1 are highlighted in gray. SNPs without the gray highlight were added in stage 2 for fine mapping.

Table 4

Haplotypes analysis for CST3 and Alzheimer's disease: 3-SNP window

SNP set[a]			Omnibus		Haplotype						
1	2	3	STAT	*p*	Haplotype			Frequency	OR	STAT	*p*
rs2424577	rs35610040	rs3787498	7.170	0.02780	G	G	A	0.172	1.960	7.500	0.00617
					G	A	G	0.196	0.758	1.290	0.25600
					A	A	G	0.625	0.815	1.200	0.27300
rs35610040	rs3787498	***rs3827142***	6.330	0.01190	G	A	A	0.172	1.960	7.500	0.00617
					A	G	G	0.818	0.544	6.330	0.01190
rs3787498	***rs3827142***	***rs5030707***	9.100	0.02800	A	A	C	0.154	1.910	6.490	0.01080
					G	G	C	0.012	2.820	1.540	0.21500
					A	A	G	0.018	1.640	0.622	0.43000
					G	G	G	0.809	0.493	8.930	0.00281
rs3827142	***rs5030707***	rs3827143	8.960	0.06220	A	C	G	0.152	2.010	7.590	0.00587
					A	G	G	0.020	1.840	0.815	0.36700
					G	G	G	0.052	0.928	0.040	0.84100
					G	C	A	0.010	1.820	0.408	0.52300
					G	G	A	0.757	0.576	6.640	0.00997
rs5030707	rs3827143	rs2424582	10.100	0.03820	C	G	G	0.152	2.010	7.590	0.00587
					G	G	G	0.020	1.020	0.001	0.97400
					G	A	G	0.019	0.212	1.950	0.16200
					G	G	A	0.052	1.090	0.058	0.80900
					G	A	A	0.742	0.613	5.260	0.02180
rs3827143	rs2424582	rs2254635	9.000	0.06120	G	G	A	0.170	2.120	9.070	0.00260
					A	G	A	0.019	0.207	1.990	0.15900
					A	A	A	0.014	0.714	0.130	0.71900
					G	A	C	0.053	1.130	0.121	0.72800
					A	A	C	0.737	0.661	3.890	0.04860
rs2424582	rs2254635	rs2405367	4.820	0.18600	G	A	A	0.175	1.900	6.850	0.00884
					A	A	A	0.014	0.874	0.024	0.87600
					G	A	G	0.014	0.717	0.141	0.70700

SNP set[a]			Omnibus		Haplotype				
1	2	3	STAT	*p*	Haplotype	Frequency	OR	STAT	*p*
					A C G	0.790	0.627	3.970	0.04640

Key: SNP, single nucleotide polymorphism.

[a]SNP(s) most strongly associated with AD are bolded and italicized, and the most significant haplotype is highlighted in gray.

Table 5

Haplotypes analysis for *MARK4* and Alzheimer's disease: 3-SNP window

SNP set[a]			Omnibus		Haplotype						
1	**2**	**3**	**STAT**	***p***	**Haplotype**			**Frequency**	**OR**	**STAT**	***p***
rs12976518	rs10445572	***rs2377324***	10.400	0.01530	A	A	G	0.278	0.548	7.040	0.00796
					G	G	A	0.386	1.610	6.520	0.01070
					G	A	A	0.086	1.510	1.660	0.19800
					A	A	A	0.241	0.816	0.860	0.35400
rs10445572	***rs2377324***	rs2240672	9.780	0.02050	A	G	A	0.274	0.552	6.780	0.00924
					G	A	A	0.048	1.120	0.070	0.79200
					G	A	G	0.344	1.610	6.530	0.01060
					A	A	G	0.324	0.992	0.002	0.96800
rs2377324	rs2240672	rs345409	8.370	0.03900	G	A	G	0.268	0.559	6.390	0.01150
					A	A	G	0.050	1.070	0.029	0.86500
					A	G	G	0.138	1.420	2.010	0.15600
					A	G	A	0.530	1.300	2.100	0.14700
rs2240672	rs345409	rs11883302	7.410	0.19200	A	G	G	0.246	0.584	4.920	0.02660
					G	G	G	0.096	1.100	0.096	0.75600
					G	A	G	0.019	1.720	0.637	0.42500
					A	G	A	0.073	0.788	0.387	0.53400
					G	G	A	0.047	1.700	1.780	0.18200
					G	A	A	0.513	1.270	1.650	0.19900

Key: SNP, single nucleotide polymorphism.

[a]SNP(s) most strongly associated with AD are bolded and italicized, and the most significant haplotype is highlighted in gray.