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Candidate genes for Alzheimer's disease are associated with individual differences in plasma levels of beta amyloid peptides in adults with Down syndrome

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Abstract

We examined the contribution of candidate genes for Alzheimer's disease (AD) on Chromosome 21 and other chromosomes to differences in Aβ peptide levels in a cohort of adults with DS, a population at high risk for AD. Participants were 254 non-demented adults with Down syndrome, 30–78 years of age. Genomic DNA was genotyped using an Illumina GoldenGate custom array. We used linear regression to examine differences in levels of Aβ peptides associated with the number of risk alleles, adjusting for age, sex, level of intellectual disability, race/ethnicity and the presence of the *APOE* ε4 allele. For Aβ42 levels, the strongest gene-wise association was found for a SNP on *CAHLM1*; for Aβ40 levels the strongest gene-wise associations were found for SNPs

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

in *IDE* and *SOD1*, while the strongest gene-wise associations with levels of the Aβ42/Aβ40 ratio were found for SNPs in *SORCS1*. Broadly classified, variants in these genes may influence APP processing (*CALHM1*, *IDE*), vesicular trafficking (*SORCS1*), and response to oxidative stress (*SOD1*).

Keywords

Down syndrome; β amyloid peptides; genetics; biomarkers

1. INTRODUCTION

Amyloid β (Aβ) plays a critical role in the development of Alzheimer's disease (AD). Amyloid β peptides Aβ40 and Aβ42 are the two major species generated by sequential proteolytic cleavage by β and γ secretases of the amyloid precursor protein (APP) (Selkoe, 2001). Brain levels of Aβ42 increase early in the development of dementia, (Cummings and Cotman, 1995, Naslund, et al., 2000) and studies of Aβ peptides in cerebrospinal fluid (CSF) have consistently shown that declining or low levels of Aβ42 and the Aβ42/Aβ40 ratio and high concentrations of tau in patients with mild cognitive impairment (MCI) are associated with higher brain Aβ load (Fagan, et al., 2006, Fagan, et al., 2009, Fagan, et al., 2007) and predict conversion to AD (Blennow and Hampel, 2003, Hansson, et al., 2007, Jack, et al., 2013). Studies of plasma Aβ have shown less consistent relationships to risk of AD than studies of CSF Aβ and inconsistent correlations between plasma and CSF Aβ peptides (Toledo, et al., 2013). Elevated plasma Aβ42 levels have been proposed as a risk factor related to both age and risk for AD. Thus, although deposition of Aβ42 in brain tissue is unlikely to result directly from increased plasma levels, both brain and plasma levels may reflect a general alteration in Aβ processing and individual differences in plasma Aβ42 peptide level may serve as a biological marker of risk, sensitive to the development and progression of AD.

Individuals with Down syndrome (DS) have increased risk for Alzheimer's disease (AD) neuropathology and clinical dementia, which has been attributed to triplication and overexpression of the gene for amyloid precursor protein, *APP*, located on chromosome 21 (Head, et al. 2012), which leads to elevated levels of Aβ peptides from an early age (Conti, et al., 2010, Head, et al., 2011, Mehta, et al., 1998, Schupf, et al., 2001, Teller, et al., 1996, Tokuda, et al., 1997). In adults with DS, high initial levels of plasma Aβ42, are associated with increased risk for AD (Coppus, et al., 2012, Head, et al., 2011, Jones, et al., 2009, Matsuoka, et al., 2009, Schupf, et al., 2007, Schupf, et al., 2001). However, there are large individual differences in initial Aβ peptide levels and a wide range of age at onset of AD within this population, suggesting a more complex underlying mechanism and a role for additional risk factors.

The factors that influence individual differences in plasma Aβ peptides are not well understood. Genetic and environmental risk factors may influence the development of AD by increasing production of Aβ or by reduced clearance or excess deposition of Aβ. Compared with individuals without DS, adults with DS could also be at increased risk for AD through triplication and overexpression of genes on chromosome 21 other than *APP*,

and genes on other chromosomes may modify this risk. Multiple genome-wide association studies (GWAS) have identified potential genetic pathways for AD (Bertram and Tanzi, 2012, Hollingworth, et al., 2011, Jun, et al., 2010, Lambert, et al., 2009a, Lambert, et al., 2013, Naj, et al., 2011) but only a few studies have examined their relation to Aβ levels (Bali, et al., 2012, Chouraki, et al., 2014, Kim, et al., 2011, Miners, et al., 2010, Reitz, et al., 2011b). Reasoning that individuals with DS may be a population group with increased sensitivity for revealing such pathways, in this study we examined the relation of candidate genes for AD to baseline levels of Aβ peptides, Aβ42, Aβ40 and the Aβ42/Aβ40 ratio in older adults with DS. The aim was to identify genetic factors associated with individual differences in level of Aβ peptides which might act as biomarkers of risk for AD.

2. METHODS

2.1 Study population

The study sample included 254 members of a community-based cohort of adults with confirmed DS, non-demented at their initial examination. Dementia status at baseline was classified using data from all available sources reviewed during a consensus conference. Following recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability (Aylward, et al., 1997, Burt and Aylward, 2000). Participants were classified into 2 groups:

1) dementia, if there was a history of progressive memory loss, disorientation, and functional decline over a period of at least 1 year and if there were no other medical or psychiatric conditions that might result in or mimic dementia present (e.g., untreated hypothyroidism, stroke) and 2) without dementia, if they were without cognitive or functional decline based on performance on neuropsychological assessments referenced to level of intellectual disability tested in young adulthood, review of medical records and interviews with informants (Silverman et al., 2004). Among participants who were non-demented at baseline, we analyzed the relation of SNPS in candidate genes to Aβ levels using plasma from the baseline visit (Schupf, et al., 2010) to identify genetics factors associated with individual difference in levels of abeta peptides which might act as biomarkers of risk. All individuals were 31 years of age and older (range 31–78) and resided in New York, Connecticut, New Jersey or northern Pennsylvania. Participants were recruited with the help of state and voluntary service provider agencies and were eligible for inclusion in the present study if: (a) a family member or correspondent provided informed consent, (b) he or she either provided consent or assent indicating willingness to participate, and (c) he or she was willing and able to provide blood samples. 76.4% of the study sample was female. The high frequency of females in the study sample reflects a focus in our research program on the relationship between menopause and risk for dementia among women with Down syndrome. 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 included evaluations of cognition and functional abilities, behavioral/psychiatric conditions and health status. 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 medical history. Past and current medical records were reviewed for all participants.

2.3 Plasma Aββ β42 and Aβ ββ40

Participants were asked to provide a 10 ml venous non-fasting blood sample (K_2EDTA lavender-top tube) at each assessment cycle. Blood draws were done between 10 am and 4 pm. Plasma levels of Aβ42 and Aβ40 were measured blind to clinical status using a combination of monoclonal antibody 6E10 (specific to an epitope present on 1–16 amino acid residues of Aβ) and rabbit antisera R165 (vs. Aβ42) and R162 (vs. Aβ40) in a double antibody sandwich ELISA as previously described. (Mayeux, et al., 2003, Mehta, et al., 1998, Schupf, et al., 2007). The detection limit for these assays was 5 pg/ml for Aβ40 and 10 pg/ml for Aβ42. Aβ40 and Aβ42 levels from each sample were measured twice using separate aliquots. Reliability between measurements was substantial for both peptides (r= . 93. and r= .97 for Aβ40 and Aβ42, respectively, p< .001), and the mean of the two measurements was used in statistical analyses.

2.4 Apolipoprotein E genotypes

Apolipoprotein E (*APOE*) genotyping employed standard PCR-RFLP methods using *Hha1* (*C*foI) digestion of an *APOE* genomic PCR product spanning the polymorphic (cys/arg) sites at codons 112 and 158. Acrylamide gel electrophoresis was used to assess and document the restriction fragment sizes (Hixson and Vernier, 1990). Participants were classified according to the presence or absence of an *APOE* ε4 allele.

Selection of Candidate Genes—An initial set of candidate genes included the top candidate genes from the ALZGENE database (http://www.alzgene.org) and additional positional candidate genes from published genome wide linkage and association studies. We used SNAP (http://www.broadinstitute.org/mpg/snap/ldsearch.php) to identify genes within the candidate regions. This process generated six 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 Down syndrome critical region-1 (*DSCR1*), 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 Supplemental Table 1 for the full list of genes). Figure 1 provides an overview of SNP selection and SNP analysis performed in the 2-stage candidate gene study.

2.5 SNP Selection

We genotyped each gene with a sufficient number of SNPs to provide dense coverage (r^2~0.8), and the selected SNPs had a relatively high minor allele frequency (MAF>0.15).

To identify tag SNPs in these genes, we applied the TAGGER program (de Bakker, 2009) to a Caucasian population available from the HapMap dataset (http://hapmap.ncbi.nlm.nih.gov/). In addition, we used SNAP (http://www.broadinstitute.org/mpg/snap/ldsearch.php) to check LD patterns across the genic region to ensure that the coverage was comprehensive. For chromosome 21, 263 SNPS from the six genes had a median minor allele frequency (MAF) of 0.30 and a median inter-marker distance of 1,914.5 base pairs. For chromosomes other than 21, 1,110 SNPS, exclusive of *APOE,* from 41 genes, had a median MAF of 0.30 and a median inter-marker distance of 1,955 base pairs. We present top strands generated from the Illumina customized platform.

2.6 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 inspected manually by viewing SNP graph cluster plots. Figure 2 shows a typical cluster plot for one of the trisomy SNPs tested (rs2830054). The minor allele was always coded as the risk allele.

2.7 Quality Control Assessment

Prior to allelic association analysis, we first checked the quality of SNP genotyping. Quality scores were determined from allele cluster definitions for each SNP as determined by the Illumina GenomeStudio Genotyping Module version 3.0 and the combined intensity data from 100% of study samples. Genotype calls with a quality score (Gencall value) of ≥0.25 were considered acceptable. For chromosome 21, the average call rate was 98%. We dropped SNPs with a call rate <90% (n=23) or SNPs that did not produce genotypes (n=9). For chromosomes other than 21, the call rate for SNPs was 99%. A total of 11 SNPs were dropped because they had GenTrain scores below threshold or had a call rate of < 98%. After the filtering process, we analyzed 231 SNPs on chromosome 21 and 1099 SNPs from chromosomes other than 21. As a further test of assay reliability, 15 randomly selected samples were genotyped in duplicate. The concordance rates for genotyped SNPs in these samples ranged from 91.8 to 100% for Chr 21 SNPs and from 95.2 to 99.6 for non-Chr 21 SNPs. We then conducted additional quality control (QC) assessments using PLINK (Purcell, et al., 2007). We excluded SNPs with the following characteristics: missing genotyping rate >5%; minimum allele frequency <1%; Hardy-Weinberg Equilibrium (HWE) test [27] at a p-value <0.000001.

2.8. Population stratification

To adjust for population stratification, we applied the multidimensional scaling (MDS) method as implemented in PLINK. Using all available SNPs that survived the QC process, genetic similarity across individuals was estimated by computing identity by state (IBS). To anchor and cross-check against individuals with known ethnic background, we also included whites (n=165), Africans (n=165) and Asians (170) from the Hapmap database

(www.hapmap.org). This analysis generated three distinct racial/ethnic clusters. These clusters were included in the multivariate model to account for ancestry.

2.9. Covariates

Covariates included age, sex, level of intellectual disability, race/ethnicity clusters from MDS analysis, and the presence or absence of an *APOE* ε4 allele. Level of intellectual disability was classified into two groups based on IQ scores obtained before onset of cognitive impairment due to onset of CMI or dementia: mild/moderate (IQ 35–70) and severe/profound (IQ <35).

2.10. Statistical analyses

Linear regression was used to examine the relationship of potential confounders, including age, sex, race/ethnicity clusters, level of intellectual disability and the presence of an *APOE* ε4 allele to peptide levels. To minimize penalties for multiple testing, we conducted a two-stage analysis to identify SNPS that are associated with levels of amyloid Aβ42 and AB40 (Figure 1). In Stage 1 (screening stage), using PLINK, we pruned SNPs to achieve a variance inflation factor (VIF) of 1.43, which is equivalent to a pairwise r^2<0.3. Using PLINK, we evaluated the allelic association between a SNP and Aβ42, Aβ40 and Aβ42/Aβ40 levels using a linear regression model after adjusting for confounders. An additive model was used based on the number of risk alleles. An empirical p-value was computed using 10,000 permutations. In Stage 2 (fine mapping stage), we focused on candidate genes where at least one SNP met an empirical p-value <0.05. To perform the fine mapping analysis, we included all SNPs that were genotyped within that gene. We repeated the linear regression model and, in addition, we computed adjusted p-values to correct for multiple testing, as proposed by Benjamini and colleagues (Benjamini and Hochberg, 1995, Benjamini and Yekutieli, 2001). The R statistical package (http://www.r-project.org/) was used to compute adjusted p-values. For the three most promising genes from the single point analysis (*CALMH1* and Aβ42, *IDE* and Aβ40, *SORCS1* and Aβ42/Aβ40 ratio), we used PLINK to conduct haplotype analysis with a 3-SNP sliding windows approach to identify contiguous regions with significant association.

3. RESULTS

3.1

Valid genotypes were obtained for 254 participants for chromosome 21 genes, and for 219 participants for non-chromosome 21 genes. The demographic characteristics of the two subsets of participants were comparable. For both subsets, mean age was 49.6 years, 76.4% of the study sample was female, 56.7% had a mild or moderate level of intellectual disability while the remainder had severe intellectual disability, and 90.6% of the sample was Caucasian. The *APOE* ε4 allele frequency was 11.3% (Table 1). Mean levels of Aβ peptides were also comparable across both sets. Mean Aβ42 level was 28.2 pg/ml (8.0–132.4) for the 254 participants with genotypes on Chromosome 21 and 27.3 pg/ml (8.0–110.7) for the 219 participants with genotypes on other chromosomes. Mean Aβ40 level was 156.0 pg/ml (24.3–491.4) and the mean Aβ42/Aβ40 ratio was 0.21 pg/ml (0.047–1.15) for both subsets of participants.

For all participants, level of intellectual disability, sex, race/ethnicity components and the presence of an *APOE* ε4 allele were not related to baseline levels of Aβ42, Aβ40 or the Aβ42/Aβ40 ratio. Aβ40 levels increased with age (r= .135, P=.031), while levels of Aβ42 and the Aβ42/Aβ40 ratio decreased with age (r= −.17, p=.008 and r= −.16, p=.009, respectively).

3.2. Aβ42

Stage 1 screening based on tagSNPs identified SNPs in *RUNX1* and *DSCR1* on chromosome 21 genes (Table 2), and identified SNPS in *MTHFR*, *MTHFD1L*, *RELN*, *CALHM1*, *SORCS1*, *SORL1*, *ACAN*, *and PCDH11X* on non-chromosome 21 genes that were associated with Aβ42 levels at empirical p-value<0.05 (Table 2). In Stage 2 fine mapping for genes on Chromosome 21, none of the SNPs for Aβ42 level achieved the adjusted gene-wiser empirical p-values using the Benjamini and Hochberg approach (Benjamini and Hochberg, 1995, Benjamini and Yekutieli, 2001) (Table 2). In Stage 2 fine mapping for non-chromosome 21 genes, two SNPs (rs177736358 and rs755577) that are ~2.5kb apart on the calcium homeostasis modulator 1 (*CALHM1*) gene were significant after correcting for multiple testing, and two SNPs (rs11814111 and rs8878183) on *SORCS1* had adjusted p-values of 0.066, approaching the threshold of 0.05, while SNPs on the other non-chromosome 21 genes failed to achieve the adjusted gene-level empirical p-values. (Table 2).

3.3. Aβ40

Stage 1 screening based on tagSNPs identified SNPs in the *APP*, *SOD1*, *DSCR1*, and *BACE2* chromosome 21 genes (Table 3) and SNPs in the *BIN1*, *RELN*, *DAPK1*, *IDE*, *ACAN*, *LDLR and PCDH11X* non-chromosome 21 genes that were associated with Aβ40 levels at empirical p-value<0.05 (Table 3). In Stage 2 fine mapping, 3 SNPs in the *SOD1* gene that were significant at the p-value of 0.05 for single point analysis barely missed the threshold p=0.05 for significance (p=0.0529) in the gene-wise analysis correcting for multiple testing, but none of the SNPS in *APP*, *DSCR1*, *or BACE2* chromosome 21 genes achieved significance in the gene-wise analysis (Table 2). 12 of 13 SNPs in the insulin degrading enzyme (*IDE*) gene, spanning 20kb, remained significant after adjusting for multiple testing while SNPS in the other non-chromosome 21 genes did not achieve gene-wise empirical p-values.(Table 3).

3.4 Aβ42/Aβ40 ratio

In Stage 1 screening, SNPs in the *APP*, *RUNX1* and *BACE2* chromosome 21 genes (Table 4) and SNPs in the *DAPK1*, *SORCS1*, *SORL1* and *LDLR* non-chromosome 21 genes reached the threshold of 0.05 for single point analysis, and were associated with variation in the level of the Aβ42/Aβ40 ratio (Table 4). In Stage 2 fine mapping, the contiguous seven SNPs for the sortilin-related VPS10 domain containing receptor 1 (*SORCS1*) gene located on 10q23-q25, which were strongly associated with the Aβ42/Aβ40 ratio in Stage 1 screening, remained significant after multiple testing adjustment (0.0337<p<0.0394) while SNPS in the other genes did not achieve statistical significance.(Table 4).

3.5 Haplotype analysis

To further characterize candidate regions that may harbor putative variants, we then performed a 3-mer sliding window haplotype analysis for the 3 promising candidate genes with the strongest support for association, *CALHM1 for* Aβ42, *IDE for* Aβ40, and *SORCS1 for* Aβ42/Aβ40. As shown in Supplemental Tables 2–4, multiple haplotypes anchoring on the SNPs that were significant from the single point gene-wise analysis strengthened the support for association.

4. DISCUSSION

We found significant SNP-wise associations with Aβ peptide levels for SNPs on 17 genes of 47 candidate genes examined, and three genes (*CALHM1*, *IDE*, and *SORCS1*) remained significant after correcting for multiple testing. These 47 candidate genes were selected from previous genome wide linkage, association and expression studies of AD in the DS and general populations. Our results extend previous findings of a relationship between SNPs in *APP*, *PICALM*, *SORLI1*, *BACE1*, *ALDH18A* and *RUNX1* and risk of AD in adults with DS (Jones, et al., 2013, Lee, et al., 2007, Margallo-Lana, et al., 2004, Patel, et al., 2011) to include examination of plasma beta amyloid peptides as a critical risk factor for AD in DS, and employing a wider range of SNPs and genes. We found significant gene-wise associations with Aβ peptide levels for SNPs in 3 genes: *CALHM1*, *IDE* and *SORCS1*. For Aβ42 levels, two SNPs (rs755577 and rs17736358) in *CAHLM1* were associated at the gene-wise level. For Aβ40 levels the strongest gene-wise associations were found for a set of SNPs on *IDE*, located 94,217038 to 94335799 bp. These were represented by a set of contiguous 3-mer haplotypes with empirical p-values ranging from 0.00015 to 0.00063 (See Supplement table 3). As an external confirmation, the 2-stage meta-analysis of the Alzheimer Disease Genetics Consortium (ADGC) dataset reported that rs2421942 was significantly associated with AD. For levels of the Aβ42/Aβ40 ratio, the strongest gene-wise associations were found for SNPs in *SORCS1*, located in the region encompassing 108,479,649 to 108,647,761bp. Although the exact same set of SNPs were associated with AD in the (ADGC) meta-analysis, rs12248379 (chr10:108562008) (p=0.00534) overlapped with haplotype A-C-C that was significant in our dataset (p=0.00329). Broadly classified, variants in these genes may influence APP processing (*CALHM1*, *IDE*) and vesicular trafficking (*SORCS1*), neurodevelopmental processes, response to oxidative stress (*SOD1*).

Processing of amyloid precursor protein (APP) by cleavage by β and γ secretases to generate Aβ peptides is central to the pathogenesis of AD (Vardarajan, et al., 2012). Missense mutations in the gene for *APP*, which increase the proteolytic conversion of APP into the fribillogenic Aβ42 peptide, have been shown to lead to early onset of AD (Goate, et al., 1991, Guerreiro, et al., Rogaeva, et al., 2007, Scheuner, et al.,1996, Younkin, 1997), but less work has been done on the relation of common SNPS in *APP* to age at onset, risk of AD, or individual differences in Aβ peptide levels (Benitez, et al., 2013, Chapman, et al., 2013, Kimura, et al., 2007, Shulman, et al., 2013). Several, but not all, studies have found a relationship between high initial levels of Aβ42 and subsequent development of AD, both among adults with Down syndrome (Coppus, et al., 2012, Head, et al., 2011, Jones, et al., 2009, Matsuoka, et al., 2009, Schupf, et al., 2007, Schupf, et al., 2001) and in the general

population (Blasko, et al., 2010, Mayeux, et al., 2003, Mayeux, et al., 1999, Pomara, et al., 2005, Schupf, et al., 2008). However, large GWAS studies of AD have not found an association between SNPs in *APP* and late onset AD (LOAD) (Bertram and Tanzi, 2012, Hollingworth, et al., 2011, Lambert, et al., 2009b, Naj, et al., 2011). A recent GWAS meta-analysis of plasma Abeta peptide concentrations in nondemented elderly also failed to find SNPs on *APP* that were associated with Aβ levels, although several suggestive loci were found on other genes; the gene most strongly associated with Aβ42 was CTXN3 (cortexin3), involved in Aβ42 secretion (Chouraki, et al., 2014). In our cohort of adults with DS, we did find SNPs on *APP* that were significantly associated with Aβ peptides in the single point analysis; however, after correcting for multiple testing at the gene-wise level, those SNPs were no longer significant. This may reflect the very high level of Aβ that is found among all adults with DS (Conti, et al., 2010, Head, et al., 2011, Mehta, et al., 1998, Schupf, et al., 2001, Teller, et al., 1996, Tokuda, et al., 1997): it may be that processing and clearance rather than generation of Aβ are more important factors associated with the individual differences we have examined. It is also possible, given the results of the single point analysis, that studies of SNPs in *APP*, using a much larger sample than we were able to study in this paper, will identify SNPs in *APP* associated with individual differences in levels of Aβ peptides.

We found that the A allele in rs755577 and the G allele in rs17736358 on *CALHM1* were associated with individual differences in Aβ42 levels. However, this SNP is located 5.8kb and 8.3kb away from rs2986017 the SNP which has been associated with increased risk and age at onset in some (Boada, et al., 2010, Dreses-Werringloer, et al., 2008, Lambert, et al., 2010), but not all (Beecham, et al., 2009, Bertram, et al., 2008, Minster, et al., 2009) prior studies. *CALHM1* is expressed in the hippocampus and encodes a calcium channel involved in APP processing. The *CALHM1* rs2986017 polymorphism has been proposed to increase Aβ levels by interfering with CALHM1-mediated Ca(2+) permeability (Dreses-Werringloer, et al., 2008). Replication in a larger dataset will be required to confirm this finding.

We found SNPs on *IDE* (insulin degrading enzyme) that were associated with individual differences in Aβ40 levels. *IDE* shares insulin and Aβ as substrates (Kurochkin and Goto, 1994). Consistent with our findings, *IDE* is up-regulated in amyloid plaques, (Bernstein, et al., 1999) and lower expression of *IDE* is found in the hippocampus in brains of elders with LOAD who are *APOE* ε4 positive (Cook, et al., 2003, Qiu and Folstein, 2006), and in persons with mild cognitive impairment (Zhao, et al., 2007), who are at highest risk for LOAD. Lower *IDE* expression has also been found to correlate with higher levels of cellular Aβ42 associated with PS1 mutations in cellular models. Several have found an association between a variant on *IDE* and plasma Aβ42 or Aβ40 levels (Carrasquillo, et al., 2010, Reitz, et al., 2012). However, data on the relation of the *IDE* locus with AD is conflicting. Some studies showed no association between the *IDE* locus and LOAD (Abraham, et al., 2001, Boussaha, et al., 2002, Nowotny, et al., 2005, Ozturk, et al., 2006, Sakai, et al., 2004), while other studies have found an association (Carrasquillo, et al., 2010, Vepsalainen, et al., 2007, Zuo and Jia, 2009).

SORCS1 is a member of the vacuolar protein sorting 10 (VPS10) domain-containing receptor protein family. VPS10 receptors are involved with APP trafficking and can

influence APP processing and Aβ production through linking APP to the endosomal retromer complex and access to secretases that cleave APP (Lane, et al., 2010, Lane, et al., 2013). SNPS in *SORCS1* have been associated with increased risk for late onset AD (Reitz, et al., 2011a, Rogaeva, et al., 2007) and also influence Aβ peptide levels where over-expression of *SORCS1* reduces γ-secretase activity and Aβ levels, and *SORCS1* suppression increases γ-secretase processing of APP and Aβ levels (Rogaeva, et al., 2007).

An interesting connection between *IDE* and *SORCS1*, the candidate genes with strongest signals in chromosome 10 in our study, is that both may be related to hyperinsulinemia and type 2 diabetes, potential important risk factors for LOAD. (Cheng, et al., 2011, Luchsinger, et al., 2004) Certain *IDE* genotypes are related to a higher risk of diabetes (Fakhrai-Rad, et al., 2000, Karamohamed, et al., 2003, Kwak, et al., 2008, Rudovich, et al., 2009), although the contribution of *IDE* to diabetes is controversial,(Groves, et al., 2003) and other studies have found no relationship.(Florez, et al., 2006, Qin and Jia, 2008). *SORCS1* may also affect insulin levels and the risk of diabetes (Clee, et al., 2006, Goodarzi, et al., 2007, Lane, et al., 2010). Diabetes and insulin resistance are also more prevalent in persons with Down syndrome (Fonseca, et al., 2005). Thus, the connection of *IDE*, *SORCS1*, insulin resistance and diabetes, Aβ and AD in Down syndrome requires further investigation.

Several genes on chromosome 21 are involved in inflammation and are overexpressed in DS, including *APP*, superoxide dismutase *(SOD-1*), Ets-2 transcription factors, Down Syndrome Critical Region 1 (*DSCR1*) stress-inducible factor, beta-site APP cleaving enzyme (*BACE-2*), and *S100β*. In this study, SNPS in *SOD1* were associated with individual differences in levels of Aβ40, but not with Aβ42 or the Aβ42/Aβ40 ratio and *SOD1* has been implicated in neurodegenerative processes (Lott, et al., 2006). Adults with DS overexpress *SOD1* and show increased free radical–mediated oxidative damage (Markesbery and Lovell, 2007, Reynolds and Cutts, 1993). Increased *SOD1* expression has been found in degenerating neurons in the brain of adults with DS (Furuta, et al., 1995). The rate of production of Aβ from *APP* may be increased in the presence of this type of oxidative damage (Dickinson and Singh, 1993, Lott and Head, 2001), where membrane damage secondary to lipoperoxidation allows abnormal cleavage of the protein (Singth and Dickinson, 2006). Zis and colleagues (Zis, et al., 2012) observed in a longitudinal study that higher SOD levels were positively associated with memory performance over a period of 4 years in 26 adults with Down syndrome, and suggested that these enzymes may have protective, antioxident effects. Further work will need to be conducted to determine the contribution of SOD levels to onset of dementia in adults with DS. Further work will need to be conducted to determine the contribution of SOD levels to onset of dementia in adults with DS.

In sum, recent GWAS and meta-analyses of genes associated with risk for AD have identified SNPS associated with several major pathways, including amyloid production, lipid/cholesterol metabolism, immune response and inflammation, vesicular trafficking/ synaptic function and neurodevelopment. Our study examined the relation of candidate genes for AD to individual differences in Aβ peptide levels among unaffected adults with Down syndrome. We found significant associations with candidate genes in several of these pathways but the strongest associations were related to Aβ processing, neuro-developmental

processes, and oxidative damage. These findings support the hypothesis that individual differences in Aβ processing or deposition, distinct from overexpression of *APP*, may act as an initial step in the pathogenesis of AD.

Our study has several limitations. Timing of blood draws was not specifically controlled for. Blood draws were done between 10 am and 4 pm. We believe that this diurnal variation is unlikely to alter the relation between $A\beta$ levels and SNPs, since timing was randomly distributed and variations would be expected to lead to non-differential bias. However, this is a potential source of increased variability in our Aβ measures.

Not all the genes associated with individual differences in Aβ peptide levels in our cohort of adults with DS have been confirmed in recent large GWAS and meta-analyses as associated with risk for AD in the general population. Our study is limited by a relatively small sample size, but examined the role of these genes in a very high risk group that is characterized by early onset of AD and by especially high levels of Aβ peptides. These findings may therefore help to clarify pathways that contribute to the development of AD, both in adults with DS and within the broader population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Abraham R, Myers A, Wavrant-DeVrieze F, Hamshere ML, Thomas HV, Marshall H, Compton D, Spurlock G, Turic D, Hoogendoorn B, Kwon JM, Petersen RC, Tangalos E, Norton J, Morris JC, Bullock R, Liolitsa D, Lovestone S, Hardy J, Goate A, O'Donovan M, Williams J, Owen MJ, Jones L. Substantial linkage disequilibrium across the insulin-degrading enzyme locus but no association with late-onset Alzheimer's disease. Human Genetics. 2001; 109(6):646–52. [PubMed: 11810277]

Aylward EH, Burt DB, Thorpe LU, Lai F, Dalton A. Diagnosis of dementia in individuals with intellectual disability. J Intellect Disabil Res. 1997; 41 (Pt 2):152–64. [PubMed: 9161927]

Bali J, Gheinani AH, Zurbriggen S, Rajendran L. Role of genes linked to sporadic Alzheimer's disease risk in the production of beta-amyloid peptides. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(38):15307–11. [PubMed: 22949636]

Beecham GW, Schnetz-Boutaud N, Haines JL, Pericak-Vance MA. CALHM1 polymorphism is not associated with late-onset Alzheimer disease. Ann Hum Genet. 2009; 73(Pt 3):379–81. [PubMed: 19472444]

Benitez BA, Karch CM, Cai Y, Jin SC, Cooper B, Carrell D, Bertelsen S, Chibnik L, Schneider JA, Bennett DA, Fagan AM, Holtzman D, Morris JC, Goate AM, Cruchaga C. Alzheimer's Disease Neuroimaging I, Genetic Environmental Risk for Alzheimer's Disease Consortium G. The PSEN1, p.E318G variant increases the risk of Alzheimer's disease in APOE-epsilon4 carriers. PLoS Genetics. 2013; 9(8):e1003685.10.1371/journal.pgen.1003685 [PubMed: 23990795]

Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B (Methodological). 1995; 57(1): 289–300.

Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. The Annals of Statistics. 2001; 29(4):1165–88.

Bernstein HG, Ansorge S, Riederer P, Reiser M, Frolich L, Bogerts B. Insulin-degrading enzyme in the Alzheimer's disease brain: prominent localization in neurons and senile plaques. Neuroscience Letters. 1999; 263(2–3):161–4. [PubMed: 10213160]

Bertram L, Schjeide BM, Hooli B, Mullin K, Hiltunen M, Soininen H, Ingelsson M, Lannfelt L, Blacker D, Tanzi RE. No association between CALHM1 and Alzheimer's disease risk. Cell. 2008; 135(6):993–4. [PubMed: 19070563]

Bertram L, Tanzi RE. The genetics of Alzheimer's disease. Prog Mol Biol Transl Sci. 2012; 107:79–100. [PubMed: 22482448]

Blasko I, Kemmler G, Jungwirth S, Wichart I, Krampla W, Weissgram S, Jellinger K, Tragl KH, Fischer P. Plasma amyloid beta-42 independently predicts both late-onset depression and Alzheimer disease. The American Journal of Geriatric Psychiatry. 2010; 18(11):973–82. [PubMed: 20808106]

Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003; 2(10): 605–13. [PubMed: 14505582]

Boada M, Antunez C, Lopez-Arrieta J, Galan JJ, Moron FJ, Hernandez I, Marin J, Martinez-Lage P, Alegret M, Carrasco JM, Moreno C, Real LM, Gonzalez-Perez A, Tarraga L, Ruiz A. CALHM1 P86L polymorphism is associated with late-onset Alzheimer's disease in a recessive model. J Alzheimers Dis. 2010; 20(1):247–51. [PubMed: 20164592]

Boussaha M, Hannequin D, Verpillat P, Brice A, Frebourg T, Campion D. Polymorphisms of insulin degrading enzyme gene are not associated with Alzheimer's disease. Neuroscience Letters. 2002; 329(1):121–3. [PubMed: 12161276]

Burt DB, Aylward EH. Test battery for the diagnosis of dementia in individuals with intellectual disability. Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Intellectual Disability. J Intellect Disabil Res. 2000; 44 (Pt 2):175–80. [PubMed: 10898382]

Carrasquillo MM, Belbin O, Zou F, Allen M, Ertekin-Taner N, Ansari M, Wilcox SL, Kashino MR, Ma L, Younkin LH, Younkin SG, Younkin CS, Dincman TA, Howard ME, Howell CC, Stanton CM, Watson CM, Crump M, Vitart V, Hayward C, Hastie ND, Rudan I, Campbell H, Polasek O, Brown K, Passmore P, Craig D, McGuinness B, Todd S, Kehoe PG, Mann DM, Smith AD, Beaumont H, Warden D, Holmes C, Heun R, Kolsch H, Kalsheker N, Pankratz VS, Dickson DW, Graff-Radford NR, Petersen RC, Wright AF, Younkin SG, Morgan K. Concordant association of insulin degrading enzyme gene (IDE) variants with IDE mRNA, Abeta, and Alzheimer's disease. PloS One. 2010; 5(1):e8764.10.1371/journal.pone.0008764 [PubMed: 20098734]

Chapman J, Rees E, Harold D, Ivanov D, Gerrish A, Sims R, Hollingworth P, Stretton A, Holmans P, Owen MJ, ODonovan MC, Williams J, Kirov G. G Consortium. A genome-wide study shows a limited contribution of rare copy number variants to Alzheimer's disease risk. Hum Mol Genet. 2013; 22(4):816–24. [PubMed: 23148125]

Cheng D, Noble J, Tang MX, Schupf N, Mayeux R, Luchsinger JA. Type 2 diabetes and late-onset Alzheimer's disease. Dementia and Geriatric Cognitive Disorders. 2011; 31(6):424–30. [PubMed: 21757907]

Chouraki V, De Bruijn RF, Chapuis J, Bis JC, Reitz C, Schraen S, Ibrahim-Verbaas CA, Grenier-Boley B, Delay C, Rogers R, Demiautte F, Mounier A, Fitzpatrick AL, Berr C, Dartigues JF, Uitterlinden AG, Hofman A, Breteler M, Becker JT, Lathrop M, Schupf N, Alperovitch A, Mayeux R, van Duijn CM, Buee L, Amouyel P, Lopez OL, Ikram MA, Tzourio C, Lambert JC. Alzheimer's Disease Neuroimaging I. A genome-wide association meta-analysis of plasma Abeta peptides concentrations in the elderly. Mol Psychiatry. 2014; 19(12):1326–35. [PubMed: 24535457]

Clee SM, Yandell BS, Schueler KM, Rabaglia ME, Richards OC, Raines SM, Kabara EA, Klass DM, Mui ET, Stapleton DS, Gray-Keller MP, Young MB, Stoehr JP, Lan H, Boronenkov I, Raess PW, Flowers MT, Attie AD. Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. Nature Genetics. 2006; 38(6):688–93. [PubMed: 16682971]

Conti E, Galimberti G, Piazza F, Raggi ME, Ferrarese C. Increased soluble APPalpha, Abeta 1–42, and anti-Abeta 1–42 antibodies in plasma from down syndrome patients. Alzheimer Dis Assoc Disord. 2010; 24(1):96–100. [PubMed: 19571731]

Cook DG, Leverenz JB, McMillan PJ, Kulstad JJ, Ericksen S, Roth RA, Schellenberg GD, Jin LW, Kovacina KS, Craft S. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. The American Journal of Pathology. 2003; 162(1):313–9. [PubMed: 12507914]

Coppus AM, Schuur M, Vergeer J, Janssens AC, Oostra BA, Verbeek MM, van Duijn CM. Plasma beta amyloid and the risk of Alzheimer's disease in Down syndrome. Neurobiology of Aging. 2012; 33(9):1988–94. [PubMed: 21958962]

Cummings BJ, Cotman CW. Image analysis of beta-amyloid load in Alzheimer's disease and relation to dementia severity. Lancet. 1995; 346(8989):1524–8. [PubMed: 7491048]

de Bakker PI. Selection and evaluation of Tag-SNPs using Tagger and HapMap. Cold Spring Harbor Protocols. 2009; 2009(6) pdb ip67. 10.1101/pdb.ip67

Dickinson MJ, Singh I. Down's syndrome, dementia, and superoxide dismutase. Br J Psychiatry. 1993; 162:811–7. [PubMed: 8392422]

Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, Pasquier F, Galimberti D, Scarpini E, Mann D, Lendon C, Campion D, Amouyel P, Davies P, Foskett JK, Campagne F, Marambaud P. A polymorphism in CALHM1 influences Ca2+ homeostasis, Abeta levels, and Alzheimer's disease risk. Cell. 2008; 133(7):1149–61. [PubMed: 18585350]

Fagan AM, Mintun MA, Mach RH, Lee SY, Dence CS, Shah AR, LaRossa GN, Spinner ML, Klunk WE, Mathis CA, DeKosky ST, Morris JC, Holtzman DM. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Abeta42 in humans. Ann Neurol. 2006; 59(3):512–9. [PubMed: 16372280]

Fagan AM, Mintun MA, Shah AR, Aldea P, Roe CM, Mach RH, Marcus D, Morris JC, Holtzman DM. Cerebrospinal fluid tau and ptau(181) increase with cortical amyloid deposition in cognitively normal individuals: implications for future clinical trials of Alzheimer's disease. EMBO Mol Med. 2009; 1(8–9):371–80. [PubMed: 20049742]

Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol. 2007; 64(3):343–9. [PubMed: 17210801]

Fakhrai-Rad H, Nikoshkov A, Kamel A, Fernstrom M, Zierath JR, Norgren S, Luthman H, Galli J. Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats. Hum Mol Genet. 2000; 9(14):2149–58. [PubMed: 10958757]

Florez JC, Wiltshire S, Agapakis CM, Burtt NP, de Bakker PI, Almgren P, Bengtsson Bostrom K, Tuomi T, Gaudet D, Daly MJ, Hirschhorn JN, McCarthy MI, Altshuler D, Groop L. High-density haplotype structure and association testing of the insulin-degrading enzyme (IDE) gene with type 2 diabetes in 4,206 people. Diabetes. 2006; 55(1):128–35. [PubMed: 16380485]

Fonseca CT, Amaral DM, Ribeiro MG, Beserra IC, Guimaraes MM. Insulin resistance in adolescents with Down syndrome: a cross-sectional study. BMC Endocr Disord. 2005; 5:6. [PubMed: 15963228]

Furuta A, Price DL, Pardo CA, Troncoso JC, Xu ZS, Taniguchi N, Martin LJ. Localization of superoxide dismutases in Alzheimer's disease and Down's syndrome neocortex and hippocampus. The American Journal of Pathology. 1995; 146(2):357–67. [PubMed: 7856748]

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Cance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 1991; 349(6311):704–6. [PubMed: 1671712]

Goodarzi MO, Lehman DM, Taylor KD, Guo X, Cui J, Quinones MJ, Clee SM, Yandell BS, Blangero J, Hsueh WA, Attie AD, Stern MP, Rotter JI. SORCS1: a novel human type 2 diabetes susceptibility gene suggested by the mouse. Diabetes. 2007; 56(7):1922–9. [PubMed: 17426289]

Groves CJ, Wiltshire S, Smedley D, Owen KR, Frayling TM, Walker M, Hitman GA, Levy JC, O'Rahilly S, Menzel S, Hattersley AT, McCarthy MI. Association and haplotype analysis of the insulin-degrading enzyme (IDE) gene, a strong positional and biological candidate for type 2 diabetes susceptibility. Diabetes. 2003; 52(5):1300–5. [PubMed: 12716770]

Guerreiro RJ, Gustafson DR, Hardy J. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. Neurobiology of aging. 2012; 33(3):437–56. [PubMed: 20594621]

Hansson O, Zetterberg H, Buchhave P, Andreasson U, Londos E, Minthon L, Blennow K. Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. Dementia and Geriatric Cognitive Disorders. 2007; 23(5):316–20. [PubMed: 17374949]

Head E, Doran E, Nistor M, Hill M, Schmitt FA, Haier RJ, Lott IT. Plasma amyloid-beta as a function of age, level of intellectual disability, and presence of dementia in Down syndrome. J Alzheimers Dis. 2011; 23(3):399–409. [PubMed: 21116050]

Head E, Silverman W, Patterson D, Lott IT. Aging and down syndrome. Curr Gerontol Geriatr Res. 2012; 2012

Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res. 1990; 31(3):545–8. [PubMed: 2341813]

Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Ruther E, Schurmann B, Heun R, Kolsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Gallacher J, Hull M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alperovitch A, Lathrop M, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Bjornsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossu P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J. E consortium, C consortium, Alzheimer's Disease Neuroimaging I. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nature Genetics. 2011; 43(5):429–35. [PubMed: 21460840]

Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Pankratz VS, Donohue MC, Trojanowski JQ. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013; 12(2):207–16. [PubMed: 23332364]

Jones EL, Hanney M, Francis PT, Ballard CG. Amyloid beta concentrations in older people with Down syndrome and dementia. Neuroscience letters. 2009; 451(2):162–4. [PubMed: 19111900]

Jones EL, Mok K, Hanney M, Harold D, Sims R, Williams J, Ballard C. Evidence that PICALM affects age at onset of Alzheimer's dementia in Down syndrome. Neurobiology of Aging. 2013; 34(10):2441.e1–5. [PubMed: 23601808]

Jun G, Naj AC, Beecham GW, Wang LS, Buros J, Gallins PJ, Buxbaum JD, Ertekin-Taner N, Fallin MD, Friedland R, Inzelberg R, Kramer P, Rogaeva E, St George-Hyslop P, Cantwell LB, Dombroski BA, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Lunetta KL, Martin ER, Montine TJ, Goate AM, Blacker D, Tsuang DW, Beekly D, Cupples LA, Hakonarson H, Kukull W, Foroud TM, Haines J, Mayeux R, Farrer LA, Pericak-Vance MA, Schellenberg GD.

Alzheimer's Disease Genetics C. Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol. 2010; 67(12):1473–84. [PubMed: 20697030]

Karamohamed S, Demissie S, Volcjak J, Liu C, Heard-Costa N, Liu J, Shoemaker CM, Panhuysen CI, Meigs JB, Wilson P, Atwood LD, Cupples LA, Herbert A. Polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes in men from the NHLBI Framingham Heart Study. Diabetes. 2003; 52(6):1562–7. [PubMed: 12765971]

Kim S, Swaminathan S, Shen L, Risacher SL, Nho K, Foroud T, Shaw LM, Trojanowski JQ, Potkin SG, Huentelman MJ, Craig DW, DeChairo BM, Aisen PS, Petersen RC, Weiner MW, Saykin AJ. Alzheimer's Disease Neuroimaging I. Genome-wide association study of CSF biomarkers Abeta1-42, t-tau, and p-tau181p in the ADNI cohort. Neurology. 2011; 76(1):69–79. [PubMed: 21123754]

Kimura R, Kamino K, Yamamoto M, Nuripa A, Kida T, Kazui H, Hashimoto R, Tanaka T, Kudo T, Yamagata H, Tabara Y, Miki T, Akatsu H, Kosaka K, Funakoshi E, Nishitomi K, Sakaguchi G, Kato A, Hattori H, Uema T, Takeda M. The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease. Hum Mol Genet. 2007; 16(1):15–23. [PubMed: 17135279]

Kurochkin IV, Goto S. Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. FEBS Lett. 1994; 345(1):33–7. [PubMed: 8194595]

Kwak SH, Cho YM, Moon MK, Kim JH, Park BL, Cheong HS, Shin HD, Jang HC, Kim SY, Lee HK, Park KS. Association of polymorphisms in the insulin-degrading enzyme gene with type 2 diabetes in the Korean population. Diabetes Res Clin Pract. 2008; 79(2):284–90. [PubMed: 17913278]

Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fievet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanche H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M, Amouyel P. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nature Genetics. 2009a; 41(10):1094–9. [PubMed: 19734903]

Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Moron FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fievet N, Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bossu P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH, JrBennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JI, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltuenen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nothen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P. European Alzheimer's Disease I Genetic Environmental Risk in Alzheimer's D Alzheimer's Disease Genetic C, Cohorts for H Aging Research in Genomic E. Meta-analysis of 74,046 individuals identifies 11

new susceptibility loci for Alzheimer's disease. Nature Genetics. 2013; 45(12):1452–8. [PubMed: 24162737]

Lambert JC, Schraen-Maschke S, Richard F, Fievet N, Rouaud O, Berr C, Dartigues JF, Tzourio C, Alperovitch A, Buee L, Amouyel P. Association of plasma amyloid beta with risk of dementia: the prospective Three-City Study. Neurology. 2009b; 73(11):847–53. [PubMed: 19752451]

Lambert JC, Sleegers K, Gonzalez-Perez A, Ingelsson M, Beecham GW, Hiltunen M, Combarros O, Bullido MJ, Brouwers N, Bettens K, Berr C, Pasquier F, Richard F, Dekosky ST, Hannequin D, Haines JL, Tognoni G, Fievet N, Dartigues JF, Tzourio C, Engelborghs S, Arosio B, Coto E, De Deyn P, Del Zompo M, Mateo I, Boada M, Antunez C, Lopez-Arrieta J, Epelbaum J, Schjeide BM, Frank-Garcia A, Giedraitis V, Helisalmi S, Porcellini E, Pilotto A, Forti P, Ferri R, Delepine M, Zelenika D, Lathrop M, Scarpini E, Siciliano G, Solfrizzi V, Sorbi S, Spalletta G, Ravaglia G, Valdivieso F, Vepsalainen S, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Hanon O, Piccardi P, Annoni G, Mann D, Marambaud P, Seripa D, Galimberti D, Tanzi RE, Bertram L, Lendon C, Lannfelt L, Licastro F, Campion D, Pericak-Vance MA, Soininen H, Van Broeckhoven C, Alperovitch A, Ruiz A, Kamboh MI, Amouyel P. The CALHM1 P86L polymorphism is a genetic modifier of age at onset in Alzheimer's disease: a meta-analysis study. J Alzheimers Dis. 2010; 22(1):247–55. [PubMed: 20847397]

Lane RF, Raines SM, Steele JW, Ehrlich ME, Lah JA, Small SA, Tanzi RE, Attie AD, Gandy S. Diabetes-associated SorCS1 regulates Alzheimer's amyloid-beta metabolism: evidence for involvement of SorL1 and the retromer complex. J Neurosci. 2010; 30(39):13110–5. [PubMed: 20881129]

Lane RF, Steele JW, Cai D, Ehrlich ME, Attie AD, Gandy S. Protein sorting motifs in the cytoplasmic tail of SorCS1 control generation of Alzheimer's amyloid-beta peptide. J Neurosci. 2013; 33(16): 7099–107. [PubMed: 23595767]

Lee JH, Chulikavit M, Pang D, Zigman WB, Silverman W, Schupf N. Association between genetic variants in sortilin-related receptor 1 (SORL1) and Alzheimer's disease in adults with Down syndrome. Neuroscience Letters. 2007; 425(2):105–9. [PubMed: 17826910]

Lott IT, Head E. Down syndrome and Alzheimer's disease: a link between development and aging. Ment Retard Dev Disabil Res Rev. 2001; 7(3):172–8. [PubMed: 11553933]

Lott IT, Head E, Doran E, Busciglio J. Beta-amyloid, oxidative stress and down syndrome. Current Alzheimer Research. 2006; 3(5):521–8. [PubMed: 17168651]

Luchsinger JA, Tang MX, Shea S, Mayeux R. Hyperinsulinemia and risk of Alzheimer disease. Neurology. 2004; 63(7):1187–92. [PubMed: 15477536]

Margallo-Lana M, Morris CM, Gibson AM, Tan AL, Kay DW, Tyrer SP, Moore BP, Ballard CG. Influence of the amyloid precursor protein locus on dementia in Down syndrome. Neurology. 2004; 62(11):1996–8. [PubMed: 15184603]

Markesbery WR, Lovell MA. Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. Arch Neurol. 2007; 64(7):954–6. [PubMed: 17620484]

Matsuoka Y, Andrews HF, Becker AG, Gray AJ, Mehta PD, Sano MC, Dalton AJ, Aisen PS. The relationship of plasma Abeta levels to dementia in aging individuals with Down syndrome. Alzheimer Dis Assoc Disord. 2009; 23(4):315–8. [PubMed: 19571732]

Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N, Mehta PD. Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology. 2003; 61(9): 1185–90. [PubMed: 14610118]

Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD. Plasma amyloid beta-peptide 1–42 and incipient Alzheimer's disease. Ann Neurol. 1999; 46(3):412–6. [PubMed: 10482274]

Mehta PD, Dalton AJ, Mehta SP, Kim KS, Sersen EA, Wisniewski HM. Increased plasma amyloid beta protein 1–42 levels in Down syndrome. Neuroscience Letters. 1998; 241(1):13–6. [PubMed: 9502204]

Miners JS, van Helmond Z, Raiker M, Love S, Kehoe PG. ACE variants and association with brain Abeta levels in Alzheimer's disease. American Journal of Translational Research. 2010; 3(1):73–80. [PubMed: 21139807]

Minster RL, Demirci FY, DeKosky ST, Kamboh MI. No association between CALHM1 variation and risk of Alzheimer disease. Hum Mutat. 2009; 30(4):E566–9. [PubMed: 19191331]

Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull WA, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nature Genetics. 2011; 43(5):436–41. [PubMed: 21460841]

Naslund J, Haroutonian V, Mohs R, Davis K, Davies P, Greengard P, Buxbaum. Correlation between elevated levels of amyloid B-peptide in the brain and cognitive decline. JAMA. 2000; 283:1571–7. [PubMed: 10735393]

Nowotny P, Hinrichs AL, Smemo S, Kauwe JS, Maxwell T, Holmans P, Hamshere M, Turic D, Jehu L, Hollingworth P, Moore P, Bryden L, Myers A, Doil LM, Tacey KM, Gibson AM, McKeith IG, Perry RH, Morris CM, Thal L, Morris JC, O'Donovan MC, Lovestone S, Grupe A, Hardy J, Owen MJ, Williams J, Goate A. Association studies between risk for late-onset Alzheimer's disease and variants in insulin degrading enzyme. Am J Med Genet B Neuropsychiatr Genet. 2005; 136B(1): 62–8. [PubMed: 15858813]

Ozturk A, DeKosky ST, Kamboh MI. Lack of association of 5 SNPs in the vicinity of the insulin-degrading enzyme (IDE) gene with late-onset Alzheimer's disease. Neuroscience Letters. 2006; 406(3):265–9. [PubMed: 16914266]

Patel A, Rees SD, Kelly MA, Bain SC, Barnett AH, Thalitaya D, Prasher VP. Association of variants within APOE, SORL1, RUNX1, BACE1 and ALDH18A1 with dementia in Alzheimer's disease in subjects with Down syndrome. Neuroscience Letters. 2011; 487(2):144–8. [PubMed: 20946940]

Pomara N, Willoughby LM, Sidtis JJ, Mehta PD. Selective reductions in plasma Abeta 1–42 in healthy elderly subjects during longitudinal follow-up: a preliminary report. The American Journal of Geriatric Psychiatry. 2005; 13(10):914–7. [PubMed: 16223971]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. American Journal of Human Genetics. 2007; 81(3):559–75. [PubMed: 17701901]

Qin W, Jia J. Down-regulation of insulin-degrading enzyme by presenilin 1 V97L mutant potentially underlies increased levels of amyloid beta 42. Eur J Neurosci. 2008; 27(9):2425–32. [PubMed: 18445230]

Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. Neurobiology of Aging. 2006; 27(2):190–8. [PubMed: 16399206]

Reitz C, Cheng R, Rogaeva E, Lee JH, Tokuhiro S, Zou F, Bettens K, Sleegers K, Tan EK, Kimura R, Shibata N, Arai H, Kamboh MI, Prince JA, Maier W, Riemenschneider M, Owen M, Harold D, Hollingworth P, Cellini E, Sorbi S, Nacmias B, Takeda M, Pericak-Vance MA, Haines JL,

Younkin S, Williams J, van Broeckhoven C, Farrer LA, St George-Hyslop PH, Mayeux R. Genetic Environmental Risk in Alzheimer Disease C. Meta-analysis of the association between variants in SORL1 and Alzheimer disease. Arch Neurol. 2011a; 68(1):99–106. [PubMed: 21220680]

Reitz C, Cheng R, Schupf N, Lee JH, Mehta PD, Rogaeva E, St George-Hyslop P, Mayeux R. Association between variants in IDE-KIF11-HHEX and plasma amyloid beta levels. Neurobiology of Aging. 2012; 33(1):199.e13–7. [PubMed: 20724036]

Reitz C, Tokuhiro S, Clark LN, Conrad C, Vonsattel JP, Hazrati LN, Palotas A, Lantigua R, Medrano M, IZJV, Vardarajan B, Simkin I, Haines JL, Pericak-Vance MA, Farrer LA, Lee JH, Rogaeva E, George-Hyslop PS, Mayeux R. SORCS1 alters amyloid precursor protein processing and variants may increase Alzheimer's disease risk. Ann Neurol. 2011b; 69(1):47–64. [PubMed: 21280075]

Reynolds GP, Cutts AJ. Free radical damage in Down's syndrome brain. Biochemical Society transactions. 1993; 21(2):221S. [PubMed: 8359470]

Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, Katayama T, Baldwin CT, Cheng R, Hasegawa H, Chen F, Shibata N, Lunetta KL, Pardossi-Piquard R, Bohm C, Wakutani Y, Cupples LA, Cuenco KT, Green RC, Pinessi L, Rainero I, Sorbi S, Bruni A, Duara R, Friedland RP, Inzelberg R, Hampe W, Bujo H, Song YQ, Andersen OM, Willnow TE, Graff-Radford N, Petersen RC, Dickson D, Der SD, Fraser PE, Schmitt-Ulms G, Younkin S, Mayeux R, Farrer LA, St George-Hyslop P. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nature Genetics. 2007; 39(2):168–77. [PubMed: 17220890]

Rudovich N, Pivovarova O, Fisher E, Fischer-Rosinsky A, Spranger J, Mohlig M, Schulze MB, Boeing H, Pfeiffer AF. Polymorphisms within insulin-degrading enzyme (IDE) gene determine insulin metabolism and risk of type 2 diabetes. J Mol Med (Berl). 2009; 87(11):1145–51. [PubMed: 19809796]

Sakai A, Ujike H, Nakata K, Takehisa Y, Imamura T, Uchida N, Kanzaki A, Yamamoto M, Fujisawa Y, Okumura K, Kuroda S. No association between the insulin degrading enzyme gene and Alzheimer's disease in a Japanese population. Am J Med Genet B Neuropsychiatr Genet. 2004; 125B(1):87–91. [PubMed: 14755451]

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med. 1996; 2(8):864–70. [PubMed: 8705854]

Schupf N, Patel B, Pang D, Zigman WB, Silverman W, Mehta PD, Mayeux R. Elevated plasma beta-amyloid peptide Abeta(42) levels, incident dementia, and mortality in Down syndrome. Arch Neurol. 2007; 64(7):1007–13. [PubMed: 17620492]

Schupf N, Patel B, Silverman W, Zigman WB, Zhong N, Tycko B, Mehta PD, Mayeux R. Elevated plasma amyloid beta-peptide 1–42 and onset of dementia in adults with Down syndrome. Neuroscience Letters. 2001; 301(3):199–203. [PubMed: 11257432]

Schupf N, Tang MX, Fukuyama H, Manly J, Andrews H, Mehta P, Ravetch J, Mayeux R. Peripheral Abeta subspecies as risk biomarkers of Alzheimer's disease. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(37):14052–7. [PubMed: 18779561]

Schupf N, Zigman WB, Tang MX, Pang D, Mayeux R, Mehta P, Silverman W. Change in plasma AB peptides and onset of dementia in adults with Down syndrome. Neurology. 2010; 75(18):1639–44. [PubMed: 21041786]

Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. Physiol Rev. 2001; 81(2):741–66. [PubMed: 11274343]

Shulman JM, Chen K, Keenan BT, Chibnik LB, Fleisher A, Thiyyagura P, Roontiva A, McCabe C, Patsopoulos NA, Corneveaux JJ, Yu L, Huentelman MJ, Evans DA, Schneider JA, Reiman EM, De Jager PL, Bennett DA. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurology. 2013; 70(9):1150–7. [PubMed: 23836404]

Silverman W, Schupf N, Zigman W, Devenny D, Miezejeski C, Schubert R, Ryan R. Dementia in adults with mental retardation: assessment at a single point in time. Am J Ment Retard. 2004; 109(2):111–25. [PubMed: 15000666]

Singth, I.; Dickinson, M. Down syndrome, dementia and superoxide dismutase. In: Prasher, V., editor. Down syndrome and Alzheimer's disease: biological correlates. Raadcliffe; Oxford: 2006. p. 79-85.

Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DM, Tabaton M, Gambetti P. Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. Nat Med. 1996; 2(1):93–5. [PubMed: 8564851]

Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A. Plasma levels of amyloid beta proteins Abeta1-40 and Abeta1-42(43) are elevated in Down's syndrome. Ann Neurol. 1997; 41(2):271–3. [PubMed: 9029078]

Toledo JB, Shaw LM, Trojanowski JQ. Plasma amyloid beta measurements - a desired but elusive Alzheimer's disease biomarker. Alzheimers Res Ther. 2013; 5(2):8. [PubMed: 23470128]

Vardarajan BN, Bruesegem SY, Harbour ME, St George-Hyslop P, Seaman MN, Farrer LA. Identification of Alzheimer disease-associated variants in genes that regulate retromer function. Neurobiology of Aging. 2012; 33(9):2231.e15–e30. [PubMed: 22673115]

Vepsalainen S, Parkinson M, Helisalmi S, Mannermaa A, Soininen H, Tanzi RE, Bertram L, Hiltunen M. Insulin-degrading enzyme is genetically associated with Alzheimer's disease in the Finnish population. J Med Genet. 2007; 44(9):606–8. [PubMed: 17496198]

Younkin SG. The AAP and PS1/2 mutations linked to early onset familial Alzheimer's disease increase the extracellular concentration and A beta 1–42 (43). Rinsho Shinkeigaku. 1997; 37(12): 1099. [PubMed: 9577655]

Zhao Z, Xiang Z, Haroutunian V, Buxbaum JD, Stetka B, Pasinetti GM. Insulin degrading enzyme activity selectively decreases in the hippocampal formation of cases at high risk to develop Alzheimer's disease. Neurobiology of Aging. 2007; 28(6):824–30. [PubMed: 16769157]

Zis P, Dickinson M, Shende S, Walker Z, Strydom A. Oxidative stress and memory decline in adults with Down syndrome: longitudinal study. J Alzheimers Dis. 2012; 31(2):277–83. [PubMed: 22561328]

Zuo X, Jia J. Promoter polymorphisms which modulate insulin degrading enzyme expression may increase susceptibility to Alzheimer's disease. Brain Res. 2009; 1249:1–8. [PubMed: 18996360]

Highlights

- Adults with DS overexpress *APP*, have increased levels of Aβ peptides and are at high risk for AD.
- The relation of variants in genes on chromosome 21 and other chromosomes to Aβ peptide levels was examined.
- Variants in *CAHLM1, IDE, SOD1 and SORCS1* were related to differences in levels of Aβ2, Aβ40 and the ABβ2/Aβ40 ratio.
- Variants influencing levels of Aβ peptides can provide insight into pathways active in the pathogenesis of AD in adults with DS.

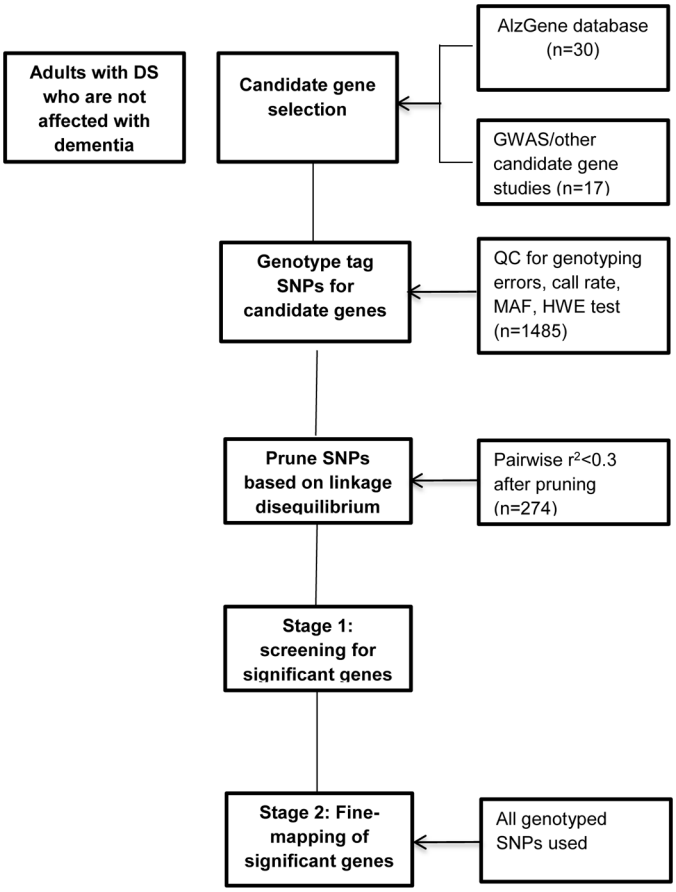

Figure 1.

Flow chart for a 2-stage candidate gene study of Abeta;42, Abeta;40, or the Abeta;42/40 ratio

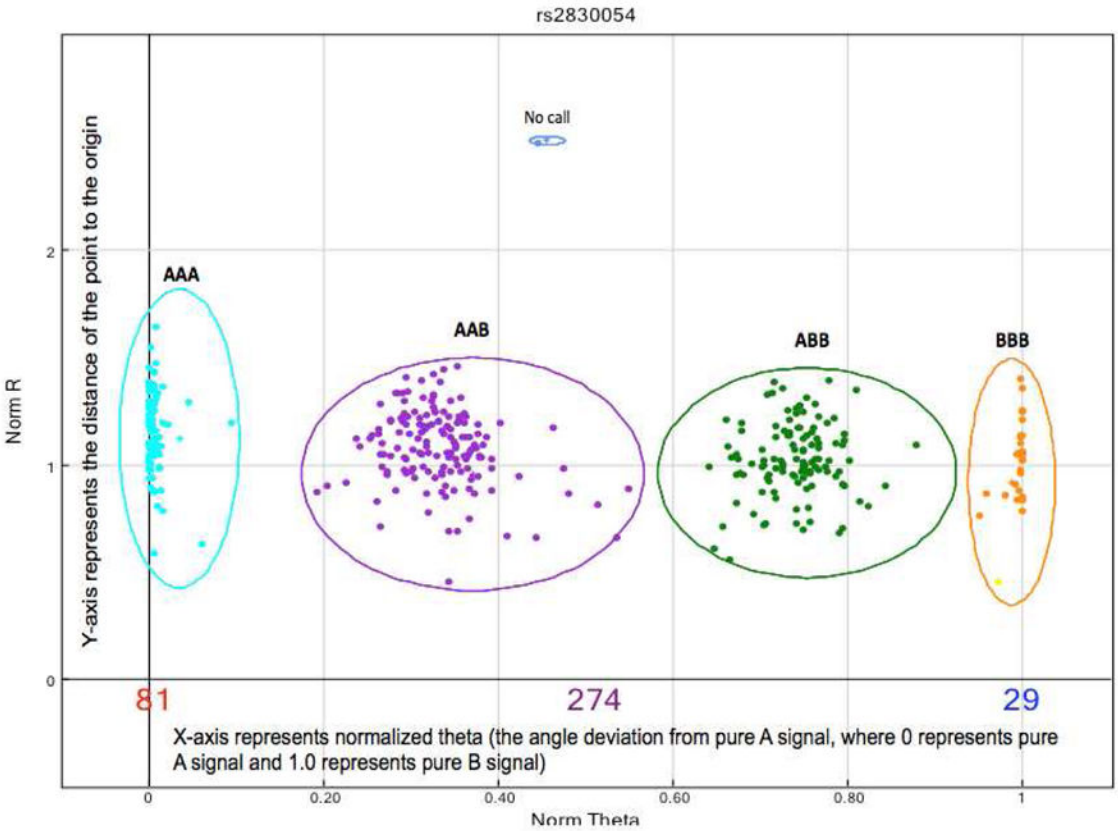

Figure 2.
Cluster plot for a SNP on chromosome 21: rs2830054 as an example

Table 1

Demographic Characteristics

Characteristic	Participants
Sample Size	254
Age (Mean ± S.D.)	49.6 ± 6.8
Sex (n, %)	
Male	60 (23.6)
Female	194 (76.4)
Level of Intellectual Disability (n.%)	
Mild/Moderate	144 (56.7)
Severe/Profound	110 (43.3)
Ethnicity (n, %)	
White	230 (90.6)
Non-White	24 (9.4)
APOE allele frequency	
E4	0.1133
E3	0.8066
E2	0.0800
Aβ Peptides (pg/ml: Mean, range)	
Aβ42	28.2 (8.0–132.4)
Aβ40	156.0 (24.3–491.4)
Aβ42/Aβ40 ratio	0.21 (0.047–1.15

Table 2

Aβ42 SNPS significant in Stage 1* and Stage 2 analysis

Chr	GENE	SNP*	Location (Hg19)	Risk Allele	MAF	BETA	SE	Empirical (Pointwise)	Genewise BH adjusted Empirical p
21	RUNX1	rs8134179	36,279,713	G	0.188	3.08	1.20	0.0107	0.7169
21	DSCR1	rs2284599	35,952,322	T	0.315	−2.01	0.95	0.0361	0.1487
21	DSCR1	rs3787720	35,904,053	T	0.352	−2.10	1.01	0.0374	0.1487
1	MTHFR	rs17037425	11,870,383	A	0.131	3.58	1.81	0.0469	0.0871
6	MTHFD1L	rs10457867	151,318,079	A	0.172	3.69	1.61	0.0205	0.7708
7	RELN	rs362710	103,177,947	G	0.267	−2.76	1.34	0.0421	0.5810
7	RELN	rs362642	103,249,871	A	0.486	2.71	1.16	0.0167	0.5762
10	**CALHM1**	**rs755577**	**105,224,066**	**A**	0.302	**3.32**	**1.30**	**0.0088**	**0.0352**
10	**CALHM1**	**rs17736358**	**105,226,538**	**G**	0.174	**4.36**	**1.51**	**0.0036**	**0.0288**
10	SORCS1	rs11814111	108,355,659	A	0.251	−3.78	1.42	0.0090	0.0665
10	SORCS1	rs878183	108,533,358	A	0.183	4.77	1.54	0.0022	0.0661
10	SORCS1	rs4918282	108,862,741	A	0.412	−2.70	1.18	0.0241	0.1004
11	SORL1	rs578506	121,323,477	G	0.482	2.86	1.17	0.0143	0.8294
15	ACAN	rs2280468	89,381,556	A	0.299	−2.76	1.33	0.0371	0.7580
X	PCDH11X	rs5984894	91,393,737	G	0.461	3.01	1.21	0.0145	0.2973
X	PCDH11X	rs2573905	91,402,220	G	0.495	3.21	1.22	0.0107	0.2973

*Positive SNPS from Stage 1 are highlighted in gray. Un-highlighted SNPS were added in Stage 2

*SNPS with adjusted empirical P < .05 based on the genewise Benjamini and Hochberg method in Stage 2 are in bold

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Table 3

Aβ40 SNPS significant in Stage 1* and Stage 2 analysis

Chr	GENE	SNP**	Location (Hg19)	Minor Allele	MAF	BETA	SE	Empirical (Pointwise)	Genewise BH adjusted Empirical p
21	APP	rs7283500	27,343,288	C	0.207	−11.61	5.45	0.0326	0.7137
21	SOD1	rs1041740	33,040,162	T	0.319	−10.61	4.51	0.0191	0.0529
21	SOD1	rs4817420	33,040,371	A	0.323	−9.5	4.49	0.037	0.0529
21	SOD1	rs12626475	33,042,929	G	0.339	−8.73	4.52	0.0529	0.0529
21	DSCR1	rs3787720	35,904,053	T	0.352	−10.88	4.47	0.0149	0.1886
21	BACE2	rs6517653	42,534,253	A	0.169	13.05	5.45	0.0174	0.2353
2	BIN1	rs1866236	127,895,655	C	0.287	17.95	6.17	0.0041	0.1148
7	RELN	rs11981312	103,289,857	A	0.351	18.53	7.07	0.0089	0.4071
7	RELN	rs12705170	103,581,271	A	0.208	−18.11	7.13	0.0118	0.4071
9	DAPK1	rs3128522	90,216,821	G	0.299	−14.01	6.45	0.0290	0.6386
10	**IDE**	**rs11187009**	**94,217,038**	**A**	**0.224**	**23.37**	**6.75**	**0.0005**	**0.0020**
10	**IDE**	**rs7899603**	**94,225,017**	**G**	**0.373**	**20.78**	**5.97**	**0.0003**	**0.0020**
10	**IDE**	**rs4646957**	**94,229,912**	**A**	**0.339**	**18.61**	**6.10**	**0.0022**	**0.0048**
10	**IDE**	**rs11187025**	**94,257,976**	**A**	**0.188**	**24.34**	**7.29**	**0.0009**	**0.0021**
10	**IDE**	**rs17445028**	**94,271,176**	**G**	**0.188**	**24.34**	**7.29**	**0.0009**	**0.0021**
10	**IDE**	**rs7078413**	**94,290,484**	**C**	**0.315**	**24.29**	**6.19**	**0.0001**	**0.0020**
10	**IDE**	**rs1832197**	**94,298,331**	**A**	**0.364**	**20.17**	**5.90**	**0.0007**	**0.0020**
10	**IDE**	**rs11187060**	**94,304,132**	**A**	**0.321**	**20.72**	**6.02**	**0.0005**	**0.0020**
10	**IDE**	**rs17445328**	**94,305,189**	**G**	**0.224**	**23.37**	**6.75**	**0.0005**	**0.0020**
10	**IDE**	**rs11187064**	**94,308,253**	**G**	**0.224**	**23.37**	**6.75**	**0.0005**	**0.0020**
10	**IDE**	**rs7076966**	**94,325,511**	**A**	**0.491**	**−14.29**	**6.37**	**0.0267**	**0.0534**
10	**IDE**	**rs7099761**	**94,335,799**	**A**	**0.480**	**20.54**	**6.20**	**0.0007**	**0.0020**
10	**IDE**	**rs11187076**	**94,336,963**	**G**	**0.423**	**22.57**	**6.46**	**0.0006**	**0.0020**
15	ACAN	rs1983332	89,409,603	G	0.290	−12.83	6.39	0.0456	0.3053
19	LDLR	rs4804570	11,256,059	G	0.224	−18.26	7.60	0.0172	0.2408
X	PCDH11X	rs5942146	91,437,953	A	0.491	−14.03	6.06	0.0181	0.2255

Chr	GENE	SNP**	Location (Hg19)	Minor Allele	MAF	BETA	SE	Empirical (Pointwise)	Genewise BH adjusted Empirical p
X	PCDH11X	rs3855797	91,704,677	A	0.246	−15.70	7.68	0.0431	0.2895

*Positive SNPS from Stage 1 are highlighted in gray. Un-highlighted SNPS were added in Stage 2

*SNPS with adjusted empirical P < .05 based on the genewise Benjamini and Hochberg method in Stage 2 are in bold

TABLE 4

Aβ42/Aβ40 SNPS significant in Stage 1* and Stage 2 analysis

Chr	GENE	SNP**	Location (Hg19)	Minor Allele	MAF	BETA	SE	Empirical (Pointwise)	Genewise BH adjusted Empirical p
21	APP	rs7283500	27,343,288	C	0.207	0.03	0.01	0.049	0.3305
21	RUNX1	rs12627198	36,420,786	A	0.156	−0.04	0.02	0.0163	0.3585
21	BACE2	rs6517653	42,534,253	A	0.169	−0.04	0.01	0.0144	0.2496
21	BACE2	rs8130833	42,556,885	G	0.278	−0.03	0.01	0.0462	0.3295
21	BACE2	rs960230	42,622,479	T	0.171	0.05	0.02	0.0056	0.2496
9	DAPK1	rs2058882	90,114,746	G	0.1878	−0.04	0.02	0.0436	0.8611
9	DAPK1	rs3095747	90,218,678	C	0.3416	−0.04	0.02	0.0152	0.8611
10	SORCS1	rs11814111	108,355,659	A	0.2511	−0.05	0.02	0.0067	0.0577
10	**SORCS1**	**rs10884341**	**108,479,649**	**A**	0.1946	**0.06**	**0.02**	**0.0022**	**0.0337**
10	**SORCS1**	**rs11193042**	**108,531,704**	**C**	0.2081	**0.06**	**0.02**	**0.0021**	**0.0337**
10	**SORCS1**	**rs878183**	**108,533,358**	**A**	0.1833	**0.07**	**0.02**	**0.0017**	**0.0337**
10	**SORCS1**	**rs11193046**	**108,536,810**	**A**	0.2104	**0.06**	**0.02**	**0.0021**	**0.0337**
10	**SORCS1**	**rs7091546**	**108,546,957**	**G**	0.2081	**0.06**	**0.02**	**0.0015**	**0.0337**
10	**SORCS1**	**rs10509823**	**108,554,591**	**G**	0.2104	**0.06**	**0.02**	**0.0021**	**0.0337**
10	**SORCS1**	**rs7916892**	**108,647,761**	**C**	0.4072	**0.05**	**0.02**	**0.0030**	**0.0394**
11	SORL1	rs578506	121,323,477	G	0.4819	0.03	0.02	0.0323	0.9796
19	LDLR	rs4804570	11,256,059	G	0.224	0.04	0.02	0.0352	0.2464

*Positive SNPS from Stage 1 are highlighted in gray. Un-highlighted SNPS were added in Stage 2

*SNPS with adjusted empirical P < .05 based on the genewise Benjamini and Hochberg method in Stage 2 are in bold

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