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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.

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

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(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.

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.

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