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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 candidates genes for Alzheimer's disease (AD) to individual differences in levels of beta amyloid peptides in adults with Down syndrom, a population at high risk for AD. Participants were 254 non-demented adults with Down syndrome, 30–78 years of age. Genomic deoxyribonucleic acid 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 and/or ethnicity, and the presence of the *APOE* ε4 allele. For Aβ42 levels, the strongest gene-wise association was found for a single nucleotide polymorphism (SNP) on *CAHLM1*; for Aβ40 levels, the strongest gene-wise associations were found for SNPs 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 amyloid precursor protein processing (*CALHM1*, *IDE*), vesicular trafficking (*SORCS1*), and response to oxidative stress (*SOD1*).

1. Introduction

Amyloid β (Aβ) plays a critical role in the development of Alzheimer's disease (AD). Aβ peptides Aβ40 and Aβ42 are the 2 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 Aβ42/Aβ40 ratio and high concentrations of tau in patients with mild cognitive impairment are associated with higher brain Aβ load (Fagan et al., 2006, 2007, 2009) 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 biological markers of risk, sensitive to the development and progression of AD.

Individuals with Down syndrome (DS) have increased risk for AD neuropathology and clinical dementia, which has been

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attributed to triplication and overexpression of the gene for *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., 2001, 2007). However, there are large individual differences in initial Aβ peptide levels and a wide range of age at the 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 reducing 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, 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 single nucleotide polymorphisms (SNPs) in candidate genes to Aβ levels using plasma from the baseline visit (Schupf et al., 2010) to identify genetic factors associated with individual difference in levels of Aβ 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 (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. 76.4% of the study sample was women. The high frequency of women 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 and/or 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 Aβ42 and Aβ40

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 enzyme-linked immunosorbent assay 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 ($r = 0.93$ and 0.97 for Aβ40 and Aβ42, respectively, $p < 0.001$), and the mean of the 2 measurements was used in statistical analyses.

2.4. Apolipoprotein E genotypes

Apolipoprotein E (*APOE*) genotyping used standard polymerase chain reaction-restriction fragment length polymorphism methods using *Hha*1 (*Cfo*I) digestion of an *APOE* genomic polymerase chain reaction 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.

2.4.1. 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 6 candidate genes on chromosome 21, and 41 genes on other chromosomes. Candidate genes on chromosome 21 included the genes for *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