Neuroglobin and Alzheimer's dementia: Genetic association and gene expression changes

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Abstract

We previously reported strong genetic linkage on chromosome 14q to Alzheimer's disease (AD) using the presence of co-morbid hallucinations as a covariate. Those results suggested the presence of a gene increasing the risk for a genetically homogeneous form of AD characterized by the absence of comorbid hallucinations. Here we report our follow up of that study through the analysis of single nucleotide polymorphisms (SNPs) in five functional candidate genes. This work provides significant evidence of association for the gene coding for neuroglobin (*NGB*), a nervous system globin known to protect cells against amyloid toxicity and to attenuate the AD phenotype of transgenic mice. On further experiments we found that *NGB* expression is reduced with increasing age and lower in women consistent with their increased risk. *NGB* expression is up-regulated in the temporal lobe of AD patients consistent with a response to the disease process, as reported for *NGB* and hypoxia. We speculate that a compromised response due to DNA variation might increase the risk for AD. Our and others' data strongly support the involvement of *NGB* in AD.
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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deterioration of memory, cognition, behavior, emotion, and intellect. With the exception of rare early onset forms with Mendelian inheritance it most commonly affects people over the age of 65. It is a major public health problem, affecting over five million Americans today, a number that could range from 11 to 16 million by the year 2050 (Alzheimer's Association data 2008; www.alz.org). Early onset forms of AD (about 5% of cases)

have an exclusively genetic etiology, with an autosomal dominant mode of inheritance and three identified causative genes *PSEN1*, *PSEN2* and *APP* (Goate et al., 1991; Levy-Lahad et al., 1995; Sherrington et al., 1995). In late-onset AD variation in the apolipoprotein E (*APOE*) gene has been shown to be a genetic risk factor (Strittmatter et al., 1993) but it is believed that many more remain to be identified (Daw et al., 2000; Jarvik et al., 1996). A number of pathogenic mechanisms have been implicated in the neurodegenerative process of AD, among the most studied being apoptosis (Bamberger and Landreth, 2002; Shimohama, 2000; Takuma et al., 2005), oxidative stress (Cecchi et al., 2002; Gibson and Huang, 2005; Nunomura et al., 2006; Reynolds et al., 2007; Shi and Gibson, 2007; Zhu et al., 2007), hypoxia (Li et al., 2007a,b; Peers et al., 2007) and inflammation (McGeer et al., 2006; Weisman et al., 2006; Wyss-Coray, 2006). Although those mechanisms are closely related to each

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other, the exact cause–effect relationship that leads to disease remains unknown.

In an effort to find the remaining genetic loci contributing to late-onset AD risk, several linkage studies have been performed (Blacker et al., 2003; Kehoe et al., 1999; Pericak-Vance et al., 2000) and have reported a large number of chromosomal regions potentially harboring risk loci. Although some of these regions appear more consistently in the literature (Bertram and Tanzi, 2004) no gene has been implicated with a certainty similar to that for APOE. In fact, recent genome scans for association have shown that it is unlikely for another locus to exist in the genome bearing a single risk variant with the effect size of *APOE* (Waring and Rosenberg, 2008). The lack of consistency and the weak results in both linkage and association studies likely reflect an underlying genetic and allelic heterogeneity. In a previous study, we addressed heterogeneity through the incorporation of covariates in a genome wide linkage analysis and detected strong linkage with a LOD score of 3.91 on chromosome 14q when the presence or absence of hallucinations was considered (Avramopoulos et al., 2005). Sequencing of multiple patients excluded the presence of mutations in *PSEN1*, which is located in the same region (Avramopoulos et al., 2005). Here we report on a follow-up study of genetic association for selected candidate genes in the 14q region that provides significant evidence for the involvement of the neuroglobin gene (*NGB*), and we further show that *NGB* has an RNA expression profile that supports its involvement in AD. Our data, in combination with the previous functional studies, make *NGB* a very interesting candidate as a genetic determinant of AD risk.

2. Materials and methods

2.1. Sample description

Genotyping sample: We initially screened five candidate genes in our linkage region using a study design that attempted to reduce heterogeneity by integrating information on the presence of hallucinations. We genotyped 99 patients with comorbid hallucinations, 125 patients without hallucinations and 152 cognitively healthy control subjects aged 58–99 years (Table 1). Cases were from the NIMH collection and were assessed for psychotic symptoms as described (Avramopoulos et al., 2005) while controls were from the collection of the Indiana cell repository (NCRAD). Our fol-

low up study on *NGB* included 351 cases from the NIMH and the Indiana repositories as well as 289 healthy controls (Table 1) aged 48–99 (median = 74, mean = 73.2), 197 from NCRAD and 92 cognitively healthy spouses of the offspring of the NIMH subjects.

The samples used for sequencing *NGB* were 24 cases from the NIMH families showing the strongest linkage on 14q and 24 healthy controls. Samples used for gene expression analyses were punches from the temporal lobe of 30 deceased patients with confirmed AD pathology and 26 controls with no brain pathology. The time between death and harvest of the brain (Post Mortem Delay; PMD) varied from 2 to 24 h. Cases were older than controls (83.3 ± 4.6 years vs. 75.1 ± 14.3 years mean $\pm$ S.D.) and included more females (22 of 30 vs. 13 of 26). Both these variables were found to correlate significantly with the gene expression and were corrected for in our model. PMD was higher in the controls (11.5 ± 5.1 h vs. 7.7 ± 4.1 h mean $\pm$ S.D.) but was not found to correlate with gene expression measurements ($p = 0.8$).

All procedures involving human subjects were in accordance with the Declaration of Helsinki and were approved by the Johns Hopkins Institutional review board.

2.2. Candidate gene identification and SNP selection

The 1 LOD interval identified in our previous linkage study spanned a 26-Mb region containing approximately 150 known genes. Through a systematic literature search on each of the genes for co-occurrence in publications with keywords relevant to AD or psychosis (dementia, Alzheimer's, psychosis, hallucinations, schizophrenia, brain, neuron, hippocampus, presenilin, amyloid, amyloid beta, secretase, apoptosis, inflammation, oxidative stress, aging, cholesterol, mitochondria) we chose five genes for follow up: dihydrolipoamide *S*-succinyltransferase (*DLST*), the hypoxia-inducible factor 1, alpha subunit (*HIF1A*), neuroglobin (*NGB*), numb homolog (*NUMB*), and sphingosine-1-phosphatase (*SGPP1*).

We downloaded reference genotype data from the HapMap project (Frazer et al., 2007; The International HapMap Consortium, 2003) genome browser (www.hapmap.org, October 2005 release) in each of the candidate genes and surrounding regions to the extent of linkage disequilibrium (LD) islands according to the Gabriel et al definition (Gabriel et al., 2002). We then analyzed the SNPs with allele frequency greater than 2% for pairwise LD and

Table 1
Description of association studies.

	Number of SNPs	Cases with psychosis	Cases without psychosis	Total cases	Controls
Original screen of 5 genes	26	99	125	224*	152
Follow-up of NGB	37	29*	53*	351†	289

Numbers of SNPs and samples used in the two steps of our association study. *These were not directly compared to controls due to study design. †Includes 269 cases with no information on the presence of psychotic symptoms.