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Research Article

Identification of Novel Gene variants in Patients with Alzheimer's Disease by Whole Exome Sequencing

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Introduction

Alzheimer's Disease (AD) affects millions of elderly people, many of the patients partially or completely lost the capability to maintain independent daily living [1-3]. Limited progress was made in the past decades in the designing intervention approaches that could effectively delay the progression of the disease. Novel leads are in urgent need. Next Generation DNA sequencing (NGS) technology has been widely used in the basic biomedical research and molecular diagnosis in clinical settings. Few NGS studies in AD were reported, and those reported focused on rare variants from a few genes, such as *APP*, *PSEN1*, *PSEN2*, *SORL1*, and *TREM2* [4-6]. Recently results from some Whole Exome Sequencing (WES) studies with specimens from AD patients were reported, but a general variant landscape is still missing [7-9]. Accumulated variants of these genes only account for the genetics of a small fraction of AD patients. Genome-Wide Association Studies (GWAS) identified several dozen AD associated genes, but most of the associations are weak [10-12]. We performed a WES study with specimens from AD patients, and we identified several dozen novel gene variants. These novel variants could potentially be causative mutations for AD or variants in association with AD.

Materials and methods

All subjects enrolled in this study were outpatients or hospitalized patients in the Jiangsu Province Geriatric Hospital from 2015 to 2018. Specimens from 36 AD patients were analyzed in this WES study. The clinicopathological data were summarized in Table S1. Diagnosis of AD was based on NINCDS-ADRDA criteria [13,14]. No data from cerebrospinal fluid analysis or PET imaging were used for diagnosis or analysis in this study. No post-mortem data were used. Most patients belong to late onset AD, and about 20% of patients were younger than 65 years old. Informed consent was obtained from all subjects. The study was approved by the Ethical Committee on Medical Research of the hospital. Considering the nature of low population frequencies of AD gene variants, data from a small size of non-AD subjects could hardly be representative and cannot serve as appropriate controls, population data from Chinese Millionome Database (CMDB, https://db.cngb.org/cmdb/) were used for comparison.

Blood specimens were drawn to tubes with EDTA, and stored at -80 C before DNA extraction. Genomic DNA extraction, library construction, human exome capturing, and NGS sequencing were as performed as described previously [15,16]. Initial data processing and variant calling were performed at Novogene **Co. Ltd** in Beijing. Human genome hg19 was used as reference sequences in BWA mapping. GATK and Samtools were used in variant calling. VCF files were annotated using ANNOVAR [15].

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Variants meeting all of the following criteria were selected for further analysis: the variant in protein coding region; the variant not listed in dbSNP database (https://www.ncbi.nlm.nih.gov/snp/, dbSNP152) or listed but with minor allele frequencies less than 1%; no frequency information available from ExAc (http://exac.broadinstitute.org/gene) or CMDB; recurrent variants or multiple variants of the same gene; the variant projected to be damaging or possibly damaging to the function of the protein by at least two of the three programs, SIFT, Polyphen-2, Mutation Taster, or the variant being a stopgain mutation; reported to be AD associated or related to brain development and function.

Results

Sequencing with each specimen generated 3-7 Gb data. Overall, the NGS sequencing data showed Q20 data over 97%, and Q30 data over 92%. Variants with sequencing quality below Q20 were not used. SNP typing results from MassARRAY and from the WES were compared. The result showed the WES method had a variant detection specificity of 100% and sensitivity of 80.5%.

We identified novel variants of 28 most relevant genes (Tables 1,2). These genes were not listed in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) as AD pathogenic genes. The genes or products of the genes in Table 1 have been studied in AD as reported in the literature. Research results of the genes in Table 2 in AD were not found in the literature, but the gene products are related to brain development and function, thus their variants may still contribute to the AD development. We found 26 out of the 36 (72.2%) AD patients carried variants in genes listed in Tables 1,2, some of them carried more than 10 variants. Some of the gene variants were recurrent, some genes were frequently affected (occurrence was 5 or more among the 36 specimens). Most of the genes in Table 1, Table 2 were located in Chromosome 1, 4, 7, or 11. The details of gene variants, functional analysis of variants, and the clinicopathological data were summarized in Table S1. Variants of genes seemingly not directly related to AD or brain development and function were summarized in Table S2. It seems that some of the gene variants showed characteristic distribution patterns as much more common in male patients or patients with advanced disease stages (Table S3). Of course, the distribution patterns were preliminary considering the limited sample size.

Discussion

Amyloid cascade hypothesis has been the center of AD pathogenesis and basis for the development of therapeutics [1]. Inflammation, especially the innate immunity, has also been recognized as a key process in the pathology of the disease [2]. However, most drug candidates for AD failed in clinical trials and those in clinical use could not stop the progression of the disease. Novel leads are in urgent need.

We identified several dozen novel gene variants by performing WES of specimens from AD patients. Most patients carried multiple gene variants. Frequently affected genes or recurrent gene variants were common. Genes in Table 1 have

Table 1: Novel variants of genes in AD with references

gene	variants	variant features*	pathway and function	References
ADAR	NM_001025107: G674C, W916C	recurrent	RNA editing	[19]
AHNAK	NM_001620: R132L, K5723E, G5871V	recurrent	blood brain barrier formation	[20]
APOB	NM_000384: P421L, A2015T, G2927W, S3301X	stopgain	lipid transportation	[21]
HMCN1	NM_031935: F46L, H985R, S3078Y	recurrent; frequent	cell adhesion and junction	[22]
HSPG2	NM_005529: R1685P, V1877L, L3754M, K4023N		extracellular matrix	[23]
IL16	NM_172217: R351I, S795I, L1285F, G1330X	stopgain	inflammation	[24]
INPPL1	NM_001567: K509E, Q817K	recurrent	inositol 5'-phosphatase, neurodegeneration	[25]
LRP1	NM_002332: D917Y, A3454S, P3486H, N3545K, W3592C	frequent	lipoprotein receptor	[26]
NTN1	NM_004822: N353K	recurrent	axon guidance and cell migration	[27]
RYR1	NM_001042723: K2653N, R3364Q, M3994I, R4174H	recurrent; frequent	Ryanodine receptor	[28]
SORL1	NM_003105: G111V, E627G	recurrent	endocytic receptor	[29]
TNC	NM_002160: E973K, R1016S, E2008G, N2039Y		extracellular matrix	[30]
TNR	NM_003285: R304L, R605C, R905L, D1079E		extracellular matrix	[22]

Recurrent: the same variant happened in multiple patients. Frequent: different variants of one gene happened multiple times and in multiple patients.

Table 2: Novel variants of genes in AD with indirect evidences.

Gene	Variants	Variant features*	Pathway and function
BAI2	NM_001703: R85H, D536Y, V985M, S1543R		brain-specific inhibitor of angiogenesis
CCDC120	NM_001163321: P129L, R628L	recurrent	mitosis, neurite growth
DNAH11	NM_001277115: G157X, E779X, Q1258X, A3660S, V3708L	stopgain; frequent	ciliary dynein heavy chain, NSC differentiation
DNAH14	NM_001373: G1553V, R2808I, T2989N, Y3532X, A3890P	stopgain; frequent	Ciliary dynein heavy chain, NSC differentiation
FAT1	NM_005245: E728Q, D817Y, D953Y, N2176S, G3802X, P4279Q	stopgain; frequent	brain development
FAT4	NM_024582: V984F, V1586M, D1784Y, S1945R, G3314S	recurrent; frequent	brain development
HTR3B	NM_006028: Y60X	recurrent	serotonin receptor
HUWE1	NM_031407: G152V, P1040Q, D2473Y, V3288I		neural proliferation and differentiation
KMT2C	NM_170606: V1437F, D2904Y, Q3591K, R4828S, R4875I, Q4877K	frequent	Histone methyltransferase, neural development
KMT2D	NM_003482: C294F, P646T, D2367Y, Q3964R		Histone methyltransferase, neural development
MATN2	NM_002380: A123V	recurrent	extracellular matrix, neuroinflammation
MX2	NM_002463: P515S	recurrent	dynamin-like GTPase, neuroinflammation
NLRC4	NM_001199138: G618W	recurrent	neuroinflammation
UBR4	NM_020765: T3161K, R3825C, L4020F, Q4500K, K4679N	frequent	neurogenesis and development
UNC13B	NM_006377: A1455E	recurrent	exocytosis and neural development

Recurrent: the same variant happened in multiple patients. Frequent: different variants of one gene happened multiple times and in multiple patients.

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been studied in AD. However, these gene variants were not reported in AD based on our literature searching. Commonly affected pathways include lipid transportation, extracellular matrix, cell adhesion and migration, inflammation and neurodegeneration. Variants identified in this study need to be studied further in AD patients as well as in general population in large sample sizes. Some of the variants might be rare gene polymorphism with or without associations with AD, and others might be true causative gene mutations that contribute greatly to the AD development. Some other variants might also contribute to the development of other cognitive diseases such as Parkinson's disease [17-30].

Our results provide valuable additions to the studies of AD genetics. In contrast to other hereditary diseases that are commonly caused by a single gene mutation or compound gene mutations (monogenic), AD may be a result of the sum of detrimental effects from multiple gene mutations, gene polymorphism, and epigenetic dysregulation, in addition to the effects from environment factors.

Additional data

Table S1: Gene variants and clinicopathological data.

Table S2: List of other gene variants.

Table S3: Characteristic distribution of gene variants

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