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].
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 variants). Some of the gene variants happened multiple times and in multiple patients. Frequent affected genes or products of the genes in Table 1 have been studied in AD with possible associations with AD (Tables 1,2). These genes were not listed in ClinVar (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.

**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 been studied in AD with possible associations with AD (Tables 1,2). These genes were not listed in ClinVar (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.

**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] |
| AHNK | NM_001620: R132L, K572E, G587IV | recurrent | blood brain barrier formation | [20] |
| APOE | 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: R166SP, V1877L, L3754M, K4023N | extracellular matrix | [23] |
| IL1 | NM_172217: R351, S795I, L1285F, G1330X | stopgain | inflammation | [24] |
| INPP1 | NM_001567: K509E, Q817K | recurrent | inositol 3-phosphatase, neurodegeneration | [25] |
| LRPI | NM_002332: D917Y, A3454S, P3464H, 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, R625C, R905L, D1079E | extracellular matrix | [22] |

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

| Gene | Variants | Variant features* | Pathway and function |
|------|----------|------------------|----------------------|
| BAI1 | NM_001703: R85H, D536Y, V985M, S1543R | brain-specific inhibitor of angiogenesis |
| CCDC120 | NM_001163321: P129L, R628L | recurrent | mitosis, neurite growth |
| DNAH1 | NM_001277711: G157X, E779X, Q1258X, A3660S, V3708L | stopgain, frequent | cilary dynene heavy chain, NSC differentiation |
| DNAH14 | NM_001373: G1553V, R2808I, T2998N, Y3532X, A3890P | stopgain, frequent | cilary dynene heavy chain, NSC differentiation |
| FAT1 | NM_005254: E728Q, D817Y, D935Y, N2176S, G3802X, P4277Q | 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, R875I, Q4877K | frequent | Histone methyltransferase, neural development |
| KMT2D | NM_003482: C294F, P646T, D3267Y, Q3964R | Histone methyltransferase, neural development |
| MATN2 | NM_002380: A123V | recurrent | extracellular matrix, neuroinflammation |
| MX2 | NM_002463: P515S | recurrent | dynamin-like GTPase, neuroinflammation |
| NLR4 | NM_001199138: G618W | recurrent | neuroinflammation |
| UBR4 | NM_020765: T3161K, R3282C, L4020F, Q4500K, K4679N | frequent | neurogenesis and development |
| UNC13B | NM_006377: A1455E | recurrent | excocytosis 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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References

1. Hardy JA, Higgins GA (1992) Alzheimer’s disease: the amyloid cascade hypothesis. Science 256: 184-185. Link: https://bit.ly/2yN1M2R
2. Heneka MT, Golenbock DT, Latz E (2015) Innate immunity in Alzheimer’s disease. Nat Immunol 16: 229-236. Link: https://bit.ly/3dzQxGv
3. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, et al. (2015) Alzheimer’s disease. Nat Rev Dis Primers 1: 15056. Link: https://bit.ly/3biakx1
4. Zhu JB, Tan CC, Tan L, Yu JT (2017) State of Play in Alzheimer’s Disease Genetics. J Alzheimers Dis 58: 631-659. Link: https://bit.ly/2WCSbn6
5. Bertram L (2016) Next Generation Sequencing in Alzheimer’s Disease. Methods Mol Biol 1303: 281-297. Link: https://bit.ly/3cn7xG
6. Bellenguez C, Charbonnier C, Grenier-Boly B, Quezne O, Le Guennec K, et al. (2017) Contribution of Alzheimer’s disease risk of rare variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls. Neurobiol Aging 59: 220 e1-220 e9. Link: https://bit.ly/3copXv
7. Jiang B, et al. (2019) Mutation screening in Chinese patients with familial Alzheimer’s disease by whole-exome sequencing. Neurobiol Aging 76: 215 e15-215 e21.
8. Park JS, Lee J, Jung ES,Kim MH, Kim IB, et al. (2019) Brain somatic mutations observed in Alzheimer’s disease associated with aging and dysregulation of tau phosphorylation. Nat Commun 10: 3090. Link: https://bit.ly/2LkniPR
9. Patel D, Mez J, Vardarajan BN, Staley L, Chung J, et al. (2019) Association of Rare Coding Mutations With Alzheimer Disease and Other Dementias Among Adults of European Ancestry. JAMA Netw Open 2: e191350. Link: https://bit.ly/2AhDgYd
10. Giri M, Zhang M, Lu Y (2016) Genes associated with Alzheimer’s disease: an overview and current status. Clin Interv Aging 11: 665-681. Link: https://bit.ly/2A9wVuC
11. Medway C, Morgan K (2014) Review: The genetics of Alzheimer’s disease, putting flesh on the bones. Neuropathol Appl Neurobiol 40: 97-105. Link: https://bit.ly/3bnr2ds
12. Raghavan N, Tosto G (2017) Genetics of Alzheimer’s Disease: the Importance of Polygenic and Epistatic Components. Curr Neurol Neurosci Rep 17: 78. Link: https://bit.ly/2Wuuo7
13. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, et al. (2007) Research criteria for the diagnosis of Alzheimer’s disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 6: 734-746. Link: https://bit.ly/2WIDWnW
14. McKhann G, Drachman D, Folstein M, Katzman R, Price D, et al. (1984) Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 34: 939-944. Link: https://bit.ly/2Zr7KV
15. Wang A, Wu L, Lin J, Han L, Bian J, Wu Y, et al. (2018) Whole-exome sequencing reveals the origin and evolution of hepatocellular carcinoma. Nat Commun 9: 894. Link: https://bit.ly/2ST4b2W
16. Wang Y, Dang X, He Q, Zhen Y, He X, Yi Z, et al. (2017) Mutation spectrum of genes associated with steroid-resistant nephrotic syndrome in Chinese children. Gene 625: 15-20. Link: https://bit.ly/2YQCqah
17. Pierzchlińska A, Białecka M, Kurzawski M, Slawek J (2018) The impact of Apolipoprotein E alleles on cognitive performance in patients with Parkinson’s disease. Neurol Neuroch Pol 52: 477-482. Link: https://bit.ly/2xR7Axh
18. Ferrari R, Wang Y, Vandrovcova J, Guerrü S, Witteolar A, et al. (2017) Genetic architecture of sporadic frontotemporal dementia and overlap with Alzheimer’s and Parkinson’s diseases. J Neurol Neurosurg Psychiatry 88: 152-164. Link: https://bit.ly/2WJEran
19. Khermesh K, D’Erchia AM, Barak M, Annese A, Wachtel C, et al. (2016) Reduced levels of protein recoding by A-to-I RNA editing in Alzheimer’s disease. RNA 22: 290-302. Link: https://bit.ly/2zpSPV
20. Manavalan A, Mishra M, Feng L, Sze SK, Akatsu H, et al. (2013) Brain site-specific proteome changes in aging-related dementia. Exp Mol Med 45: e39. Link: https://bit.ly/2zwPOeb
21. Wingo TS, Cutler DJ, Wingo AP, Le NA, Rabinovici GD, Miller BL, et al. (2019) Association between polymorphisms in Interleukin-16 gene and age at death in patients with Alzheimer disease. JAMA Neurol 76: 809-817. Link: https://bit.ly/3cpyp23z
22. Gao H, Tao Y, He Q, Song F, Saffen D (2015) Functional enrichment analysis of three Alzheimer’s disease genome-wide association studies identities DAB1 as a novel candidate liability/protective gene. Biochim Biophys Res Commun 463: 490-495. Link: https://bit.ly/2WQpikZ
23. Ilivonen S, Helisalmi S, Mannermaa A, Alafuzoff I, Lehtovirta M, et al. (2003) Heparan sulfate proteoglycan 2 polymorphism in Alzheimer’s disease and correlation with neuropathology. Neurosci Lett 352: 146-150. Link: https://bit.ly/2Aiq9pJ
24. Anvar NE, Saliminejad K, Ohadi M, Kamali K, Daneshmand P, et al. (2015) Contribution of Alzheimer’s disease risk of rare variants in TREM2, SORL1, and ABCA7 in 1779 cases and 1273 controls. Neurobiol Aging 59: 220 e1-220 e9. Link: https://bit.ly/3copXv
25. Lim JW, Kim SK, Choi SY, Kim DH, Gadhe CG, et al. (2018) Identification of crizotinib derivatives as potent SHIP2 inhibitors for the treatment of Alzheimer’s disease. Eur J Med Chem 157: 405-422. Link: https://bit.ly/2ZYqRB9

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26. Hou H, Habib A, Zi D, Tian K, Tian J, et al. (2017) Low-Density Lipoprotein Receptor-Related Protein-1 (LRP1) C4408R Mutant Promotes Amyloid Precursor Protein (APP) alpha-Cleavage in Vitro. Neuromolecular Med 19: 300-308. Link: https://bit.ly/2SRo0HQ

27. Lourenço FC, Galvan V, Fombonne J, Corset V, Llambi F, et al. (2009) Netrin-1 interacts with amyloid precursor protein and regulates amyloid-beta production. Cell Death Differ 16: 655-663. Link: https://bit.ly/2AF2YMt

28. Bruno AM, Huang JY, Bennett DA, Marr RA, Hastings M, et al. (2012) Altered ryanodine receptor expression in mild cognitive impairment and Alzheimer’s disease. Neurobiol Aging 33: 1001 e1-1001 e6. Link: https://bit.ly/2WIkzEt

29. Vardarajan BN, Zhang Y, Lee JH, Cheng R, Bohn C, et al. (2015) Coding mutations in SORL1 and Alzheimer disease. Ann Neurol 77: 215-227. Link: https://bit.ly/3blyuWj

30. Mi Z, Halfter W, Abrahamson EE, Klunk WE, Mathis CA, Mufson EJ, et al. (2016) Tenascin-C Is Associated with Cored Amyloid-beta Plaques in Alzheimer Disease and Pathology Burdened Cognitively Normal Elderly. J Neuropathol Exp Neurol 75: 868-876. Link: https://bit.ly/3cy9zQE