Segregation of a rare TTC3 variant in an extended family with late-onset Alzheimer disease

ABSTRACT

Objective: The genetic risk architecture of Alzheimer disease (AD) is complex with single pathogenic mutations leading to early-onset AD, while both rare and common genetic susceptibility variants contribute to the more widespread late-onset AD (LOAD); we sought to discover novel genes contributing to LOAD risk.

Methods: Whole-exome sequencing and genome-wide genotyping were performed on 11 affected individuals in an extended family with an apparent autosomal dominant pattern of LOAD. Variants of interest were then evaluated in a large cohort of LOAD cases and aged controls.

Results: We detected a single rare, nonsynonymous variant shared in all 11 LOAD individuals, a missense change in the tetratricopeptide repeat domain 3 (TTC3) gene. The missense variant, rs377155188 (p.S1038C), is predicted to be damaging. Affecteds-only multipoint linkage analysis demonstrated that this region of TTC3 has a LOD score of 2.66 in this family.

Conclusion: The TTC3 p.S1038C substitution may represent a segregating, rare LOAD risk variant. Previous studies have shown that TTC3 expression is consistently reduced in LOAD patients and negatively correlated with AD neuropathology and that TTC3 is a regulator of Akt signaling, a key pathway disrupted in LOAD. This study demonstrates how utilizing whole-exome sequencing in a large, multigenerational family with a high incidence of LOAD could reveal a novel candidate gene.

GLOSSARY

AD = Alzheimer disease; ADGC = Alzheimer’s Disease Genetics Consortium; APOE = apolipoprotein E; APP = amyloid precursor protein; ESP = Exome Sequencing Project; FTD = frontotemporal dementia; LOAD = late-onset AD; MAF = minor allele frequency; PSEN = presenilin; SNV = single-nucleotide variant; TTC3 = tetratricopeptide repeat domain 3; WES = whole-exome sequencing.

Alzheimer disease (AD) is the most common form of dementia in the elderly, accounting for up to 75% of all dementia patients.1 Mutations in the amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) genes have been shown to cause autosomal dominant, early-onset AD.2–4 Late-onset AD (LOAD) is etiologically more complex and presents with intermediate to high heritability (58%–79%).5 The apolipoprotein E (APOE) gene contributes significantly to LOAD risk; the APOE ε4 allele confers an increased risk to LOAD, while the rare APOE ε2 allele is protective.6–9 In addition to the APOE gene, genome-wide association studies identified 19 further loci that confer low individual, but reproducible risk to LOAD.10 Moreover, rare variants in TREM2 have been shown to contribute to LOAD risk.11 It seems likely that additional, yet unknown, rare variants with intermediate to large effects may

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contribute to the complex genetic architecture of LOAD. We identified a large, multiplex LOAD family with an autosomal dominant pattern of inheritance and negative for pathogenic variants in known AD genes. We performed whole-exome sequencing (WES) in 11 affected family members and prioritized rare variants that segregated with disease. A single alteration in tetra-tricopeptide repeat domain 3 (TTC3) was identified in all 11 LOAD individuals. Our study shows that WES in a single extended family with a high LOAD burden can lead to the identification of rare genetic variants that may contribute to risk for AD.

METHODS Standard protocol approvals, registrations, and patient consents. All individuals ascertained in this study provided written informed consent prior to their participation. If the study participant was not competent to provide consent, then written consent was obtained from the immediate next of kin or his or her legal representative. All participants were ascertained using the protocol approved by the appropriate institutional review board. Oversight of the study falls under the University of Miami (IRB #2007307).

Collection and characterization of an extended LOAD family. We identified a large family (no. 1229) that was consistent with an autosomal dominant inheritance pattern of LOAD. Family 1229 has 15 affected individuals, 3 of whom have a neuropathologically confirmed diagnosis of LOAD. All affected individuals meet the standard National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria for LOAD with a disease onset of 65 years of age or older.12 Besides direct neurologic examinations, a battery of neuropsychometric testing was performed to monitor preclinical symptoms of LOAD and comorbidities (Parkinsonism, depression) that are known to influence the course of preclinical AD. The family was evaluated to determine APOE ε genotypes and screened for high-risk variants in APP, PSEN1, and PSEN2.

Genome-wide genotyping and linkage analysis. Genotype data were obtained for 9 LOAD-affected individuals of family 1229 using the Human OmniExpress BeadChip array (Illumina, San Diego, CA). Genotype quality control comprised the exclusion of single-nucleotide variants (SNVs) with call rates below 98%. The B allele frequency plots and the log R ratio plots were used to search for potential copy number variants surrounding our top variant. These data were analyzed with MERLIN to determine an affected-only multipoint linkage LOD score with LOAD in family 1229 assuming an autosomal dominant model.13 Two-point age-dependent linkage LOD score with LOAD in family 1229 was performed using an age curve as previously described.14

Whole-exome sequencing. WES was performed in all 11 affected individuals of family 1229. WES used 3 μg of DNA from each sample. Exome capture and sequence library construction were performed on a Sciclone G3 NGS Workstation (Caliper Life Sciences, Hopkinton, MA) using the SureSelect Human All Exon 50 Mb Kit (Agilent Technologies, Santa Clara, CA) and the Paired-End Multiplexed Sequencing library kit (Illumina) for sequence library preparation. Two exome sample libraries were sequenced per lane on a HiSeq 2000 Sequencing System (Illumina) in paired-end 2 × 100 base pair runs. In addition, WES of 373 non-AD samples of European ancestry were also run and analyzed at the John P. Hussman Institute for Human Genomics in order to get minor allele frequency (MAF) estimates and control for WES artifacts.

SNV and insertion-deletion calling and annotation. Sequencing data were processed using the Illumina RTA base calling pipeline v1.8. Alignment to the human reference genome (hg19) was executed with the Burrows-Wheeler Aligner and variant calling performed with the Genome Analysis Toolkit (GATK).15,16 GATK parameters for variant quality control included duplicate sequence read removal, base quality score recalibration (QUAL >50), and variant quality score recalibration (VQSLOD >0).17 SNVs passing quality measurements were annotated with SeattleSeq (http://snp.gs.washington.edu/SeattleSeqAnnotation137/). We define rare SNVs and insertions-deletions (indels) as those with a MAF <1% in dbSNP139 (http://www.ncbi.nlm.nih.gov/snp/), 1000 Genomes Project populations, the 5,379 European and African-American samples from the NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (http://evs.gs.washington.edu/ EVS/), and 373 Caucasian exomes processed at the John P. Hussman Institute for Human Genomics.18,19 Variants were evaluated for phylogenetic conservation in mammals with the Genomic Evolutionary Rate Profiling score and PhastCons.20,21 A Genomic Evolutionary Rate Profiling score >3 and a PhastCons score >0.7 were considered phylogenetically conserved. A variant’s predicted deleteriousness to protein function was assessed by the Likelyhood Ratio Test, the Mutation Assessor, Mutation Taster, PolyPhen-2 (HumDiv), and PROVEAN/SIFT programs using the default score cutoffs for deleteriousness.22–26

SNV validation by Sanger sequencing. Ten rare SNVs identified by WES that passed quality control and were shared in >80% (≥9/11) of affected individuals of family 1229 were evaluated by Sanger sequencing in the 11 affected and 5 unaffected members of this family.

Evaluating the TTC3 variant in a LOAD cohort. The TTC3 variant was evaluated using a custom TaqMan genotyping assay (Life Technologies, Carlsbad, CA). A large cohort of 6,669 LOAD cases and 5,585 controls (≥60 years) of European ancestry from the Alzheimer’s Disease Genetics Consortium (ADGC) cohort were used. Power calculations were performed with the Genetic Power Calculator.27

RESULTS Clinical and neuropathologic characterization. DNA samples were collected from family 1229 from 11 affected and 5 unaffected members of at least 65 years old (figure 1, table e-1 at Neurology.org/ng). Clinically, each of the 11 affected individuals presented with late-onset dementia (range: 70–85 years old). The initial course of disease followed a typical AD pattern, with early forgetfulness and progressive worsening in areas of cognitive function such as short-term memory, orientation, executive function as reflected in activities of daily living, and language retrieval (word finding).

Patients were assessed for features suggestive of alternate dementing pathologies, such as dementia

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with Lewy bodies, frontotemporal dementia, and vascular dementia. Abnormal features suggestive of a frontotemporal etiology such as frontal release signs, early changes in personality, behavior, or language changes were not present in any family members. A positive snout reflex was noted approximately 9 years into the disease course in one individual (0122), but no other individual exhibited frontal release signs or symptoms. The late appearance of a single frontal release sign is not suggestive of a diagnosis of frontotemporal dementia. A second individual (9006) exhibited mild parkinsonian features (masked facies, hunching, micrographia, poor balance) when examined at age 84, ten years into the disease course. The later onset of comorbid parkinsonian features is a known entity in AD.

Four affected individuals underwent autopsy with subsequent neuropathologic results (table e-1). All individuals demonstrated classic Braak and Braak pathology, ranging in stage from III to V.

**Complete segregation of the rare TTC3 p.S1038C substitution with LOAD.** WES in 11 LOAD members of family 1229 resulted in an average coverage of 83% (±0.04% SD) of the human exome sequence at a minimal depth of 20 reads per base pair. On average, 11,227 (±305 SD) exonic SNVs were called per exome, for a total of 32,784 unique SNVs across all 11 affected family members. The majority of disease-causing variants identified to date are nonsynonymous, protein-altering SNVs including nonsense, missense, and splice site variants as well as small insertion-deletion polymorphisms (insertions/deletions). Of all exonic variants detected in family 1229, about half (16,363 SNVs) were found to be nonsynonymous.

Family 1229 is characterized by a suggestive autosomal dominant pattern of LOAD inheritance (figure 1). We therefore hypothesized that a single (or a few) highly penetrant disease variant, with considerable individual risk, could explain the high burden of LOAD observed in this family. Statistical power estimates for the detection of LOAD variants suggest that published genome-wide association studies would likely have identified loci that harbor variants with aMAF >1% in the general population. Thus, we further filtered for rare variants (MAF <1%), which reduced the candidate variants to 2,897 SNVs. Among these rare and nonsynonymous variants, only a single variant, rs377155188, was identified in all 11 affected family members; this variant is in the TTC3 gene and encodes for a serine to cysteine substitution at amino acid position 1038 (figure 1; table 1).

We evaluated the remaining rare, nonsynonymous variants. None were shared in 10 of 11 affected family members, but 9 variants were shared in 9 affected family members (table 1). All 10 variants in at least 9 LOAD family members were confirmed by traditional Sanger sequencing (table 1).

The TTC3 variant, rs377155188, is extremely rare in European Americans (MAF = 0.00012) and absent in African Americans according to the NHLBI ESP. The p.S1038C substitution in the TTC3 protein is predicted to be damaging by 5 distinct algorithms. Furthermore, it is located in a highly conserved site.
The TTC3 heterozygous genotypes identified through WES were validated by Sanger sequencing in all 11 affected individuals. Wild-type genotypes were obtained for 4 out of 5 unaffected family members ≥65 years old (figure 1). Individual no. 9018 was the only aged and unaffected heterozygous carrier of TTC3 rs377155188. However, this individual did not receive a formal cognitive examination as did the 3 other unaffected family members. This individual’s negative affection status of LOAD has been based on family history data after the individual’s death at age 76. This person’s age at death is only 1 year above the average age of LOAD onsets in this family (table e-1); thus, given the absence of a formal examination, this carrier may still have been presymptomatic at age 76.

We then evaluated the prevalence of rs377155188 by genotyping 12,254 unrelated individuals (6,669 LOAD cases and 5,585 controls) of European ancestry from the ADGC. Over all individuals, rs377155188 showed a low frequency (MAF = 0.020%), as has been reported by the ESP for Americans of European descent (MAF = 0.012%).16 We observed 5 heterozygous individuals in total. Four were LOAD cases, all of whom carried the APOE e3/e4 genotype. Only a single cognitively normal control carried the TTC3 variant (0.018%). This individual was last examined at age 84 and also carried a protective APOE e2/e3 genotype. These genotypes resulted in an allelic association odds ratio of 3.35, which did not reach statistical significance (Fisher exact test, ρ = 0.248). Power calculations show that we are underpowered to detect a significant effect in our cohort (49.6% power to achieve significance at α = 0.05; assuming a disease frequency of 0.10), given the extremely rare allele frequency of the rs377155188 variant in our cohort and the general population.27

Disruption of the TTC3 variant may, like APOE, contribute to LOAD risk; however, it is unlike APP, PSEN1, and PSEN2 and may not be sufficient to initiate AD pathogenesis.

LOAD is a common, complex, and age-related disease. Additional factors, such as the aggregation of several genetic risk variants, might contribute to LOAD. Individual 122 presented with the TTC3 risk allele and an APOE e3/e3 genotype, demonstrating that the presence of an APOE e4 is not required to confer AD risk in this family (figure 1; table e-1). In addition, the family has 9 e3/e4 AD carriers and a single e4/e4 affected individual. However, the high abundance of APOE e3/e4 genotypes in family 1229 may not be sufficient to explain the apparent autosomal dominant inheritance pattern of LOAD. The average age of LOAD onset among affected family members was 75.7 years. A study representative of the US population determined the prevalence of LOAD at ≥70 years of age to be 9.7% (95% confidence interval: 7.6%–11.9%).28 The LOAD risk of APOE e3/e4 carriers is about 3-fold higher than that found in e3/e3 carriers, while e4/e4 carriers have a 15-fold higher risk of AD.4 We would expect ~4 pheno-copies attributable to the APOE e4 allele in family 1229 by assuming that the single e4/e4 carrier and available for 9 LOAD-affected members of family 1229 to calculate the affecteds-only multipoint linkage score under an autosomal dominant model of inheritance. Two regions were identified with a LOD score greater than 2: chr19:48.89–51.07 Mb (LOD = 2.49) and chr21:38.08–40.12 Mb (LOD = 2.66). Of the top variants of interest, only the one in TTC3 on chromosome 21q22.13 fell within a linkage region. The genotyping data were also used to determine whether a copy number was located on chromosome 21 surrounding the top variant of interest, but none was found (figure e-1). In addition, age-dependent penetrance analysis of the TTC3 variant confirmed the affecteds-only results (LOD = 2.70).

### DISCUSSION
We performed WES in an extended, multigenerational family with an autosomal dominant inheritance pattern of LOAD. Disease segregation analyses identified the TTC3 p.S1038C substitution as the only rare, nonsynonymous variant completely shared among all LOAD family members. It reached a suggestive multipoint linkage LOD score of 2.66. Moreover, it was absent in 3 aged family members with confirmed normal cognition (figure 1). A fourth aged family member carried the variant in TTC3, but died at age 76, very close to the average age at onset for the 11 AD relatives with the variant, 75.7 years old. Therefore, it is possible that if this individual had lived longer, he may have developed AD. Thus, the TTC3 variant may, like APOE, contribute to LOAD risk; however, it is unlike APP, PSEN1, and PSEN2 and may not be sufficient to initiate AD pathogenesis.

### Table 1  Top rare LOAD candidate variants identified in family 1229

| Gene   | Genomic location (hg38) | dbSNP     | Nucleotide | Amino acid change |
|--------|-------------------------|-----------|------------|-------------------|
| GAPVD1 | chr9:125302647          | rs55779102 | G→A        | Val284Met         |
| NLRP5  | chr19:56027918          | rs34175666 | G→A        | Arg562His         |
| OSMR   | chr5:38917567           | rs34324145 | T→A        | Val437Asp         |
| TP53RK | chr20:46686994          | —         | insA       | Leu174fs*196      |
| TTC3   | chr21:37162006          | rs377155188 | C>G       | Ser1038Cys        |
| ZNF142 | chr2:218648790          | rs139412557 | G→A       | Arg373His         |
| ZNF284 | chr19:44086203          | rs199523025 | G→A       | Arg242His         |
| ZNF587 | chr19:57859762-57859764 | —       | delTCT     | Leu451del         |
| ZNF780A| chr19:40074599          | rs142531830 | T→A       | Cys631Ser         |
| ZNF93  | chr19:19933965          | rs145491369 | G→A       | Gly337Glu         |

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30% of the 9 ε3/4 carriers suffered from LOAD due to their APOE genotype. Thus, it is highly unlikely that APOE ε3/4 accounts for the heavy burden of AD in this family.

TTC3 is an appealing LOAD candidate gene because of its involvement in pathways implicated in LOAD; functional studies in neuronal cell lines have shown that TTC3 plays an important role in ubiquitination, Akt signaling, negative cell cycle control, and inhibition of neuronal differentiation.30–31 In addition, the TTC3 protein carries conserved domains predicted to be involved in microtubule and actin-related processes of the cytoskeleton, apoptosis, cellular stress response, and mitochondrial function.32–36 Therefore, TTC3 might act in multiple LOAD-relevant pathways. Moreover, these processes are intertwined with each other with respect to normal neuronal and synaptic functioning and Aβ and protein tau pathology.32,37–39 The identification of additional AD families with rare, nonsynonymous variants potentially contributing to disease would strengthen TTC3 as an AD candidate gene.

During human embryogenesis, TTC3 becomes predominantly expressed in neuronal tissue with high expression in cortical layers of the brain.40,41 Human postmortem brain microarray data demonstrate that cortical TTC3 expression is consistently lower in LOAD cases compared to controls without dementia.42–43 Moreover, TTC3 expression is negatively correlated with LOAD neuropathology markers Braak stage and frontal atrophy in the prefrontal cortex of LOAD cases. This pattern suggests that TTC3 activity might protect against LOAD; thus, variants that reduce TTC3 expression could contribute to LOAD risk. The p.S1038C substitution may alter the TTC3 protein’s conformation and thereby its function via the formation of bisulfide bonds with other cysteine residues, for instance in the short Cys-His–rich motif of the C-terminal H2 ring finger domain of the protein.44–45 A domain required for ubiquitin ligase activity, TTC3 is an E3 ubiquitin ligase that acts as a specific terminal regulator of Akt signaling, a core cellular stress response, and mitochondrial function.46–49 TTC3 becomes predicted to be involved in microtubule and actin-related processes of the cytoskeleton, apoptosis, cellular stress response, and mitochondrial function.50,51 Therefore, TTC3 might act in multiple LOAD-relevant pathways. Moreover, these processes are intertwined with each other with respect to normal neuronal and synaptic functioning and Aβ and protein tau pathology.32,37–39 The identification of additional AD families with rare, nonsynonymous variants potentially contributing to disease would strengthen TTC3 as an AD candidate gene.

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Segregation of a rare \textit{TTC3} variant in an extended family with late-onset Alzheimer disease

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