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Featured Article

Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer’s disease

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Abstract

Introduction: A minority of patients with sporadic early-onset Alzheimer’s disease (AD) exhibit de novo germ line mutations in the autosomal dominant genes such as APP, PSEN1, or PSEN2. We hypothesized that negatively screened patients may harbor somatic variants in these genes.

Methods: We applied an ultrasensitive approach based on single-molecule molecular inversion probes followed by deep next generation sequencing of 11 genes to 100 brain and 355 blood samples from 445 sporadic patients with AD (>80% exhibited an early onset, <66 years).

Results: We identified and confirmed nine somatic variants (allele fractions: 0.2%–10.8%): two APP, five SORL1, one NCSTN, and one MARK4 variants by independent amplicon-based deep sequencing.

Discussion: Two of the SORL1 variant might have contributed to the disease, the two APP variants were interpreted as likely benign and the other variants remained of unknown significance. Somatic variants in the autosomal dominant AD genes may not be a common cause of sporadic AD, including early onset cases.

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Keywords: Mosaicism; Post-zygotic; Mutation; Alzheimer, Prion-like

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1. Background

In the vast majority of the Alzheimer’s disease (AD) cases, the disease is considered as a complex disorder with a high genetic component as part of a multifactorial determinism (for review, see [1]). However, AD can be inherited as an autosomal dominant trait in a few families, with highly penetrant pathogenic genetic variants in the APP, PSEN1, or PSEN2 genes. These variants are sufficient to cause the disease, usually before the age of 66 years (early-onset Alzheimer’s disease [EOAD]). APP encodes the precursor of the amyloid-β (Aβ) peptide, the aggregation of which triggers AD pathophysiology. Aβ is generated following the cleavage of APP by the β-secretase (encoded by BACE1) and the γ-secretase complex, the catalytic subunit of which is encoded by PSEN1 or PSEN2. APP, PSEN1, and PSEN2 pathogenic variants are typically identified in families with autosomal dominant EOAD, that is, at least two generations showing at least one relative affected by EOAD. However, patients with sporadic EOAD, that is, negative family history, have also been reported to carry a pathogenic variant in these genes. Recently, 18/129 (14%) patients with sporadic EOAD and an age of onset before 51 years were reported to present a pathogenic PSEN1 variant or an APP duplication [2], although it was only 2/90 (2.2%) in patients with a relatively later onset (51–65) [3]. Importantly, the mutation occurred de novo in all 10 cases where DNA from the unaffected parents was available [2]. In addition, whole exome sequencing (WES) of EOAD patients and their unaffected parents revealed de novo germline mutations in two novel genes: VPS35 and MARK4 [4]. Overall, no pathogenic variants are found in a majority of the patients with sporadic AD undergoing screening for mutations in the known genes [3–5].

It has been hypothesized for decades that post-zygotic or even somatic, brain-specific, variants could cause the disease in a proportion of sporadic AD patients but remain undetected by standard sequencing techniques [6,7]. Recent advances in sequencing technologies currently allow the accurate assessment of this hypothesis for the first time (for review see [8]). For instance, deep sequencing of APP, PSEN1, PSEN2, and MAPT was recently applied to DNA isolated from the brain of 72 sporadic AD patients and 58 controls [9]. In another study, WES was performed in brain-blood paired samples of 17 sporadic AD patients (average depth of coverage: 60.8×) [10]. Although some somatic variants could be detected, no clear pathogenic variant was identified in these studies. Of note, the majority of the previously published patients exhibited a late onset of AD (after 65 years). One could hypothesize that, similar to inherited or de novo germline pathogenic variants, somatic variants with high penetrance could be associated with an early onset.

The first sequencing study of single neurons from nondiseased human brains recently revealed a high load of somatic genetic variations. The number of somatic single nucleotide variants could be as high as 1500 per neuronal genome [11,12]. Interestingly, most of the variants that were present in more than 5%–10% of the neurons were also detected in tissues originating from all three embryonic layers. This suggests that, if brain tissue is not available for sequencing, sequencing DNA isolated from other tissues including blood can allow the detection of post-zygotic variants. Whatever the tissue of detection and allelic ratios, assessing the pathogenicity of a given variant still requires accurate interpretation. Regarding AD, we found one example of a post-zygotic pathogenic PSEN1 variant detected in 8% of the blood cells and 14% in the brain cells of an EOAD patient [13].

Given the knowledge on seeding and spreading of neuro-pathological lesions in AD brains [14], we hypothesized that patients without a germline pathogenic variant in AD autosomal dominant genes may harbor post-zygotic or somatic variants. The primary aim of this study was to assess the presence of post-zygotic or somatic variants in APP, PSEN1, and PSEN2 in patients with sporadic AD using single-molecule molecular inversion probes (smMIPs). The smMIP technology uses molecular barcodes (unique molecular identifiers [UMI]) to allow for molecule-specific deep sequencing. This is therefore an ultrasensitive technique for the detection of low-level mosaics [15,16]. Our secondary aim was to assess the presence of post-zygotic or somatic variants in 8 additional genes, namely BACE1, NCSTN, APH1A, APH1B, PSENEN, SORL1, VPS35, and MARK4. We applied molecule-specific deep sequencing of this panel of 11 genes to DNA isolated from blood (355 samples) or from brain (100 samples) from 445 sporadic AD patients from France, the UK, and the Netherlands (Table 1).

2. Methods

We included 445 patients fulfilling the National Institute on Aging–Alzheimer’s Association criteria for probable AD or a definite diagnosis of AD (i.e., high AD neuropathologic change according to National Institute on Aging–Alzheimer’s Association criteria [17]) and a negative family history, one positive control carrying a pathogenic PSEN1 variant, and 52 cognitively normal controls. All cases recruited by the French National Reference Center for Young Alzheimer Patients (CNRMJAJ, Rouen, France) from multiple French centers exhibited an early onset (<66 years), the cases recruited by the Netherlands Brain Bank exhibited either an early onset or, when the age at onset was not available, age at death was before 76 years, and cases recruited by the Medical Research Council (MRC) brain bank were not selected in the light of ages of onset; nine of them had an early onset. Among cases, DNA was isolated from blood (n = 355 samples) and/or from brain tissue (n = 100 samples) (Table 1, Supplementary Tables S1–4). DNA was
isolated from blood for all 52 controls. All cases except those from the Netherlands Brain Bank (Netherlands Institute for Neuroscience, Amsterdam; open access: www.brainbank.nl) were previously negatively screened for germline pathogenic variants in APP, PSEN1, and PSEN2, either by whole exome or by Sanger sequencing [18,19]. All participants or their legal representatives provided written informed consent for genetic analyses and/or for a brain autopsy and for the use of the material and clinical information for research purposes. Ethical approval for the genetic analysis of postmortem brain tissue was obtained from the ethical review board of each participating center. For details on inclusion, see Supplementary Methods.

We designed and set up an ultrasensitive smMIP assay aiming at sequencing the coding regions of 11 genes including the three autosomal dominant AD genes (APP, PSEN1, and PSEN2), the genes recently identified in a trio-exome sequencing study in sporadic EOAD cases VPS35 and MARK4, the risk factor gene SORL1, and, as an exploratory study, BACE1 encoding the β-secretase, and the genes encoding the other members of the γ-secretase complex NCSTN, PSENEN, APH1A, and APH1B. After rebalancing the concentration of the smMIP pool following a first test run, we performed four independent runs of sequencing on an Illumina NextSeq sequencer following a first test run, we performed four independent runs of sequencing on an Illumina NextSeq sequencer. All cases, the mean age at onset, and the mean age at death are provided in Supplementary Methods.

### Table 1

| Study                  | N patients (only blood) | N patients (only brain) | N patients (blood + brain) | Total N patients | Mean age at onset (range) | Mean age at death (range) |
|------------------------|-------------------------|-------------------------|---------------------------|------------------|--------------------------|--------------------------|
| Rouen CNRMAJ, France   | 347                     | 2                       | 2                         | 351              | 54.42 (44–65)            | NA                       |
| MRC Brain Bank, UK     | 0                       | 80                      | 0                         | 80               | 69.9 (53–82)             | 85 (71–99)               |
| Netherlands Brain Bank | 0                       | 8                       | 6                         | 14               | 56.4 (48–63)             | 66.9 (57–75)             |
| Total                  | Total blood samples: 355 form 355 patients | Total brain samples: 100 from 98 patients | 445                      | 445              |                          |                          |

Abbreviation: MRC, Medical Research Council.
*One sample from cerebellum and one sample from frontal cortex for one patient, one sample from an unspecified region for the second patient.
\[\text{Among the 29/80 patients with available information.}\]
\[\text{Among the 12/14 patients with available information.}\]
Table 2: Somatic variants identified in patients

| Patient ID       | Age at onset | Gender | Sample | Chromosome | Position | Genesymbol | p. nomenclature | Protein nomenclature | p. nomenclature | Minor Allele Frequency | Validation | VAF | VA F (ADS) (%) | VAF | VA F (PCRR1) (%) | ROU-1496-001 | ROU-1347-001 | ROU-0085-001 | ROU-0778-001 | ROU-0609-001 | EXT-0482-001 | EXT-0772-001 |
|------------------|--------------|--------|--------|------------|----------|------------|----------------|---------------------|------------------|----------------------|------------|-----|----------------|-----|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| C7               | 72           | M      | Blood  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |
| C8               | 72           | M      | Brain  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |
| EXT-0482-001     | 58           | M      | Blood  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |
| EXT-0772-001     | 50           | F      | Blood  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |
| ROU-0609-001     | 55           | M      | Blood  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |
| ROU-0778-001     | 60           | M      | Blood  | chr1:174725599 | c.1125A>C | APP       | p.Glu379Glu    | p.Glu379Glu        | Prob. Dam.       | 0.15                  | PolyPhen 2, SIFT | 0.29        | 0.05 | 0.05           | 0.10 | 0.05           | NA           | NA          | NA          | NA          | NA          | NA          | NA          |

Abbreviations: ADS, amplicon deep sequencing; p. nomenclature, protein nomenclature; VAF, variant allele fraction; VAF, variant allele fraction; PolyPhen 2, probably damaging; Delet., deleterious; DC, disease causing; Pol., polymorphism; NA, not available.

In four samples, from one particular sequencing run, additional variants were identified with allelic fraction in the ranges of 1% to 3%. However, we considered these results as putative DNA contamination because the variants were known as common polymorphisms in variant databases (minor allele frequency > 1%), each putatively contaminated sample harbored at least two of these variants, and they were detected as germline heterozygous or homozygous in other samples from the same run, all samples initially belonged to a single plate, before capture. The presence of DNA contamination was further assessed using the same technique based on the pileup formats as for candidate somatic variants, in all four runs, taking into account nucleotide changes that correspond to known SNPs. No additional contamination was identified.

3.3. Interpretation of probably germ line APP, PSEN1, and PSEN2 variants

After variant calling by GATK and SeqNext, followed by annotation and variant interpretation, we accurately detected the probably germline heterozygous PSEN1 variant included as a positive control in one brain sample from the MRC brain bank (Supplementary Table S6). No probable germline (allelic ratio 25%–100%) variant was rated as pathogenic or likely pathogenic in these genes in cases. Of note, we confirmed the presence of four known heterozygous missense variants of unknown significance (class 3 following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommendations [21], one in PSEN1, and three in PSEN2) in two French patients (blood samples) and two patients from the MRC brain bank (brain samples) (Supplementary Table S6), including the p.V101M PSEN2 variant that has been previously reported in the brain of a patient with sporadic AD [9], also as a probably germline variant. Additional variants were detected in all three genes, but they were

putative contribution to AD. No candidate somatic variant was detected in PSEN1 and PSEN2 across all samples.

The other somatic variants were located in SORLI (n = 5, including one missense, three synonymous, and one intronic variant), NCSTN (n = 1, missense) and MARK4 (n = 1, synonymous). One of the SORLI somatic variants (NM_003105.5:c.2207G>A, VAF = 3.61% in blood) was annotated as missense and predicted damaging by 3/3 in silico prediction tools among Mutation Taster, PolyPhen 2, and SIFT (strictly damaging). The other SORLI somatic variants were synonymous (n = 3) or intronic (n = 1). Of note, one of the synonymous variants was predicted to strongly enhance a cryptic 5’ splicing site (NM_003105.5:c.2475G>A, VAF = 0.36% in blood, MaxEntScan score +202%) and hence might disrupt the SORLI coding sequence. The intronic SORLI mutation was close to a canonical splice site (c.5605-3C>T) although splicing prediction tools suggested a weak or absent effect.

In four samples, from one particular sequencing run, additional variants were identified with allelic fraction in the ranges of 1% to 3%. However, we considered these results as putative DNA contamination because the variants were known as common polymorphisms in variant databases (minor allele frequency > 1%), each putatively contaminated sample harbored at least two of these variants, and they were detected as germline heterozygous or homozygous in other samples from the same run, all samples initially belonged to a single plate, before capture. The presence of DNA contamination was further assessed using the same technique based on the pileup formats as for candidate somatic variants, in all four runs, taking into account nucleotide changes that correspond to known SNPs. No additional contamination was identified.
classified as benign or likely benign based on their predicted effect, variant frequencies in controls, and previous reports.

3.4. Probable germline VPS35 and MARK4 variants

VPS35 and MARK4 are candidate genes for autosomal dominant EOAD given the observation of de novo germline mutations in two sporadic EOAD patients, and subsequent in vitro studies showing biochemical defects consistent with AD pathophysiology [4].

We identified one rare nonsynonymous variant in VPS35 and five in MARK4, all with a VAF suggestive of a germline origin (Supplementary Table S7). Of note, the c.2320C>T, p.(Ser661Asn); c.230G>A, p.(Val767Gln); c.1347C>T, p.(Pro449Leu); c.1553C>T, p.(Pro518Leu)). One variant was predicted damaging by all three assessment tools (c.1033C>T, p.(Pro344Leu); c.1553C>T, p.(Pro518Leu)). One variant was predicted damaging by PolyPhen 2, SIFT, and Mutation Taster, in 17 patients and no control (Supplementary Table S8). These categories of variants have been shown to increase the risk of EOAD [18,23], and three variants (two novel) were found in novel patients, identified from brain tissues.

3.6. Probable germline variants in BACE1 and genes encoding members of the α-secretase complex

We detected 11 rare nonsynonymous variants in 12 patients in BACE1 (n = 2), NCSTN (n = 4), APH1A (n = 1), APH1B (n = 3), and PSENEN (n = 3) (Supplementary Table S9). These variants were detected in 16 blood samples and two brains samples and the VAF suggested their germline origin. All but one were missense. A frameshift variant was detected in the APH1B gene. However, this gene is not under strong constraint against loss of function, similar to PSEN2, judging by the probability of loss of function intolerance established from Exome Aggregation Consortium data [20]. All were detected in patients.

4. Discussion

In this study, we screened 11 genes for somatic mutations in 355 blood samples and 100 brain samples from 445 patients with AD, of which 372 (83.5%) exhibited an early onset (<66 years). In total, we identified nine somatic variants with variant fractions ranging from 0.2% to 10.8%. These variants were detected in multiple DNA copies and are more likely clonal than recurrent mutational events. The coverage statistics, together with the validation of all variants detected, including all six with an allelic ratio below than 1% (range 0.22%-0.48%), support the ultrasensitivity of our detection method. We did not find any candidate post-zygotic or low-level somatic variant in the three established autosomal dominant AD-causative genes APP, PSEN1, and PSEN2 that could be interpreted as likely pathogenic. Given the high sensitivity of the assay, we consider our screen as negative regarding likely pathogenic variants in the coding region of these genes.

We could find only one example in the literature of an AD patient with a post-zygotic causative variant in PSEN1 [13]. In this study, a patient with EOAD starting at the age of 27 years was found to have inherited a pathogenic mutation in PSEN1 from her affected mother, who presented a disease onset at the age of 52. The mutation was present in 8% of the mother’s blood cells and 14% of her brain cells, suggesting that the mutation occurred as a post-zygotic event in the mother and that it was present in variable proportions of cells in multiple tissues including the mother’s oocytes [13]. Of note, the majority of our patients presented an early onset of sporadic AD (83.5%), and therefore this is the largest series of sporadic EOAD patients screened for pathogenic somatic variants causative for AD to date. The assessment of the somatic variant hypothesis in sporadic AD has been performed only recently, using deep sequencing [9] or brain-paired WES [10], in patients with a later onset on average than in our study. To our knowledge, our screen is the first to leverage UMIs to allow single-molecule tracing
and even better sensitivity. Taken together, we consider that somatic variants in APP, PSEN1, and PSEN2 are not a common cause of sporadic AD, even in patients with an early onset. We acknowledge, however, that somatic variants might still be present as even more rare events in brain regions, which have not been assessed. Indeed, this and previous studies focused only on one or two brain regions per individual. The interpretation of putative region-specific variants may however be difficult. In addition, our assay did not allow the identification of mosaic copy number variations.

As part of our gene panel, we also sequenced the VPS35 and MARK4 genes. They were each previously hit by one de novo germline mutation in sporadic EOAD patients [4]. The effect of these variants was studied in vitro, and the location where the mutation occurred in the protein could be highly specific, given the results of functional assays. Despite the identification of a synonymous somatic variant in MARK4 (VAF = 0.43%), we could not identify any putatively damaging variant in the corresponding exons as a germline or a somatic variant.

Germline protein truncating and rare missense predicted to be strictly damaging SORL1 variants significantly increase the risk of EOAD [18, 23]. We detected five SORL1 somatic variants (VAF ranging from 0.63% to 7.91%). Among them, one was missense and classified as strictly damaging. It was detected in a blood sample of an EOAD patient and could, if present in the brain tissue, contribute to the genetic determinism of AD in this patient. Among the other SORL1 somatic variants, one was predicted to enhance a cryptic 5′ splicing site and could disrupt SORL1 coding sequence. If so and if present in the brain tissue, it could also contribute to the genetic determinism of AD in this patient. SORL1 rare damaging variants were originally identified in EOAD probands with a positive family history of EOAD, with no pathogenic APP, PSEN1, or PSEN2 variant [24]. However, the paucity of segregation data still precludes the classification of SORL1 as a putative Mendelian gene and association studies showed a role as a risk factor (for review, see [1]). Our results suggest that the other genes TREM2 and ABCA7, the rare damaging variants of which having been shown to increase the risk of AD, should also be screened for post-zygotic and somatic variation.

We included in our assay the candidate genes BACE1 encoding the β-secretase and the other genes encoding the other proteins from the γ-secretase complex (in addition to PSEN1 and PSEN2). We detected one somatic variant in NCSTN, which was present in ~22% of the sequenced cells from the blood of one EOAD patient (VAF = 10.8%). This variant introduced a missense that was predicted damaging by SIFT but not by PolyPhen2 and Mutation Taster. It has been observed in 12 individuals from the gnomAD database (minor allele frequency = 4.9 × 10⁻³) [20]. The visualization of the BAM files of the three variant carriers available in the gnomAD website suggested that this variant was compatible with a heterozygous variant with germline origin, which is not consistent with the hypothesis of a damaging effect when carried as a post-zygotic event. Interestingly, we also detected 12 variants in 11 patients that were probably present in the germline. All were detected in patients. To our knowledge, there is no evidence of rare variants in these genes segregating in families further than by chance, or of a significant association of rare variants with AD. This study was not designed as an association study, and these genes were not reported among the latest large association studies including our own data from France [18]. By including these genes that play a key role in Aβ generation in the context of the γ-secretase complex, we made the hypothesis that the absence of damaging variants segregating in families in the literature could be explained by a putative intolerance (abnormal development, lethality, and other diseases). Post-zygotic damaging variants might be better tolerated and putatively increase the generation of Aβ through increased β or γ-cleavage or its regulation. We did not find such candidates somatic variants in our study. These genes remain biological candidates currently lacking genetic evidence.

In conclusion, we used single-molecule deep sequencing in brain and/or blood samples of 445 patients with sporadic AD and could detect nine somatic variants with allelic ratios as low as 0.2%. Although we detected a few putatively damaging SORL1 somatic variants, we did not detect any candidate post-zygotic or somatic variant that could be interpreted as pathogenic or likely pathogenic in the three known autosomal dominant AD genes. Our results, together with a previous report [9], challenge the hypothesis that somatic mutations in key AD genes would cause a significant proportion of AD with a sporadic presentation. We conclude that somatic variation in these genes is most likely not a frequent cause of sporadic AD.

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Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2018.06.3056.

RESEARCH IN CONTEXT

1. Systematic review: Attention toward the somatic variant hypothesis is growing. This hypothesis states that a proportion of patients with Alzheimer’s disease could have developed the disease because of somatic mutations in the brain, leading to pathological lesions that would later spread into the brain. However, we could find only one published example. Advances in sequencing technologies allow the assessment of this hypothesis since very recently only.

2. Interpretation: We assessed this hypothesis using an ultrasensitive molecule-specific deep sequencing approach in young patients. Nine somatic variants were identified, and some of them could have contributed to the development of the disease. However, no pathogenic variant was found in the known autosomal dominant genes, thus challenging the hypothesis.

3. Future directions: Other techniques could be applied to detect other genomic variations such copy number variations. In addition, genetic variants in a small proportion of cells - not detectable by our technique - could be a future research direction.

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