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Assessment of Mendelian and risk factor genes in Alzheimer disease: a prospective nationwide clinical utility study and recommendations for genetic screening

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Abstract

Purpose. To assess the likely pathogenic/pathogenic (LP/P) variants rates in Mendelian dementia genes and the moderate-to-strong risk factors rates in patients with Alzheimer disease (AD).

Methods. We included 700 patients in a prospective study and performed exome sequencing. A panel of 28 Mendelian and 6 risk-factor genes was interpreted and returned to patients. We built a framework for risk variant interpretation and risk gradation and assessed the detection rates among early-onset AD (EOAD, age of onset (AOO) ≤ 65 years, n=608) depending on AOO and pedigree structure and late-onset AD (LOAD, $66 < \text{AOO} < 75$, n=92).

Results. Twenty-one patients carried a LP/P variant in a Mendelian gene (all with EOAD, 3.4%), 20/21 affected *APP*, *PSEN1* or *PSEN2*. LP/P variant detection rates in EOAD ranged from 1.7% to 11.6% based on AOO and pedigree structure. Risk factors were found in 69.5% of the remaining 679 patients, including 83 (12.2%) being heterozygotes for rare risk variants, in decreasing order of frequency, in *TREM2*, *ABCA7*, *ATP8B4*, *SORL1* and *ABCA1*, including 5 heterozygotes for multiple rare risk variants, suggesting non-monogenic inheritance, even in some autosomal-dominant-like pedigrees.

Conclusion. We suggest that genetic screening should be proposed to all EOAD patients and should no longer be prioritized based on pedigree structure.

Keywords

Alzheimer disease, risk variant, pathogenic variant, exome, clinical utility

Introduction

Several countries are implementing genomic medicine plans in public healthcare, mostly for patients with rare diseases. Early-onset dementia is a clinical indication for exome/genome sequencing^{1,2}. Early-onset Alzheimer Disease (EOAD, age of onset (AOO) <66 years) is the first cause of early-onset dementia³ and accounts for 5-10% of all Alzheimer Disease (AD) patients⁴. It thus represents a potentially large source of genetic screening requests and can be considered somewhere between rare and common diseases. Unlike many other rare early-onset neurodegenerative disorders, the cause of EOAD is not monogenic in the majority of cases⁵. In addition, the clinical utility of exome/genome sequencing or even extended gene panels has not been assessed in large prospective AD series and it remains unclear which patients should be prioritized, if some should be.

Overall, pathogenic variants in *APP*, *PSEN1* or *PSEN2* causing monogenic forms of AD are encountered in less than 0.1% AD patients⁵. They generally affect young patients with average ages of onset of 50.9, 44.4, and 53.9 years, respectively⁶. Among EOAD patients, a large diversity of pathogenic variant detection rates has been reported, depending on inclusion criteria^{6-9 10,11}. Monogenic causes probably represent less than 20% of all EOAD cases. In non-monogenic AD, genetics factors are thought to play a significant role, as suggested by twin studies¹². Most risk variants are shared between EOAD and late-onset AD (LOAD) cases, with diverse effects and frequencies. The most important AD genetic risk factor is the $\epsilon 4$ allele of the *APOE* gene, with a frequency of ~14% in individuals from European ancestry and AD lifetime risks of ~20% and ~50% at the age of 85, respectively for heterozygotes and homozygotes¹³. In addition, significant progress has been made in the understanding of the genetics of complex forms of AD during the last decade. Large-scale case-control studies using either DNA chips¹⁴ or exome/genome sequencing¹⁵ identified common and rare variants influencing the risk of AD with a broad spectrum of effects. Common variants are associated with low (<1.3) odds ratios (OR), precluding a clinical use, while polygenic risk scores (PRS), gathering the effect of multiple common variants seem to be primarily driven by the *APOE* genotype¹³ and miss rare variants. Rare coding variants with a larger effect (OR ranging from 1.5 to more than 27) have recently been reported, with a stronger effect among EOAD patients. Some of these rare risk variants, in the *SORL1*, *TREM2*, *ABCA7*, *ABCA1* or *ATP8B4* genes, may be worth using in a clinical setting¹⁵⁻¹⁸. Indeed, clinical genetics interpretation is generally restricted to Mendelian genes. Yet, it may be seen as questioning to report a “negative” result to a patient who could elsewhere be heterozygote for a strong risk factor. Although risk variants cannot be used for genetic counseling, they still contribute to the disease etiology in patients with positive carrier status and thus represent a valuable source of information for patients and families seeking for responses to questions regarding disease etiology. The strongest EOAD rare risk factors appears to be loss-of-function (LOF) *SORL1* variants, with an average OR of 27.5^{16,19,20}. This even questioned whether *SORL1* should be considered as an autosomal dominant AD gene²¹, but we

recently showed that penetrance is not complete by age 70, except in the context of APOE- ϵ 4 homozygosity²². The case of other genes with rare risk variants is clearer from that perspective, given their lower OR compared to *SORL1* LOF variants.

In a prospective study, called ECASCAD (Exome – Clinical Application of Sequencing in Alzheimer Disease), we included 700 AD patients from France and proposed exome sequencing as a first-tier genetic test. We interpreted rare variants in Mendelian dementia genes and built a clinical interpretation framework for AD-associated risk variants. Results were explained to patients and/or caregivers by referring clinicians following a personalized recommendation based on the genetic report. In absence of a monogenic cause, results of *APOE* genotype and rare risk factors were reported to the patients. We report the detection rate of autosomal dominant AD (likely) pathogenic variants, those in AD differential diagnoses, and risk factors, based on pedigree structure and AOO in probands. Based on this prospective series as well as data from the literature and our own retrospective series, we propose recommendations on clinical indications for genetic screening.

Methods

Patient enrollment and study procedures

Patients were included from 40 French centers (see supplementary information for detailed procedures). Diagnoses were based on IWG-2 criteria²³ and an AD pathophysiological process was assessed by CSF AD biomarkers or amyloid PET if CSF was not available. Inclusion criteria were as follows: (i) a diagnosis of typical or atypical AD with AD pathophysiological process, (ii) first symptoms noticed at the age of 65 or before, whatever the family history, (iii) absence of an already known monogenic cause. Patients were not included in case of: (i) AD CSF biomarkers not consistent with an AD pathophysiological process, (ii) a differential diagnosis better explained the symptoms, (iii) previous genetic assessment using sequencing techniques, except for *APOE* genotyping (in case of known *APOE* genotype upon inclusion, patients were included regardless of the result), (iv) a likely pathogenic / pathogenic (LP/P) variant was already known in the family, and (v) if one of the parents was born in a non-European country or self-reported as being of non-European descent, as current knowledge on rare risk factors relies mainly on patients of European ancestry. In addition, five centers were allowed to include patients with AOOs until 75 years so that we could extend the clinical utility study to patients with LOAD (66-75 years). While in these age categories, we do not expect pathogenic variants in monogenic dementia genes, except a few variants with reduced penetrance, some of these patients may still have questions on disease etiology and/or family history, although genetic tests are usually not requested by the patients or not accepted by physicians, stating that there is no clinical indication. Although this part of the study was not powerful enough to drive conclusions, we opened the inclusions with the aim to measure how many patients would request such an analysis, among LOAD patients, as an exploratory add-on to the study.

All patients or their legal representatives provided informed written consent for genetic analyses. This study was approved by the *CPP-Ouest III* ethics committee (notification 2018-A02359-46). Patients were included from December 2018, until n=700 inclusions were reached (June 2021).

Study procedures are presented in Figure 1 and in supplementary information. Briefly, patients underwent at least 2 visits, one upon inclusion, and one for the results delivery, by the same physician, who was also generally in charge of clinical follow-up. Specific information was provided before inclusion. To help the clinicians, a standardized and personalized information accompanied the genetic report. Templates of letters to referring physicians, key points summaries, and templates of letters to patients are available upon request (in French).

Clinical interpretation of variants

We chose to perform exome sequencing to access the coding sequence of a panel of genes with good sensitivity and specificity including single nucleotide variants (SNV), short insertions and

deletions (indels) and Copy Number Variants (CNVs) and to allow the reinterpretation of existing data in the future in light of the discovery of novel genes without the need to resequence. Exome sequencing and bioinformatics pipelines are displayed in supplementary information and Figure 1. Rare (allele frequency <1%) SNV, indels and CNVs in a list of 28 Mendelian dementia genes were interpreted following the American College of Medical Genetics and Genomics – Association for Molecular pathology (ACMG-AMP) recommendations²⁴ by an expert geneticist. Variants classified as class 4 (likely pathogenic, LP) or class 5 (pathogenic, P) were returned to patients. Some of the class-3 variants (uncertain significance) were also returned, when additional investigations might help reclassifying them in the future.

Regarding risk factors, in the absence of existing consensus or interpretation guidelines, we built a framework for variant interpretation and classification (Figure 2 and supplementary information). After literature review, we first restricted the analysis to a list of validated AD-risk genes, which we defined as genes with exome-wide significance of a burden of rare, selected coding variants associated with AD or single rare variants with genome-wide significance and with at least one replication and OR>1.5. Then, we defined stringent criteria for classification as a definite AD risk factor and finally classified those into three categories of effect (modest, moderate, and strong) based on published odds ratios (supplementary table 4).

Retrospective estimation of overall pathogenic variant detection rates among EOAD patients

To compare *APP*, *PSEN1* and *PSEN2* LP/P variant detection rates in EOAD overall with published data, and because most published LP/P variant detection rates were obtained from smaller series, we estimated the LP/P variant detection rates among EOAD patients since the beginning of genetic screening in France in our national center. Overall, we consider that we have prospectively or retrospectively sequenced all probands whose DNA has been sent to our center since the 1990s and until November 2022, among those with (i) a neuropathological diagnosis, (ii) a clinical diagnosis of AD with supporting AD pathophysiology or (iii) a typical AD diagnosis, in absence of biomarkers available. Exome programs in a research setting were focused on individuals from European ancestry, to match with available data in terms of control individuals for case-control analyses, explaining why we restricted our estimates to this population.

Results

Patient enrollment

Over 30 months, we included 700 patients from 40 French centers. Of them, 608 (87%) presented EOAD and 92 (13%) had LOAD (66<A00<76 years); 422 (60.3%) were female and 278 (39.7%) were male. All but 5 patients had CSF biomarkers available and consistent with an AD pathophysiological process; these 5 patients showed positive amyloid PET scans. Among EOAD patients, 258 (42.4%) were sporadic or family history was unknown and 350 (57.6%) had a positive history, including 230 for whom the medical history of AD consisted of one or several late-onset cases and 120 with at least one relative with EOAD (Figure 3). Among the latter, 95 pedigrees showed that EOAD spanned at least two generations, thus suggesting possible autosomal dominant EOAD. Among LOAD patients, 61 (66.3%) had a positive family history, and 31 (33.7%) were sporadic cases or family history was unknown. There was no significant difference in the rates of positive family history between EOAD and LOAD cases. The percentage of males was 40% among EOAD patients and 36% among LOAD patients (Non Significant NS).

Variants in Mendelian genes

All results were delivered to the referring clinician after a median of 5.6 months (range: [0.8-11.1]); only 5 patients had a delay from inclusion to genetic report >10 months, for technical reasons). Following clinical interpretation of an *in silico* gene panel of 28 genes associated with monogenic causes of dementia, we identified 21 patients (3%) harboring a likely pathogenic or pathogenic (LP/P) variant considered as the cause of the disease, all in EOAD patients: 14 in *PSEN1*, 4 in *APP*, 2 in *PSEN2* (Table 1) and one patient exhibited a pathogenic variant NM_007375.3:c.1144G>C p.(Ala382Pro) in an AD differential diagnosis gene, *TARDBP*. Re-examining clinical information allowed us to reconsider the diagnosis towards Fronto-Temporal Lobar Degeneration (FTLD) for the latter patient, who presented behavioral symptoms and borderline amyloidopathy based on CSF biomarkers (supplementary information).

In addition, four LP/P variants in Mendelian genes were considered, all in EOAD patients, and were interpreted as putatively contributing to the phenotype but not necessarily causing AD (supplementary information, supplementary table 1). Overall, 7 class-3 variants (uncertain significance) were returned to patients. One of them (in *PSEN1*) could later be reclassified as benign following mRNA assessment, and another one (in *MAPT*) led to question the AD diagnosis (supplementary table 2).

Rates of LP/P variants based on pedigree structure

All 21 LP/P variants considered as causing dementia were found in EOAD patients (3.5%), including 12/120 patients with a family history of EOAD (familial-early, 10%), which represented 11/95 (11.6%) among pedigrees with EOAD spanning at least 2 generations (Figure 3).

In EOAD patients with a family history of LOAD (familial-late) or unknown AOO in the affected relative, only 3 LP/P variants were detected, in addition to the above-mentioned *TARDBP* pathogenic variant. This includes a patient carrying NM_000447.3:c.850A>G p.(Arg284Gly) variant in *PSEN2*, with a history of LOAD in the father (DNA not available, death at 92 years of age) and one patient carrying NM_000021.4:c.346A>G p.(Thr116Ala) variant in *PSEN1*, with uncles and aunts affected by LOAD and living unaffected parents (DNA NA, aged 88 and 87 respectively at last visit of the proband). As both variants were expected to be fully penetrant, we hypothesize that these variants may be unrelated to the family history and could eventually be *de novo* variants (DNV), although we could not demonstrate it. The third case had a family history of AD with unknown AOOs in the mother and maternal uncles and aunts; his father was unknown, thus causing a censoring effect. He carried a NM_000021.4:c.806G>A p.(Arg269His) variant in *PSEN1*, already associated with some diversity of AOO, including ages > 65 years. Overall, no direct link between the LP/P variants and family history could be established in these three EOAD cases with a family history of LOAD.

Among sporadic EOAD patients, 5/258 (1.9%) showed a LP/P variant, including one DNV in the *APP* gene in a patient with an AOO of 42 years. The other four patients had AOOs ranging from 52 to 58 (Table 1). All four variants were either novel (NM_000021.4:c.289G>A p.(Val97Met) in *PSEN1* and NM_000447.3:c.365C>T p.(Thr122Met) in *PSEN2*) or described in only one patient before (NM_000021.4:c.800C>T p.(Pro267Leu) in *PSEN1*, in an autosomal dominant EOAD family proband²⁵, and NM_000021.4:c.1309A>G p.(Ile437Val) in *PSEN1*, in a sporadic patient⁹), thus with insufficient information on penetrance.

Rates of risk factors

Among patients without a monogenic cause of dementia (n=679), 154 (22.7%) were APOE-ε4 homozygous and 278 (40.1%) were heterozygous. Eighty-three patients (12.2%) carried at least one rare risk factor, including 5 probands carrying 2 of them (0.7%). Of these 83 patients, 14 (16.8%) were also APOE-ε4 homozygous and 34 (4.2%) were heterozygous, thus accumulating at least two risk factors and 12/83 were LOAD patients. Overall, we found at least one risk factor in 470/679 (69.2%) patients. Patients with at least one risk factor were enriched in the LOAD group (93%, as compared to 84% among EOAD patients, p=0.0009) and this was mainly due to the APOE4 allele which was present in 77% of LOAD patients and 61% of EOAD patients (p=0.0004; no significant difference in proportions

of rare risk variants between EOAD and LOAD patients). Patients with at least one risk factor were more susceptible to show a positive family history (64% compared to 44%, $p=1.4 \times 10^{-6}$) and there was no significant difference in sex ratios.

The gene showing the largest number of risk variants was *TREM2*, with 11 NM_018965.4:c.185G>A p.(Arg62His) (R62H) and 18 NM_018965.4:c.140G>A p.(Arg47His) (R47H) patients carrying this variant (supplementary table 3). One of them was homozygous for the *TREM2* R47H variant and he also carried a missense variant of uncertain significance in *SORL1*, NM_003105.6:c.418G>A p.(Asp140Asn) (not reported). *ABCA7* was the second most frequent gene, with 25 patients carrying at least one *ABCA7* truncating variant. One patient harbored 2 truncating *ABCA7* variants (AOO 65 years, family history of LOAD), but we could not determine if variants were in *cis* or in *trans*. The most recurrent *ABCA7* truncating variant (n=8) was the splice region NM_019112.4:c.5570+5G>C variant, with a demonstrated LOF effect¹⁸. The second most recurrent *ABCA7* variant, NM_019112.4:c.2126_2132del p.(Glu709Alafs*86), was found in 5 patients and previously reported in diverse ethnicities^{18,26,27}. The third gene in frequency was *ATP8B4*, with 20 patients being heterozygous for the NM_024837.4:c.1183G>A p.(Gly395Ser) (G395S) variant with a modest effect. Nine patients carried a *SORL1* truncating variant, including one patient also harboring an *ABCA7* truncating variant and an *APOE-ε3/ε4* genotype; this patient had an AOO of 65 years and had a family history of EOAD. Except one doubleton, all *SORL1* truncating variants were singletons. Three patients carried a variant considered as a risk factor in *ABCA1*, including one truncating variant and two missense variants.

In addition to the already-mentioned patients, two carried multiple rare risk factors: one patient with a *TREM2* R47H variant and an *ATP8B4* G395S variant, in addition to an *APOE-ε3/ε4* genotype (AOO 61, family history of EOAD) and one patient with an *ABCA7* truncating variant and an *ATP8B4* G395S variant, in addition to an *APOE-ε3/ε4* genotype (AOO 62, sporadic case). Overall, 5 patients harbored two rare risk factors, if counting the *TREM2* R47H homozygote double, and 3 of them carried these variants in an *APOE-ε3/ε4* context (Supplementary Figure).

When focusing on patients with a pedigree suggestive of autosomal dominant EOAD (EOAD spanning two generations), 74/84 (88.1%) carried at least one risk factor, and 12/84 (14.3%) carried at least one rare risk factor, suggesting that EOAD may be oligogenic in these patients too.

Retrospective estimation of overall pathogenic variant detection rates among EOAD patients

To expand these prospective findings and with the aim to contribute to the discussion on genetic screening recommendations in AD, we performed a retrospective analysis of all EOAD unrelated probands from European descent assessed by exome or Sanger sequencing (or both) since the discovery of the *APP*, *PSEN1* and *PSEN2* genes, until November 2022, in our national reference center. Of 2,069 patients, 255 exhibited a LP/P variant, thus the overall LP/P variant detection rate is 12.3% (95% confidence interval [10.9-13.7]).

Journal Pre-proof

Discussion

In this prospective study, we assessed how a large genetic screen in AD patients may lead to reporting clinically-relevant genetic variants to patients, including Mendelian dementia genes and risk factors, in a clinical setting. There was no major issue in implementing exome sequencing for this rather large series, on a nationwide scale, demonstrating that it is possible and meaningful to propose exome sequencing to basically any patient with EOAD, despite the relatively high frequency of AD as compared to other causes of early-onset dementia.

Clinical utility may be divided into two main outputs, i.e. LP/P variant detection rates in Mendelian genes and risk factor detection rates.

We previously assessed LP/P variant detection rates in *APP*, *PSEN1* and *PSEN2* genes in retrospective series⁹⁻¹¹. In a first series, we focused on EOAD patients with a positive family history of EOAD, so that cases spanned at least 2 generations. The LP/P variant detection rate was 77%¹¹. Here, after restriction to the same definition of pedigrees, we reached an 11.6% variant detection rate, despite the use of a more sensitive technology. Several hypotheses may explain this difference. First, during the inclusion period of the ECASCAD study, our center received samples from individuals with EOAD, not included in the ECASCAD study because a LP/P variant was already known in their family, whether or not the referring clinician was aware of this, the link with a known family was made possible thanks to the family database of our center, acting as a unique center for EOAD genetics since the 1990s. Overall, 15 patients from 15 different families were assessed by targeted sequencing of the familial pathogenic variant during the same inclusion period as the ECASCAD study. If these variants had not been known from the laboratory, these patients would have been considered as probands and would have been included in the ECASCAD study, thus reaching a 24% detection rate in the category of EOAD spanning two generations, still somewhat away from the expected 77%. Second, since more than two decades, presymptomatic testing can be requested, so that some of the asymptomatic heterozygotes for LP/P variants may have become symptomatic in the meantime, obviously not requesting another genetic test after symptoms onset and not adding up to the above-mentioned number of symptomatic relatives. Third, the recruitment of EOAD probands has evolved since our 2012 report, with an average AOO of EOAD patients sent for genetic testing of 54.3 years before 2012 and 57.3 years here ($p < 0.001$) and a more stringent selection of cases of sequencing prior to the 2000s (e.g. requirement of a family history positive for EOAD and spanning 3 generations rather than 2 at the beginning of the lab activity, or a bias towards a lower proportion of *APOE* $\epsilon 4$ -positive probands). Thus, despite unbiased prospective inclusion of cases here, the prospective variant detection rate seems to deeply depend on the history of genetics screening in the country. We hypothesize that the majority of families of French ancestry with fully penetrant variants may already be known, and that families

with non-French ancestry, atypical pedigrees and less penetrant variants may still be a source of novel families with a LP/P variant, to which should be added *de novo* variants.

We previously established national recommendations for *APP*, *PSEN1* and *PSEN2* screening, which were: diagnosis of EOAD in the proband and presence of a family history of EOAD (whatever the generation) or the AOO in the proband is <51 years. In the latter category of patients, we previously detected a 12.3% LP/P variant detection rate in a retrospective study¹⁰, most of them were *de novo* variants after assessing parental DNA, when available. Here, only 1/31 (3.2%) patient fulfilling these criteria was heterozygous for an *APP* pathogenic variant, which was also confirmed to be *de novo*.

In our previous national recommendations, patients with an AOO between 51 and 65 and a negative family history or a family history of LOAD were not prioritized for genetic screening in a diagnostic setting. After retrospective sequencing of such cases, we previously identified 1.2-2.2% LP/P variant detection rates⁹. Here, the rate of LP/P variants in patients with sporadic EOAD and AOO between 51 and 65 or patients with EOAD and a family history of LOAD was 1.5%, which is thus consistent with our previous report.

To better compare our LP/P variant detection rate in *APP*, *PSEN1* and *PSEN2* with the literature, we gathered data from our national center for all EOAD probands with available sequencing data since the discovery of these genes. We found a LP/P variant detection rate of 12.3% [10.9-13.7]. Although this seems consistent with previous reports in other countries,^{7,8} this can still be slightly overestimated because of hard selection of cases at the beginning of the studies in the 1990s.

In some common disorders, in which monogenic causes can be hidden in a minority of patients (e.g. cancers, cardiovascular diseases), prioritization algorithms based on *a priori* probabilities of finding a pathogenic variant are often used. For actionable diseases, wider indications of genetic screening are proposed, up to universal reflexive testing, not to miss therapeutic opportunities²⁸. Although AD is not highly actionable, it appears as critical for genetic counseling, not to miss a LP/P variant in an EOAD proband requesting a genetic analysis. In addition, in anticipation to putative preventive treatments in presymptomatic individuals with monogenic AD variants²⁹, which could even be extended to oligogenic combinations of moderate-to-strong risk factors someday, and following promising clinical trials based on the use of recently proven effective molecules as disease-modifying treatments³⁰⁻³², variant detection rates should no longer be the main argument for proposing a genetic test in the context of EOAD. In addition, it seems, from the current study, that there is no longer a big advantage, in terms of variants detection rate, to prioritize genetic screening based on AOO and pedigree structure, among EOAD patients. Indeed, after more than 25 years of genetics screening of EOAD patients in France, the lowest LP/P variant detection rate among novel EOAD patients was 1.7% and the highest, 11.6%, depending on AOO and pedigree structures. Thus, we consider that all EOAD

patients should be offered a genetic screen, provided that the clinician requesting the analysis is trained in such a prescription and results delivery.

Although the inclusion of LOAD patients was too limited, preventing us from generalizing our results, we did not identify any LP/P variant in this AOO category. Some *APP*, *PSEN1* or *PSEN2* variants have already been identified in patients with an AOO > 65 years, with extremely low variant detection rates. For example, in the Alzheimer Disease European Sequencing (ADES) study encompassing patients from Europe and patients from the USA (Alzheimer Disease Sequencing Project, ADSP), 0.3% of the LOAD patients carried a LP/P variant in the discovery stage of this study¹⁵. Some of these variants were known to be associated with reduced penetrance and later AOO. Thus, genetic screen in LOAD patients should rely on a case-by-case basis and sequencing is not widely recommended.

Interestingly, the rate of LP/P variants in genes causing differential diagnoses was extremely low, suggesting that using IWG-2 criteria drastically reduces the likelihood of non-AD diagnoses. In addition, the only patient with a redirection of the diagnosis towards FTLD was questionable because of borderline evidence of amyloidopathy and uncommon phenotype for AD. Besides, another patient, with a class-3 *MAPT* variant, showed normal A β 42 levels following a second lumbar puncture (supplementary table 3). Although a final diagnosis of FTLD cannot be confirmed in the latter patient based on genetic arguments, the diagnosis is still uncertain and not in favor of AD anymore. This study therefore underlines the importance on relying on etiological biomarkers in atypical and typical presentations.

The second aim of our study was to assess the rate of clinically relevant genetic risk factors. Although the clinical impact may appear as limited for patients, because risk factors cannot be used for genetic counseling, variants with OR>1.5, and most likely variants >2 and even more if >5 may still be clinically meaningful. Here, we developed a framework for the classification of AD risk factors in a clinical setting and identified that 69.2% of the probands without a monogenic cause exhibited at least one definite AD risk factor. This included 12.2% of patients carrying so-called rare risk factors, thus showing that such risk factors affect a significant proportion of patients and may thus be considered as rather common in EOAD and even among young LOAD patients. Such results suggest oligogenic inheritance in some patients, especially in case of identification of multiple risk factors, and this includes families with a pedigree suggesting autosomal dominant inheritance. We suggest that identifying one or multiple moderate to strong risk factors in EOAD patients may help families understand the etiology of the disease, although they are not sufficient to fully explain AD occurrence in the cases. Patients with early-onset dementia and their caregivers or relatives often wonder about

the etiology of such an elderly-associated disease occurring at a young age. Delivering etiological factors, even if not actionable, may thus be useful. In addition, risk variants bring some pieces of evidence that AD may eventually be non-monogenic, combined with a negative screen of Mendelian genes, thus providing some reassurance to families regarding recurrence risk in relatives, not reaching 50% for first-degree relatives. In addition, if the future of AD management includes prevention strategies, better understanding the role of such moderate-to-strong risk factors appears to be useful.

Of note, we used stringent criteria for variant selection, so that we returned only definite risk factors to patients. A number of variants of uncertain significance were identified but not reported. Indeed, if gene-based tests provided clear evidence of an AD-risk association of burdens of rare, predicted damaging missense and truncating variants in the selected genes, coming back to the individual and the variant levels should rely on ACMG-AMP-like arguments, which did not allow most of the missense variants to reach sufficient evidence to be reported to patients.

We report here the results of a prospective study until the delivery of the result. Of note, some of the variants may be reclassified in the future (more specifically, variants of uncertain significance, as for example one of the *PSEN1* variants later reclassified as likely benign here), and some novel genes may be considered, at least among risk factor genes. Thus, producing and interpreting such exome sequencing data should be accompanied by a service of results reinterpretation, especially for variants of uncertain significance, but also for negative results, upon request of the patient/family and referring physician.

One weakness of our study is the limitation to individuals from European ancestry. Although we regret that limitation, providing the information on risk variants requires (i) the replication of the results of association studies in the same ethnicities as patient and (ii) estimates of odds ratios in large series with inclusion criteria as close as possible to those used in the clinic. Unfortunately, association studies have mainly been performed on individuals from European ancestry, especially the latter large exome sequencing study that unveiled the role of rare variants in *ABCA1* and *ATP8B4*¹⁵. For the latter two genes, there is indeed only one study, with results replicated within this study, but all datasets are based on individuals with European ancestries. For other genes, such as *ABCA7* or *TREM2*, pieces of information are available in African-Americans, for example, as recently reviewed³³. However, most results on rare variants remain not significant after multiple testing correction, probably due to insufficient power, or odds ratios are not computed. The increased number and size of genetic studies in other ethnicities will allow the reduction of such limitations in the future. However, only risk variants seem to be affected by such limitations. There is no reason that monogenic dementia variant detection rates would be affected, and our overall results are consistent with the literature in diverse ethnicities^{7,8}. Thus, our recommendations on genetic screening of monogenic dementia genes in AD may well apply to any ethnicity.

Another limitation is that we did not consider risk factors with odds ratios below 1.5, most of them having been identified in genome-wide association studies based on DNA chips¹⁴. Such variants can be combined into polygenic scores, which could also provide part of the etiological explanation. However, beyond the fact that most of these variants are not in coding exons – and thus not detectable by exome sequencing – polygenic scores deeply rely on the large effect of the *APOE4* allele, which was taken into account in our study. The combined effect of non-*APOE* related variants remains modest. In addition, we can speculate that information on polygenic scores may be more difficult to understand for patients and families.

Here, we used exome sequencing as a cost-effective tool to (i) accurately detect variants in the selected gene list and (ii) allow the reinterpretation of results in light of the discovery of novel genes in the future, without the need to resequence. Sequencing only a gene panel would be expected to provide similar results, with a limitation for future reanalyses, while proposing genome sequencing remains more costly, with very limited gains in terms of variant detection. Gene panels might be seen as more powerful for CNV detection than exome sequencing, but we previously showed similar performances when CNVs affect at least two targets (i.e. two exons), as the design of baits for exon capture in panels and exomes is usually very similar, with the exception of panels that capture introns, although such a design is not common³⁴. Some smaller CNVs, i.e. single-exon CNVs, may be missed by bioinformatics tools when working from exon capture, but in a similar manner between panel and exome sequencing. For these reasons, we do not recommend using preferentially gene panel or exome sequencing or genome sequencing, as the availability of these techniques depends on the organization of genetic testing in a given country and results are expected to be comparable.

In conclusion, after decades of genetic screening of autosomal dominant genes in EOAD patients, we recommend to analyze dementia genes by sequencing all probands with EOAD, whatever the family history. The clinical utility of the identification of LP/P variants in Mendelian genes is clear, given the consequences for genetic counseling, and it may be even higher if preventive treatments become available in the future. The clinical utility of moderate-to-strong risk factors relies on the need for some patients and families to receive some (partial) answers on disease etiology and on reducing the likelihood of a missed LP/P variant in a Mendelian gene. The clinical impact of returning risk factors according to patients and clinicians should be assessed specifically in anticipation of putative clinical use.

Data availability

All variants of clinical interest are available in tables and supplementary tables.

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Author contributions

Conceptualization: G.N., D.W.; Data curation: G.N., D.W., A.Z., M.L., O.Q., S.R., A.-C.R., A.B., C.S., C.C.; Formal analysis: G.N., D.W., A.Z., M.L., O.Q., S.R., A.-C.R., A.B., C.S., C.C.; Funding acquisition: G.N., D.W.; Methodology: G.N., D.W.; Project administration: G.N., D.W.; Supervision: G.N., D.W.; Writing-original draft: G.N., D.W.; Writing-review & editing: G.N., D.W.; Validation: G.N., D.W.; Investigation: G.N., A.Z., M.L., O.Q., S.R., A.C.R., A.Bon., C.S., R.O., F.Sa., A.Bol., J.F.D., D.A., P.A., S.A., A.C.B., G.B., M.Ba., Y.B., S.B., M.Be., K.B., S.B., C.B.B., P.B., J.C., L.C.P., P.C., M.P.C., V.C., Y.C., J.C., E.C., F.C.C., L.C., P.C., B.C., C.C., B.D., S.D., V.d.I.S., A.d.L., D.D., F.D., V.D., C.D., E.D., M.D.F., J.D., A.D., F.E.B., M.F., A.G., A.G.S., O.G., M.G., C.Gr., S.G., J.G., C.Gu., V.G.P., S.H., C.R.H., C.Ha., G.H., C.He., C.Ho., T.J., S.J., L.K., P.K.S., J.L., H.M.L., B.L., I.L.B., G.L.G., A.L., T.L., R.L., A.L., M.A.M., E.M., C.M., O.M., A.M., R.M., E.M.R., S.M., H.M., A.M., J.N., C.N., P.O., C.P., J.P., F.P., A.P., N.P., V.P., H.P.C., M.R. A.R.S., C.R.J., D.S., M.Sar., M.Sau., F.Se., M.T., C.Th., Q.T., C.Ti., C.Tu., L.V.D., O.V., N.V., N.W., C.C., D.W.

Ethics declaration

This study has been approved by the ethics committee *CPP-Ouest III* (notification 2018-A02359-46).

Conflicts of interest

The authors have no conflict of interest to declare

Figures Legends

Figure 1. Study procedures

Figure 2. Framework for the interpretation and classification of risk factors in Alzheimer disease

Truncating variants are defined as nonsense, canonical splice site or frameshift variants predicted to trigger nonsense-mediated decay (not concerning the last coding exon or 50 last bp of the penultimate exon) and not affecting small (<100bp) in-frame exons for splice site variants or single exon deletions.

Strength of effect is classified based on OR and appears in bold: modest if $1.5 < OR < 2$, moderate if $2 < OR < 5$ and strong if $OR > 5$

TREM2 R47H: NM_018965.4:c.140G>A p.(Arg47His)

TREM2 R62H: NM_018965.4c.185G>A p.(Arg62His)

ATP8B4 G395S: NM_024837.3:c.1183G>A p.(Gly395Ser)

Figure 3. Rates of AD and other dementia Mendelian genes based on ages of onset and pedigree structure.

LOAD: late-onset Alzheimer Disease ($66 < \text{onset} \leq 75$ years) EOAD: Early-Onset Alzheimer Disease ($\text{onset} \leq 65$); Familial-early: at least one affected relative presents EOAD; Familial-late: no known relative with EOAD, at least one affected relative presenting LOAD; AOO: Age of onset; DNV: De Novo Variant. Black filled symbols indicate EOAD; grey filled symbols indicate LOAD. LP/P: likely pathogenic or pathogenic variants considered to be the cause of dementia.

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ID	Sex	APOE	Gene	Variant class	Variant nomenclature	ACMG-AMP variant classification scores	AD Clinical presentation	AOO (y)	Disease duration (y)	MMSE	1 st symptoms to MRI (y)	Brain MRI	Family history
EFA-055-001	Female	34	<i>PSEN1</i>	4 (LP)	NM_000021.4:c.289G>A p.(Val97Met)	PM1, PM2, PP3, PP4	amnesic AD	58	4	14	4	Insular and parietal bilateral atrophy	Sporadic
EFA-075-001	Female	34	<i>PSEN1</i>	4 (LP)	NM_000021.4:c.800C>T p.(Pro267Leu)	PM1, PM2, PM5, PP3, PP4	amnesic AD	55	6	18	6	Moderate diffuse cortical atrophy (S:2-2) associated with numerous occipital microbleeds	Sporadic
EFA-084-001	Female	33	<i>APP</i>	5 (P)	NM_000484.4:c.2140A>G p.(Thr714Ala)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	42	5	8	4	Slight diffuse cortical atrophy	Sporadic (de novo variant)
EFA-105-001	Female	34	<i>APP</i>	5 (P)	NM_000484.4:c.2148C>G p.(Ile716Met)	PS1, PS3, PM1, PM2, PP3, PP4	amnesic AD	63	6	22	5	Hippocampal bilateral atrophy (S:3-3)	Familial - EOAD
EFA-142-001	Female	23	<i>PSEN1</i>	4 (LP)	NM_000021.4:c.346A>G p.(Thr116Ala)	PM1, PM2, PP3, PP4	amnesic AD	54	2	22	2	Left hippocampal atrophy (S:1-0) and superficial cortical hemosiderosis	Familial - LOAD
EFA-216-001	Male	23	<i>PSEN1</i>	5 (P)	NM_000021.4:c.1171G>T p.(Val391Phe)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	61	4	19	3	Diffuse cortical atrophy predominantly in parietal cortex	Familial - EOAD (2 generations)
EFA-218-001	Male	33	<i>TARDBP</i>	5 (P)	NM_007375.4:c.1144G>C p.(Ala382Pro)	PS1, PS4, PM2, PP2, PP3	Frontal variant of AD ^a	55	3	29	3	Temporal bilateral atrophy	Familial - unknown AOO for relatives
EFA-303-001	Female	33	<i>PSEN1</i>	5 (P)	NM_000021.4:c.640C>T p.(His214Tyr)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	50	9	0	4	Diffuse cortical atrophy predominantly in left hemisphere	Familial - EOAD (2 generations)
EFA-355-001	Female	34	<i>APP</i>	5 (P)	NM_000484.4:c.2149G>A p.(Val171Ile)	PS1, PS3, PM1, PM2, PP3, PP4	amnesic AD	48	3	14	3	Diffuse cortical atrophy predominantly in medial temporal cortex	Familial - EOAD (2 generations)
EFA-378-001	Male	22	<i>PSEN1</i>	5 (P)	NM_000021.4:c.791C>T p.(Pro264Leu)	PS1, PS3, PM1, PM2, PP3, PP4	posterior cortical atrophy	45	9	9	7	Occipital and parietal bilateral atrophy	Familial - EOAD (2 generations)
EFA-444-001	Female	33	<i>PSEN1</i>	5 (P)	NM_000021.4:c.551A>G p.(Glu184Gly)	PS1, PM1, PM2, PM5, PP3, PP4	amnesic AD	53	4	12	4	Normal	Familial - EOAD (2 generations)
EFA-460-001	Male	44	<i>APP</i>	5 (P)	NM_000484.4:c.2149G>A p.(Val171Ile)	PS1, PS3, PM1, PM2, PP3, PP4	amnesic AD	44	3	19	2	Bilateral parietal and temporal atrophy extended to left posterior precuneus	Familial - EOAD (2 generations)
EFA-492-001	Male	34	<i>PSEN2</i>	5 (P)	NM_000447.3:c.850A>G p.(Arg284Gly)	PS1, PS3, PM2, PP3, PP4	amnesic AD	52	7	12	4	Moderate diffuse atrophy (S:1-2)	Familial - LOAD
EFA-500-001	Female	33	<i>PSEN1</i>	5 (P)	NM_000021.4:c.1133G>A p.(Gly378Glu)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	33	1	25	1	Right parietal occipital atrophy	Familial - EOAD (2 generations)
EFA-570-001	Female	33	<i>PSEN1</i>	5 (P)	NM_000021.4:c.360A>C p.(Glu120Asp)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	posterior cortical atrophy	44	4	19	3	Bilateral parietal occipital atrophy	Familial - EOAD (2 generations)
EFA-601-001	Male	33	<i>PSEN1</i>	5 (P)	NM_000021.4:c.806G>A p.(Arg269His)	PS1, PM1, PM2, PM5, PP3, PP4	amnesic AD	55	3	17	2	ND (CT scan showing moderate cerebrovascular leukopathy)	Familial - unknown AOO for relatives
EFA-618-001	Male	34	<i>PSEN1</i>	5 (P)	NM_000021.4:c.344A>G p.(Tyr115Cys)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	42	9	20	4	Normal	Familial - EOAD (2 generations)
RFA-015-001	Female	34	<i>PSEN1</i>	5 (P)	NM_000021.4:c.1174C>G p.(Leu392Val)	PS1, PS3, PM1, PM2, PM5, PP3, PP4	amnesic AD	46	3	26	2.5	Mild temporo-frontal atrophy	Familial - EOAD (2 generations)
RFA-051-001	Female	33	<i>PSEN1</i>	4 (LP)	NM_000021.4:c.1225G>A p.(Ala409Thr)	PM1, PM2, PP3, PP4	amnesic AD	55	22	0	13	Parietal bilateral atrophy	Familial - EOAD (2 generations)
RFA-059-001	Female	44	<i>PSEN1</i>	5 (P)	NM_000021.4:c.1309A>G p.(Ile437Val)	PS3, PM1, PM2, PP3, PP4	amnesic AD with behavioral modifications	57	3	9	2	Parietal bilateral atrophy	Sporadic
RFA-079-001	Male	34	<i>PSEN2</i>	4 (LP)	NM_000447.3:c.365C>T p.(Thr122Met)	PM1, PM2, PP3, PP4	amnesic AD	57	3	18	3	Hippocampal atrophy	Sporadic

Table 1. Patients with a monogenic variant considered as the cause of dementia

^aPatient initial diagnosis was frontal variant of AD due to CSF biomarkers, then was redirected to behavioral fronto-temporal lobar degeneration after exome sequencing

LP: likely pathogenic, P: Pathogenic, ACMG-AMP: American College of Medical Genetics and Genomics - Association for Molecular Pathology

MMSE: mini-mental state examination, MRI: magnetic resonance imaging, ND: not done

S:XX-XX refers to left and right Scheltens' scale hippocampal atrophy score on brain MRI, respectively

**Enrollment:
N=700 during 30 months**

- **AD with age of onset ≤ 65 or ≤ 75 whatever the family history**
- **Positive CSF biomarkers (or amyloid PET)**



**Information & request for the genetic analysis
Data collection: clinical, imaging and CSF data**



Upon inclusion: DNA isolation and preparation

Exome sequencing

Agilent sureselect V6+UTR
Illumina >100-120x average
NovaSeq6000



**Receipt of raw data
Bioinformatics analyses**



**Identification of SNVs, indels,
copy number variations (deletions, duplications)**



Targeted interpretation of genetic variants in:

- **Autosomal dominant AD genes: *APP, PSEN1, PSEN2***
- **Other dementia Mendelian genes**
- **AD risk factor genes: *TREM2, SORL1, ABCA7, ABCA1, ATP8B4, APOE***



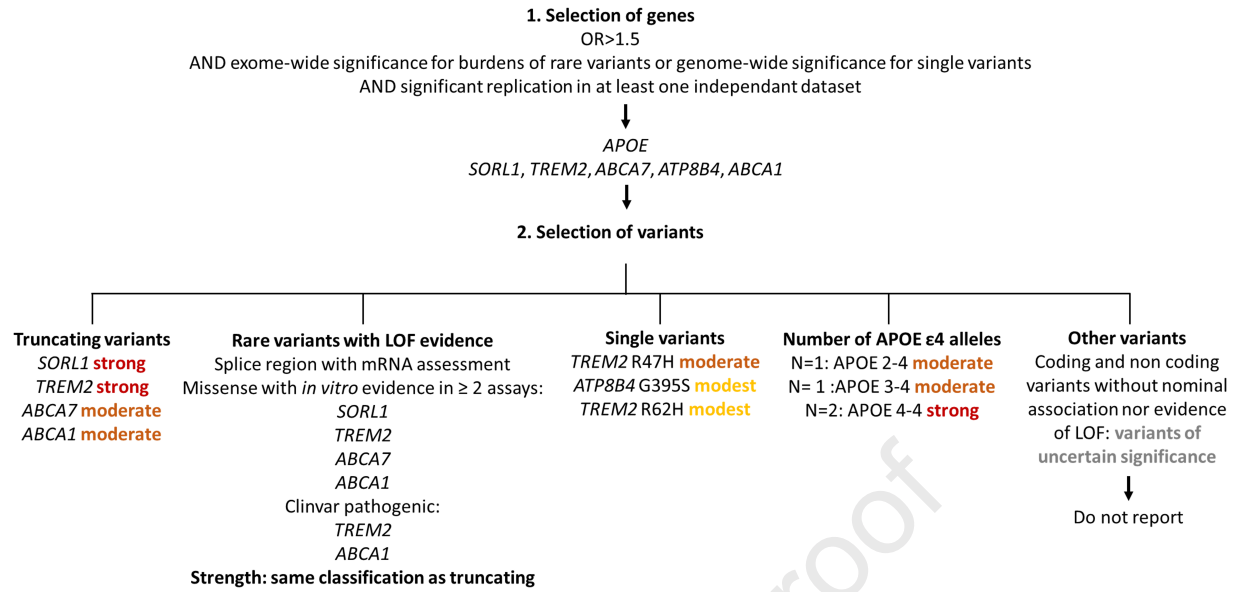
Confirmation by an independent technique (rate: 100%)

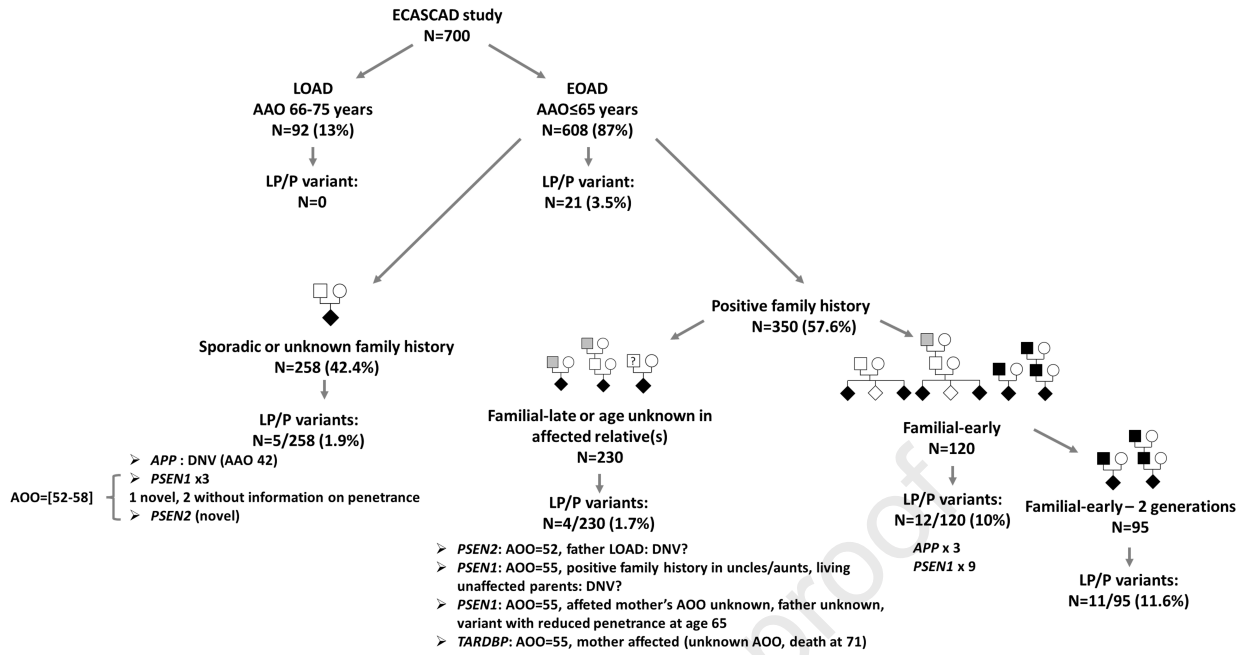


**Genetic report with standardized & personalized information:
Genetic result + keypoint summary letter + patient letter**



Report to patient by referring physician





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The authors have no conflict of interest to declare

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