BRAIN
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Aberrant splicing of PSEN2, but not PSEN1, in individuals with sporadic Alzheimer's disease

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Alzheimer's disease is the most common neurodegenerative disease, characterized by dementia and premature death. Early-onset familial Alzheimer's disease is caused in part by pathogenic variants in presenilin 1 (PSEN1) and presenilin 2 (PSEN2), and alternative splicing of these two genes has been implicated in both familial and sporadic Alzheimer's disease. Here, we leveraged targeted isoform-sequencing to characterize thousands of complete PSEN1 and PSEN2 transcripts in the prefrontal cortex of individuals with sporadic Alzheimer's disease, familial Alzheimer's disease (carrying PSEN1 and PSEN2 variants), and controls. Our results reveal alternative splicing patterns of PSEN2 specific to sporadic Alzheimer's disease, including a human-specific cryptic exon present in intron 9 of PSEN2 as well as a 77 bp intron retention product before exon 6 that are both significantly elevated in sporadic Alzheimer's disease samples, alongside a significantly lower percentage of canonical full-length PSEN2 transcripts versus familial Alzheimer's disease samples and controls. Both alternatively spliced products are predicted to generate a prematurely truncated PSEN2 protein and were corroborated in an independent cerebellum RNA-sequencing dataset. In addition, our data in PSEN variant carriers is consistent with the hypothesis that PSEN1 and PSEN2 variants need to produce full-length but variant proteins to contribute to the onset of Alzheimer's disease, although intriguingly there were far fewer full-length transcripts carrying pathogenic alleles versus wild-type alleles in PSEN2 variant carriers. Finally, we identify frequent RNA editing at Alu elements present in an extended 3′ untranslated region in PSEN2. Overall, this work expands the understanding of PSEN1 and PSEN2 variants in Alzheimer's disease, shows that transcript differences in PSEN2 may play a role in sporadic Alzheimer's disease, and suggests novel mechanisms of Alzheimer's disease pathogenesis.

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Introduction

Ranking as the most common form of both dementia and neurodegeneration, Alzheimer's disease is progressively debilitating and uniformly fatal. Almost six million Americans have the disease—a number that will continue to escalate as our population ages—and still there remains no effective treatment. To better develop targeted interventions, we sought to uncover the complex transcriptional changes that may take place in Alzheimer's disease. Here, we study transcripts produced by presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*).

Originally identified through linkage analyses, autosomal dominant variants in *PSEN1*, *PSEN2*, and amyloid precursor protein (*APP*) lead to early-onset Alzheimer's disease (onset <65 years of age).[1–3] These three risk genes are mechanistically united in the following way: either of the two presenilins form part of the γ-secretase complex, which cleaves APP to liberate a product called amyloid beta (Aβ) peptide. Though the mechanisms by which variants in *PSEN1* and *PSEN2* lead to Alzheimer's disease are still debated, it is commonly thought that changes in *PSEN1* or *PSEN2* bias APP cleavage to form versions of Aβ peptide that are more prone to aggregation. Aggregation of Aβ, in turn, is a hallmark pathology of Alzheimer's disease.[4–7]

In addition to playing a role in early-onset Alzheimer's disease, variants in *PSEN1* and *PSEN2* can also increase risk for, or cause, late-onset Alzheimer's disease.[8–12] It is generally thought that all disease-causing variants in these genes impair normal function of the γ-secretase complex, resulting in abnormal cleavage of APP and aggregation of Aβ. Tellingly, of all identified Alzheimer's disease-related *PSEN1* variants (there are now over 200),[13] not one leads solely to a truncation or absence of protein product.[14] *PSEN2* variants are not as common as those in *PSEN1* (19 identified so far),[13] but they similarly do not appear to lead to haploinsufficiency. These observations have led to a controversial hypothesis that *PSEN1* and *PSEN2* produce full-length but variant proteins in the context of Alzheimer's disease. Determining whether this hypothesis is true would notably increase our understanding of Alzheimer's disease pathogenesis.

Recently, we characterized the impact of an Alzheimer's disease-related *PSEN2* variant, PSEN2 K115Efs.[15] This variant is a 2 bp deletion, which should lead to a frameshift and premature stop codon, and therefore be the first known case of a loss-of-function variant in *PSEN2*. Instead, however, we detected a novel intronic splice acceptor site that added 77 bp to the transcript, which would restore the reading frame and generate a full-length PSEN2 product with 25 extra amino acids.[16] This transcript was specific to patient brain tissue and was not observed in fibroblasts. We also observed the same intron retention splicing pattern in brain tissues from patients with sporadic Alzheimer's disease (i.e. lacking the 2 bp deletion) and observed use of this alternative splice site in RNA-sequencing data of anterior frontal cortex in the Mount Sinai Brain Bank, leading us to hypothesize that alternative splicing of familial Alzheimer's disease risk genes may also play a role in sporadic Alzheimer's disease.

The reading frame of PSEN2 K115Efs could also be restored by the omission of exon 6. Alternative splicing leading to skipping of *PSEN2* exon 6 occurs under hypoxic conditions—a condition increasingly implicated in Alzheimer's disease[17]—and the truncated splicing product is elevated in the brains of patients with sporadic Alzheimer's disease.[18] This splicing event is thought to decrease the unfolded protein response and increase γ-secretase activity.[19] Splicing is altered by high mobility group AT-hook 1 (*HMGA1*),

which binds exon 6 and causes it to be skipped, resulting in a premature stop codon and thus a truncated protein product.[20,21] PSEN2 K115Efs acts in a manner similar to *PSEN2* transcripts with exon 6 spliced out—that is, both form similar truncated products—but together, they form an almost full-length PSEN2 product, lacking exon 6.

In this study, we set out to characterize full-length transcripts of *PSEN1* and *PSEN2* in brain tissue from individuals with familial and sporadic Alzheimer's disease as well as controls using PacBio Isoform-Sequencing (Iso-Seq). PacBio Iso-Seq is a state-of-the-art technique that uses single molecule, real-time (SMRT) sequencing to completely sequence all targeted mRNAs up to 20 kilobases long.[22] The long-read capability of PacBio Iso-Seq is the only way to fully capture all exons in combination with pathogenic variants in *PSEN1* (2776 bp) and *PSEN2* (2249 bp) transcripts. Unlike short-read sequencing, it allows us to identify complete novel transcripts and alternative splicing events, as well as quantify differences in transcript abundance between samples.

Materials and methods

Samples used

The University of Washington Alzheimer's Disease Research Center (UW ADRC) Neuropathology Core provided post-mortem prefrontal cortex samples from the 20 cases listed in Table 1. All samples were collected following informed consent approved by the UW Institutional Review Board. Control individuals were recruited through the Adult Changes in Thought study from Kaiser Permanente Washington,[23] a population-based cohort study in which participants 65 years or older undergo cognitive screening every 2 years. Tissues used for this study were collected during rapid brain autopsy and flash frozen in liquid nitrogen or supercooled isopentane. Here, two controls, two sporadic Alzheimer's disease and two *PSEN1* samples were frozen in isopentane.

RNA extraction and Iso-Seq

RNA was extracted from brain tissue samples using the RNeasy Lipid Tissue Mini Kit (Qiagen). Then 300 ng of RNA was then converted to cDNA, hybridized and prepared for Iso-Seq following a protocol from PacBio called 'Iso-Seq Express Capture Using IDT xGen Lockdown Probes'. Briefly, RNA was converted into cDNA and amplified using a combination of the NEBNext Single Cell/ Low Input cDNA Synthesis & Amplification Module (New England Biolabs), the Iso-Seq Express Oligo Kit (PacBio), and barcoded primers (found in Appendix 3 of the PacBio protocol). RNA integrity number scores for each sample varied on the basis of the quality of the original tissue. Samples were then pooled into two groups based on higher or lower RNA integrity number scores (researchers were blinded to sample identity), and these two pools were hybridized to a custom panel of xGen Lockdown Probes (Integrated DNA Technologies) using the xGen Lockdown Hybridization and Wash Kit (Integrated DNA Technologies). In total, the probe panel contained 181 probes tiling both exonic and intronic regions of *PSEN1* (128 probes) and *PSEN2* (53 probes) at approximately equal intervals (Fig. 1A and Supplementary Table 1). After 15 cycles of amplification of the captured cDNA, pools were combined (after confirming that both DNA pools had similar quality control scores) and submitted to UW PacBio Sequencing Services for library preparation and running on PacBio Sequel II for 1 SMRT Cell 8M with a

Table 1 Samples used in study

Cohort	Age at death (years)	Sex	PMI (hours)	Cognitive status	PSEN variant, if applicable	APOE status	Braak score	CERAD score
Control	88	F	3.50	No dementia		ε3/ε3	III	0
Control	90	M	5.00	No dementia		ε3/ε3	III	0
Control	88	F	3.18	No dementia		ε2/ε3	II	0
Control	90	F	4.50	No dementia		ε2/ε3	III	0
Control	78	M	10.02	No dementia		ε3/ε3	0	0
Control	83	F	2.67	No dementia		ε3/ε3	III	0
PSEN	43	M	2.50	Dementia	PSEN1, I143T	ε3/ε4	VI	3
PSEN	55	M	3.12	Dementia	PSEN1, S212Y	ε2/ε4	VI	3
PSEN	58	M	6.33	Dementia	PSEN2, N141I	ε3/ε3	VI	3
PSEN	73	M	5.50	Dementia	PSEN2, N141I		VI	3
PSEN	67	F	10.18	Dementia	PSEN2, K115Efs	ε3/ε3	VI	3
PSEN	40	F	4.65	Dementia	PSEN1, V272A		VI	3
PSEN	38	M	9.85	Dementia	PSEN1, M146L		VI	3
Sporadic	90+	M	6.00	Dementia		ε3/ε4	VI	3
Sporadic	90+	F	4.50	Dementia		ε3/ε3	V	3
Sporadic	88	F	3.33	Dementia		ε3/ε3	VI	3
Sporadic	84	F	4.47	Dementia		ε2/ε4	V	3
Sporadic	90+	F	5.50	Dementia		ε3/ε4	VI	3
Sporadic	81	F	4.50	Dementia		ε3/ε4	VI	3
Sporadic	60	M	8.68	Dementia			VI	3

APOE = Apolipoprotein E; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; F = female; M = male; PMI = post-mortem interval; PSEN = presenilin.

30-h video time, using SQII v.2.0 polymerase and v.2.0 sequencing chemistry.

Iso-Seq analysis

We received full-length transcript information in FASTQ files, which were initially assessed using the standard Iso-Seq Analysis pipeline in SMRT Link (PacBio, v.10.1.0.119588). Briefly, barcoded reads were sorted, and barcodes and poly-A sequences were clipped using the Lima SMRT Analysis tool (v.2.2.0). Reads with a quality score of 20 or greater were mapped to the human GRCh38 genome using pbmm2 (v.1.3.0) and minimap2 (v.2.23) to generate a set of BAM files.

We counted the number of reads per sample with a transcript origin in PSEN1 (GRCh38 coordinates chr14:73,136,507–73,223,691) and PSEN2 (chr1:226,870,616–226,896,098). Of these, we quantified the number of reads that contained both canonical and alternative splice isoforms. To obtain total reads, we counted the number of reads that mapped to transcript coordinates. To ascertain full-length PSEN1 and PSEN2 transcripts, we extracted reads that contained the expected start and stop codon sequences. For uniquely mapped reads, all identical isoforms were counted as one.

RNA editing analysis

Iso-Seq reads that mapped to the AluJb (chr1:226896582–226896815) element in the PSEN2 3′ untranslated region (UTR) were assessed for the number of guanine residues at each adenine position. The percentage of total A-to-G changes was then quantified.

RNA-seq analysis

Mayo Clinic sample RNA-seq BAM files from the cerebellum and temporal cortex were downloaded from Accelerating Medicines Partnership Program for Alzheimer's Disease, Synapse ID syn5550404. Percentage splice inclusion of alternative splice products was measured by calculating junction spanning reads across canonical and alternative splice junctions, confirmed by quantification from Sashimi plots generated by Integrated Genomics Viewer. Due to increased read depth at these positions, RNA editing was calculated across both AluJb (chr1:226896582-226896815) and AluY (chr1:226898626-226898913) elements in the PSEN2 3′ UTR.

Statistical analysis

Statistical analyses were performed using Prism v.9.2.0 (GraphPad Software). The Shapiro–Wilk test ($n < 8$) or D'Agostino–Pearson omnibus K2 test ($n > 8$) was first used to determine whether data were normally distributed. A one-way ANOVA was used to compare groups of more than two with parametric distribution. If the ANOVA gave $P < 0.05$, this analysis was then followed by Tukey's multiple comparisons. To analyse correlations, a Spearman correlation coefficient was used for non-parametric data and a Pearson correlation coefficient was used for parametric data.

Data availability

RNA sequence reads from the Mayo Clinic are available from synapse.org with accession number syn5550404.

Results

Obtaining long-read transcripts of PSEN1 and PSEN2

To observe PSEN1 and PSEN2 transcripts in individuals with Alzheimer's disease and controls, we first prepared cDNA from 20 samples. These samples were composed of prefrontal cortex tissues, from six non-demented donors with low or absent Alzheimer's disease neuropathological change (referred to as 'controls'), seven individuals with both familial Alzheimer's disease and a PSEN1 or PSEN2 variant, and seven individuals with neuropathologically confirmed sporadic Alzheimer's disease (Table 1). Of the seven samples from individuals with PSEN1 or PSEN2 variants, four had PSEN1 variants (all different), and three had PSEN2
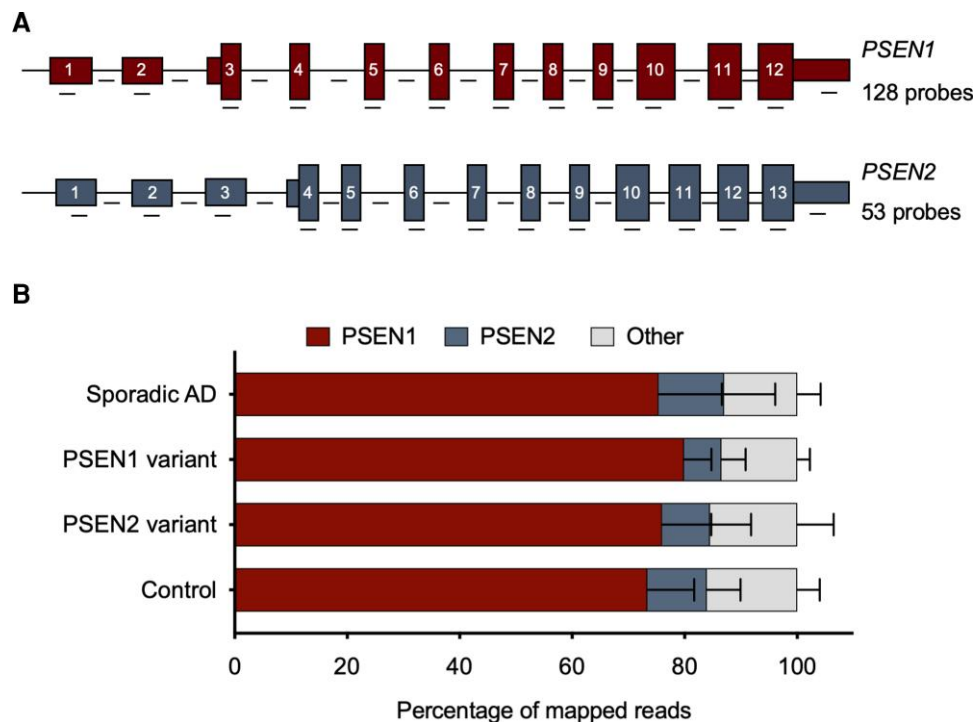
Figure 1 Iso-Seq reads mapping to PSEN1 and PSEN2. (**A**) Schematic of probe location and number of probes used per gene. (**B**) Iso-Seq reads mapping to *PSEN1* and *PSEN2*. Reads that mapped to *PSEN1* and *PSEN2* were not significantly different across cohorts (*P* = 0.62 for *PSEN1* and *P* = 0.53 for *PSEN2*, one-way ANOVA). Bars are mean and standard deviation. Sporadic Alzheimer's disease (AD) *n* = 7, PSEN variant carrier *n* = 7, control *n* = 6.

variants, one of which was the K115Efs variant and two of which were N141I variants. Together, we refer to these samples as 'PSEN variant carriers'. As noted later in this study, we did observe that one of our sporadic Alzheimer's disease samples carried a *PSEN1* variant of unknown significance, and we continued to consider this sample sporadic, based on the known literature of this variant of unknown significance (the individual's late age of death at >90 years old also supports this decision). These samples were all obtained within a post-mortem interval of less than 12 h. Controls and sporadic Alzheimer's disease samples were reasonably age- and sex-matched. Due to the early-onset nature of familial Alzheimer's disease, we could not obtain age-matched controls for the *PSEN* variant carriers (Table 1).

We obtained *PSEN1*- and *PSEN2*-specific transcripts from the cDNA by hybridization capture, and then submitted these samples for SMRT sequencing. We favoured the use of hybridization probes over PCR amplification of transcripts to ensure that we would be able to observe all alternative splice isoforms, including truncated isoforms. Our hybridization probes corresponded to both coding sequence and genomic region (Supplementary Table 1), so that we could identify potential intron retention products. The number of probes used per gene and a schematic of probe location is provided in Fig. 1A. A step in cDNA conversion involving selection for poly-A tails suggests that our findings here reflect transcripts that have undergone mRNA processing.

Alignment to PSEN1 and PSEN2

On average, we obtained 81 034 ± 36 374 mapped reads per sample (range 28 444–167 125). Of these, 75.8 ± 8.68% mapped to *PSEN1* (range 53.8–86.1%) and 9.86 ± 6.91% mapped to *PSEN2* (range 2.69–31.5%). Values were not significantly different across cohorts (Fig. 1B; *P* =

0.62 for *PSEN1* and *P* = 0.53 for *PSEN2*, one-way ANOVA). Analysis of uniquely mapped reads instead of total reads led to similar results (Supplementary Fig. 1). The higher mapping rate for *PSEN1* may reflect an increased abundance of *PSEN1* in the frontal cortex or could be due to the higher number of probes used for *PSEN1* mRNA versus *PSEN2* mRNA (Fig. 1A). The reads that did not map to *PSEN1* or *PSEN2* were evenly distributed throughout the genome, with no evidence for biased enrichment of a particular gene when analysing unique reads (Supplementary Fig. 2A), and some preferential amplification of proteolipid protein 1 (*PLP1*) and myelin basic protein in just two samples when analysing total reads (Supplementary Fig. 2B). The distribution of reads was comparable between cohorts.

PSEN1 isoforms

The generation of appropriately spliced and full-length *PSEN1* was largely efficient, with ~75% of reads encoding for an in-frame protein product. A minimum of 16 000 canonical full-length transcripts of *PSEN1* that encoded for an in-frame protein product were found in each sample. Importantly, in the PSEN variant carriers, roughly half of full-length transcripts of *PSEN1* were observed to contain the pathogenic variant.

Two predominant splice isoforms were detected, corresponding to the omission or inclusion of 12 bp (the amino acids VRSQ) at the end of exon 3 of 12 (first coding exon) of the *PSEN1* transcript (Fig. 2A). The abundance of the transcript without these 12 bp (463 aa; ENST00000357710.8) versus with these nucleotides (467 aa; ENST00000324501.10) was comparable across sporadic Alzheimer's disease, PSEN variant carriers and controls, with short and long isoforms each occurring at the same rate (Fig. 2B; *P* = 1.00, one-way ANOVA). Transcripts missing the 12 bp are thought to be the alternative transcripts, as these nucleotides are conserved in *PSEN2* transcripts.
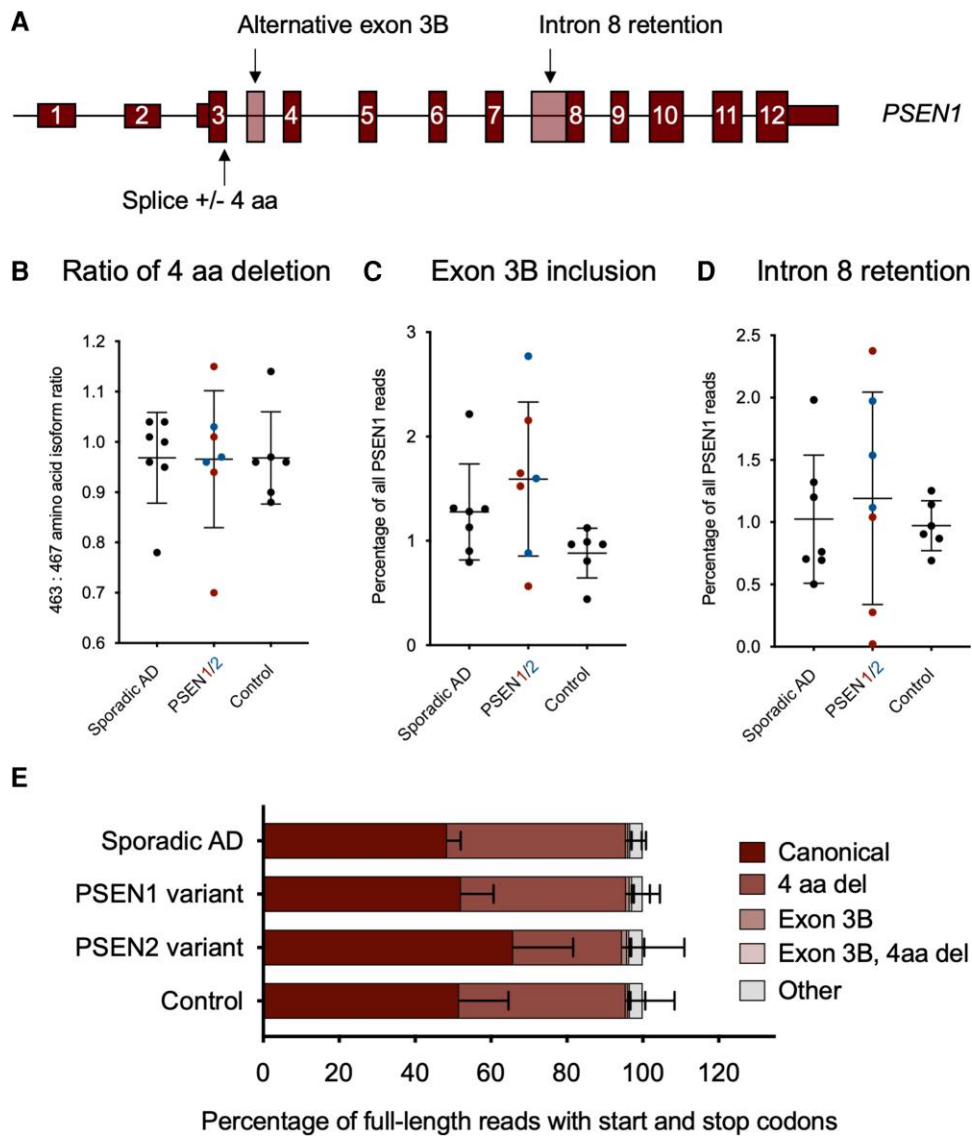
Figure 2 Isoforms of PSEN1. (**A**) Schematic showing the three most predominant isoforms identified for PSEN1. (**B**) Ratio of 4 amino acid (aa) deletion to inclusion isoform of PSEN1. Abundance (percentage of total PSEN1 transcripts) of PSEN1 (**C**) exon 3b inclusion isoform, and (**D**) intron 8 retention isoform, across cohorts. No isoform exhibited significantly different ratio or abundance across cohorts ($P > 0.05$, one-way ANOVA, for each). (**E**) Distribution of various PSEN1 isoforms as a percentage of all full-length transcripts with canonical start and stop codons. All bars are mean and standard deviation. Sporadic Alzheimer's disease (AD) $n = 7$, PSEN variant carrier $n = 7$, control $n = 6$.

The third most common isoform from our analysis, however, was not annotated. This isoform of PSEN1 included a 130 bp alternatively spliced exon in intron 3 (GRCh38 chr14:73,160,009–73,160,138) (Fig. 2A). The extra exon (exon 3B) is composed of portions of AluJb and L1MB2 repeat elements and introduces a frameshift and premature termination codon at amino acid 61. If an internal methionine start codon is used, the N-terminally truncated protein product would be 384 amino acids long. The inclusion of exon 3B remained the same between controls, PSEN variant carriers, and sporadic Alzheimer's disease samples (Fig. 2C; $P = 0.084$, one-way ANOVA). One of the next most abundant isoforms contains an alternative splice acceptor site in intron 8 that leads to an intron retention product and is present in ~1% of reads for all samples. Again, this number remained the same across all three cohorts (Fig. 2D; $P = 0.79$, one-way ANOVA). All full-length transcripts carrying each of these alternative splice events in an otherwise canonical sequence are

quantified as a percentage of total full-length PSEN1 isoforms observed across cohorts in Fig. 2E. Analysis of uniquely mapped reads gave similar results for all PSEN1 isoforms (Supplementary Fig. 2).

In one sample annotated as sporadic, we identified a 3 bp deletion in exon 4 of PSEN1 (p.Asp40del; GRCh38 coordinates chr14:73,170,825–73,170,827). This 3 bp deletion has been previously observed in Alzheimer's disease cases[24,25] and is present in gnomAD (v.3.1.2) in 18 individuals (allele frequency of 0.0001183), indicating that it is currently a variant of unknown significance. In a recent systematic study, this variant was deemed a risk factor for Alzheimer's disease.[26]

PSEN2 isoforms

Alignment of Iso-Seq reads to PSEN2 revealed a more heterogeneous population of transcripts than those observed for PSEN1

(Fig. 3A). The generation of an appropriately spliced and full-length *PSEN2* was slightly less efficient than for *PSEN1*, with 62% of reads encoding for an in-frame protein product. At least 882 canonical full-length transcripts of *PSEN2* were found in each sample. Like *PSEN1*, full-length transcripts contained both wild-type and pathogenic variant alleles for each of the PSEN variant carriers.

Again, after the canonical *PSEN2* transcript, the second most abundant transcript that spanned from start to stop codons was one that contained an alternative splice site—an alternative acceptor site in this instance—at the beginning of exon 11 of 13, which resulted in the removal of 3 bp (1 amino acid) from the total protein product. Sporadic Alzheimer's disease samples, controls and PSEN variant carriers all had similar levels of this isoform, with the short isoform occurring less often than the long isoform (Fig. 3B; $P = 0.11$, one-way ANOVA).

Also, like *PSEN1*, the third most abundant transcript was non-productive (i.e. a premature stop codon is introduced). A 1657 bp transcript was detected in 4.8% of full-length reads across all samples. This alternative exon appears between exons 9 and 10, herein referred to as exon 9B (Fig. 3A). The exon is 310 bp, flanked by canonical splice sites, contains stop codons in all three reading frames and is predicted to produce a protein truncated at 321 amino acids. Reads mapping to the exon 9B region accounted for over 50% of total *PSEN2* reads for one sporadic sample and were significantly more abundant in sporadic Alzheimer's disease than in controls ($P = 0.0007$) or PSEN variant carriers ($P = 0.0013$), while PSEN variant carriers exhibited similar levels as controls (Fig. 3C; $P = 0.88$, one-way ANOVA followed by Tukey's multiple comparisons). A known isoform of *PSEN2* (ENST00000487450.1) lists the presence of exon 9B, though mRNA abundance stemming from this isoform is low based on GTEx data.[27] We observed several other transcript isoforms that contained exon 9B in combination with intron retention and alternative splice products present in introns 8 and 9, many of which were enriched in sporadic Alzheimer's disease (Supplementary Fig. 3). Exon 9B inclusion was not correlated with age at death in sporadic or any other cases [Supplementary Fig. 4; $r = 0.24$, P (two-tailed) = 0.30, Spearman correlation].

A fourth *PSEN2* isoform was also detected corresponding to the inclusion of 77 bp of intronic sequence prior to exon 6 (Fig. 3A). We previously identified the presence of this 77 bp intron product in the context of a 2 bp deletion.[16] This transcript is a full-length mRNA including start and stop codons and all exons in between, but due to a frame shift, is not predicted to form a full-length protein product. Again, sporadic Alzheimer's disease samples had a significantly higher abundance of this 77 bp intron product as compared to controls ($P = 0.0050$) or PSEN variant carriers ($P = 0.017$), while PSEN variant carriers exhibited similar levels as controls (Fig. 3D; $P = 0.76$, one-way ANOVA followed by Tukey's multiple comparisons). Average percentages were 6.59% for sporadic Alzheimer's disease, 1.91% in PSEN (although three samples had zero reads) and 0.79% in controls. The highest level of the 77 bp intron product in the PSEN variant carriers was observed in the *PSEN2* 2 bp deletion (PSEN2 K115Efs; highlighted in Fig. 3D), which corroborates our previous work.[16]

Exon 6 skipped products are not a common feature of *PSEN2* Iso-Seq data

An exon 6 skipped product, termed PS2V, is the subject of several previous reports.[18,21,28,29] This exon skipping is induced by hypoxia and mediated by binding of HMGA1.[20,21] Here, we find few examples of reads that skip exon 6, including in the 2 bp deletion sample,

where deletion of exon 6 would restore full-length protein production (minus 48 aa).[16] Abundance of transcripts exhibiting exon 6 skipping remained the same across cohorts (Fig. 3E; $P = 0.28$, one-way ANOVA). A notable exception to this lack of PS2V was in the two carriers of the *PSEN2* N141I pathogenic variant. In each, 8.26–9.64% of all reads that mapped to *PSEN2* contained a splice product skipping exon 6 (highlighted in Fig. 3E). Interestingly, amino acid 141 is in the middle of exon 6, suggesting that the nucleotide variant may influence alternative splicing of exon 6 (Fig. 3A). The binding site consensus sequence of HMGA1 is CUGCUACAAG at the end of exon 6 (amino acids 163–166), distal to the N141 codon (Supplementary Fig. 5).

In one of the *PSEN2* N141I carriers, we identified a heterozygous position (rs1046240) in exon 5. Using this information, we could deduce that all 479 reads that skipped exon 6 also contained the alternate allele at rs1046240 and therefore were in *cis* with the 141I allele. For the second N141I individual, however, we did not identify heterozygous positions in the mRNA that could be leveraged for a similar analysis.

Finally, full-length *PSEN2* transcripts carrying each of these mentioned alternative splice events on their own were quantified as a percentage of all full-length *PSEN2* isoforms (containing canonical start and stop codons and an otherwise canonical transcript) observed across cohorts in Fig. 3F. These data include a splice isoform that skips exon 9, though this isoform maintains the *PSEN2* reading frame and is not significantly different between cohorts (Supplementary Fig. 3). In contrast to *PSEN1*, the proportion of full-length reads for *PSEN2* is significantly lower in sporadic Alzheimer's disease cases relative to controls ($P = 0.0055$) or PSEN variant carriers ($P = 0.013$, one-way ANOVA followed by Tukey's multiple comparisons). Of note, the 'other' group in Fig. 3F includes various combinations of alternative exons and intron retention products whose commonality involves the sharing of exon 9B sequence (Supplementary Fig. 3). Analysis of uniquely mapped reads led to similar results for all *PSEN2* isoforms (Supplementary Fig. 2).

Allelic bias at PSEN1 and PSEN2 pathogenic variant positions

Long-read Iso-Seq also afforded us the ability to examine allelic bias in PSEN variant carriers. An unresolved question from our previous work was whether the 2 bp deletion could produce a full-length in-frame product in combination with the 77 bp insertion in intron 6. Aligning reads from this individual revealed that the wild-type allele accounted for 88% of the reads (2858 reads for full-length *PSEN2* and 98 reads including the 77 bp intronic sequence). Of the 412 reads with a 2 bp deletion, about half spliced to the canonical exon 6 junction and continued to the last exon of the transcript, which is predicted to yield a truncated 124 amino acid protein product (Fig. 4A). However, 190 additional reads included the 77 bp product, generating a full-length transcript with 25 additional amino acids inserted after exon 5.

The *PSEN2* 2 bp deletion allele (i.e. the K115Efs variant) only contributes to 12% of total *PSEN2* transcripts, therefore some nonsense-mediated decay is probably active. The other two *PSEN2* samples (both with the N141I variant) displayed only 35–37% of transcripts with pathogenic variants, further indicating that decreased stability of the variant allele (probably due to nonsense-mediated decay) may take place. For *PSEN1*, the pathogenic variant was present in transcripts in roughly equal amounts at 42–51% of the time (Fig. 4B).
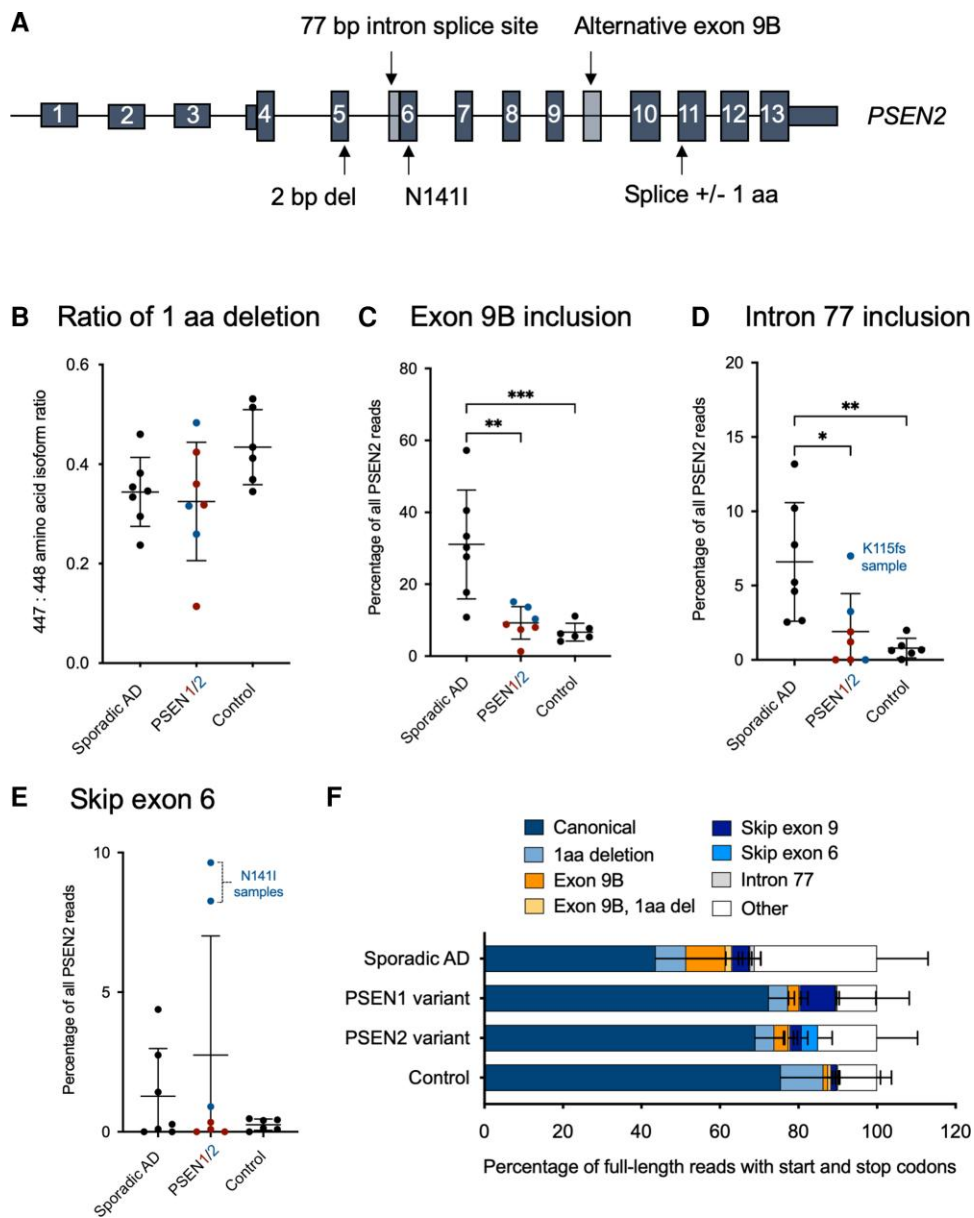
Figure 3 Isoforms of PSEN2. (**A**) Schematic showing the three most predominant isoforms identified for *PSEN2*, as well as the 77 bp intron splice site and the location of the N141I variant. (**B**) Ratio of 1 amino acid (aa) deletion to inclusion isoform of *PSEN2*. Abundance (percentage of total *PSEN2* transcripts) of *PSEN2* (**C**) exon 9b inclusion isoform, (**D**) intron 77 inclusion isoform, and (**E**) exon 6 skipping events, across cohorts. (**F**) Distribution of various *PSEN2* isoforms as a percentage of all full-length transcripts with canonical start and stop codons. *P*-values were determined by a one-way ANOVA, followed by Tukey's multiple comparisons. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. All bars are mean and standard deviation. Sporadic Alzheimer's disease (AD) $n = 7$, PSEN variant carrier $n = 7$, control $n = 6$.

Confirmation of PSEN1 and PSEN2 alternative splicing events in independent RNA-seq datasets

To determine whether the *PSEN2* exon 9B splice product is a prominent feature of Alzheimer's disease, we queried RNA-seq datasets made available through the Accelerating Medicines Partnership Program for Alzheimer's Disease. We determined that the exon 9B splice products were highest in the cerebellum from the Mayo Clinic,[30] which corresponds to increased expression of *PSEN2* in the cerebellum and cerebellar hemisphere relative to other brain regions based on GTEx data.[27] The Mayo Clinic dataset contains RNA-seq data from the cerebellum for 82 individuals with sporadic Alzheimer's disease, 76 controls, 25 individuals with pathological

ageing (Aβ deposits but no cognitive impairment) and 81 individuals with progressive supranuclear palsy. Exon 9B was included 17.4% of the time on average in Alzheimer's disease samples, significantly higher than in controls (9.81%, $P < 0.0001$) and in the pathological ageing cohort (11.0%, $P = 0.024$) but not different than progressive supranuclear palsy samples (Fig. 5A; 18.6%, $P = 0.87$, one-way ANOVA followed by Tukey's multiple comparisons).

In addition, we observed both exon 6 skipping and 77 bp intron inclusion alternative splice events at exon 6. Alternative splicing at exon 6 was higher in Alzheimer's disease samples versus controls (4.37% versus 2.52%; $P = 0.045$), as well as in progressive supranuclear palsy versus controls (4.42% versus 2.52%; $P = 0.037$), but not for Alzheimer's disease versus pathological ageing ($P = 0.54$) or
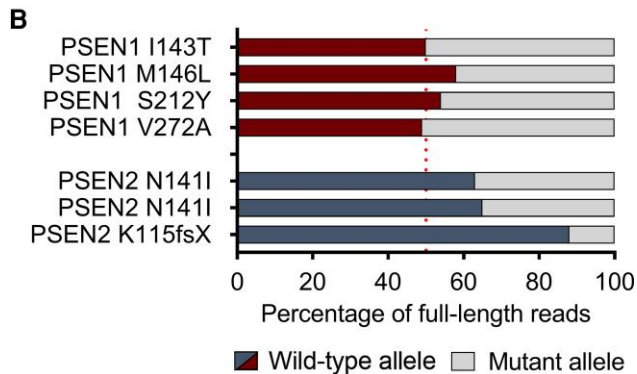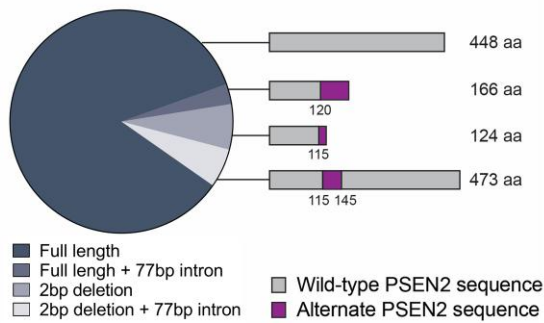
A PSEN2 2bp deletion isoforms

Figure 4 Allelic bias at *PSEN1* and *PSEN2* pathogenic variant positions. (**A**) Percentage of *PSEN2* isoforms with or without the 2 bp deletion and 77 bp intron retention. (**B**) Percentage of transcripts with the wild-type allele or the PSEN variant (mutant allele) present. Sporadic Alzheimer's disease $n = 7$, PSEN variant carrier $n = 7$, control $n = 6$. aa = amino acids.

progressive supranuclear palsy (Fig. 6B; $P = 1.00$, one-way ANOVA followed by Tukey's multiple comparisons). As in our Iso-Seq data, the 1 amino acid deletion splice product was not significantly different in *PSEN2* in Alzheimer's disease versus other samples (Supplementary Fig. 6A; $P = 0.059$) nor was splicing different for the *PSEN1* 4 aa deletion product (Supplementary Fig. 6B; $P = 0.75$), or inclusion of alternative exon 3B (Supplementary Fig. 6C, $P = 0.70$, one-way ANOVA). We found that exon 9B inclusion did not correlate with age at death [Supplementary Fig. 7; P (two-tailed) > 0.10 for all cohorts].

RNA from the temporal cortex was also sequenced for many of the same samples in the Mayo Clinic dataset (82 Alzheimer's disease, 74 controls, 28 pathological ageing and 80 progressive supranuclear palsy). Here, we found fewer reads that mapped to the exon 9B splice junction, with several samples having no reads at all. Alzheimer's disease samples averaged 3.09% splicing to exon 9B relative to 2.34% in controls, 1.75% in pathological ageing and 2.25% in progressive supranuclear palsy (Fig. 5C). Levels of this transcript were not significantly different between cohorts ($P = 0.15$, one-way ANOVA). The number of *PSEN2* exon 6 skipping or 77 bp intron inclusion events likewise were minimal in the temporal cortex, and not significantly different between cohorts (Fig. 5D; $P = 0.90$, one-way ANOVA).

An extended *PSEN2* 3′ UTR is subject to RNA editing

During our analysis of *PSEN2* N141I transcripts, we observed a series of A-to-G changes present in the 3′ UTR sequence. Upon further inspection of these variants, we found they were scattered across all reads in a non-allelic manner (i.e. they were not specific to one haplotype). The A-to-G nature suggested instead that these are RNA editing events, reinforced by their enriched abundance at AluJb and AluY short interspersed nuclear elements in the 3′ UTR sequence. Adenosine deaminases acting on RNA-mediated editing of adenosine to inosine nucleosides can be detected by the presence of A-to-G mismatches in the cDNA as reverse transcription enzymes incorporate guanidine residues at inosine positions.

We identified two 3′ UTRs for *PSEN2* (a short 3′ UTR of 507 bp and long 3′ UTR of 2976 bp; Fig. 6A). The long 3′ UTR included the Alu elements and is observed about one-third of the time across samples. The level of RNA editing at Alu elements in the 3′ UTR of *PSEN2* was comparable in sporadic Alzheimer's disease cases, PSEN variant carriers and controls (Fig. 6B; $P = 0.69$, one-way ANOVA). However, the larger dataset from the Mayo Clinic also permitted evaluation of RNA editing at Alu sites in the 3′ UTR of *PSEN2*. Here, we found that RNA editing was significantly enriched in both Alzheimer's disease and progressive supranuclear palsy samples relative to controls ($P < 0.0001$ for both), and in progressive supranuclear palsy relative to pathological ageing samples (Fig. 6C; $P = 0.011$, one-way ANOVA followed by Tukey's multiple comparisons). We detected the presence of both individual reads with several edits, and individual residues with several edits (examples shown in Fig. 6D).

Motivated by our identification of RNA editing in the 3′ UTR of *PSEN2*, we revisited alternative exons 3B in *PSEN1* and 9B in *PSEN2*, both of which include sections of repetitive elements. The *PSEN1* exon 3B sequence includes portions of AluJB and L1MB2 long interspersed nuclear element (LINE) repeats. There are several RNA edits in this region, although the level of editing is low across sporadic AD, PSEN variant carriers and controls. The alternative *PSEN2* acceptor site in exon 9B is in an L2 LINE. While we did not detect RNA editing events, it is possible that some of the adjacent intronic sequence is edited, thus strengthening the splice acceptor sequence.

Discussion

In this study, we used long-read Iso-Seq to identify transcript variation in *PSEN1* and *PSEN2* in cases of sporadic and familial Alzheimer's disease. Our Iso-Seq findings for *PSEN1* match its established gain-of-function role in Alzheimer's disease. We identified equivalent expression of wild-type and variant alleles for four different *PSEN1* pathogenic variants that were analysed. We observed a high degree of splicing fidelity—even in aged individuals—with little to no splicing differences between sporadic Alzheimer's disease versus controls (Fig. 2). In contrast, our findings illuminate a previously under-appreciated role for *PSEN2* alternative splicing in Alzheimer's disease. Patients with the N141I Volga German pathogenic variant were biased towards skipping of exon 6 and loss of the variant allele, skewing the ratio of mapped reads towards wild-type *PSEN2*. The skipping of exon 6—which would lead to a premature termination codon—indicates that a partial loss-of-function property of *PSEN2* may be implicated. How the variant might promote exon 6 skipping is currently unknown, though the variant does not overlap an established binding site for HMGA1, which can promote exon 6 skipping. In addition, we found increased expression of the wild-type *PSEN2* allele in a carrier of the K115Efs variant, while the *PSEN2* isoforms associated with the 2 bp deletion were often linked to a 77 bp intron retention product, leading to an in-frame protein product and confirming our previous work.[16]
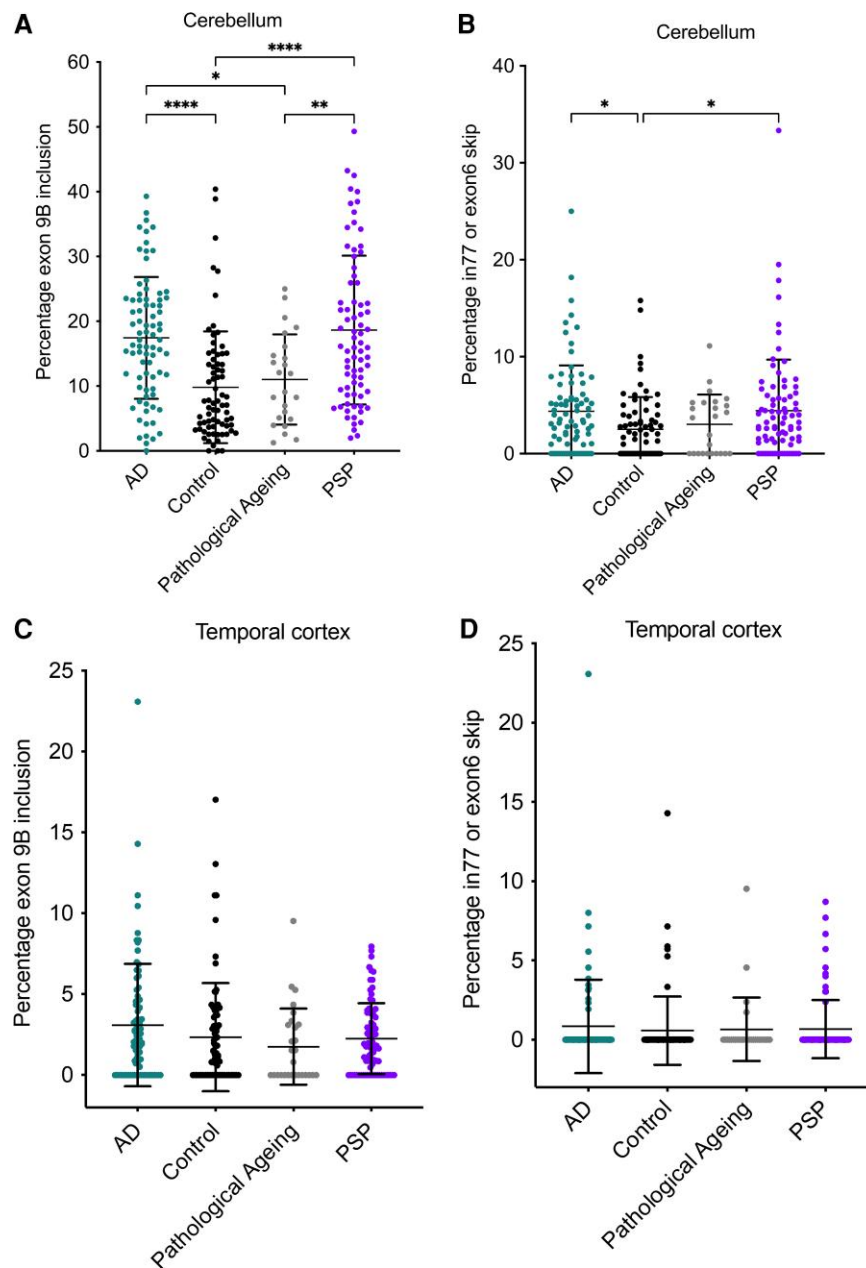
Figure 5 RNA splicing in Mayo Clinic RNA-seq samples. Proportion of spliced reads in cerebellum samples for *PSEN2* (**A**) exon 9B, and (**B**) 77 bp intron retention product or exon 6 skipping. Proportion of spliced reads in temporal cortex samples for *PSEN2* (**C**) exon 9B, and (**D**) 77 bp intron retention or exon 6 skipping products. PSP = progressive supranuclear palsy. P-values were determined by a one-way ANOVA, followed by Tukey's multiple comparisons. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. All bars are mean and standard deviation. Sporadic Alzheimer's disease (AD) $n = 82$, control $n = 76$, pathological ageing $n = 25$, progressive supranuclear palsy $n = 81$ for cerebellum samples. Sporadic AD $n = 82$, control $n = 74$, pathological ageing $n = 28$, progressive supranuclear palsy $n = 80$ for temporal cortex samples.

We also identified significant transcript differences in sporadic Alzheimer's disease cases versus controls and pathogenic PSEN variant carriers, including more non-productive transcripts (Fig. 3). While we would have predicted that individuals with pathogenic variants in *PSEN2* had the most disrupted splicing of *PSEN2* mRNA, instead we found that it was in the sporadic Alzheimer's disease cases that demonstrated the greatest abundance of non-productive intron 77 retention and exon 9B products (Fig. 3). In general, many alternative splice events were observed for *PSEN2* that were enriched in sporadic Alzheimer's disease. Our results are consistent with the hypothesis that PSEN1 and PSEN2 produce full-

length but variant proteins to contribute to Alzheimer's disease. In addition, these data indicate that alternative splicing of *PSEN2* is not unique to Alzheimer's disease variants, but rather that transcript differences in *PSEN2* may play an important role in the onset of sporadic Alzheimer's disease.

PSEN1 and PSEN2 are highly similar (65% identical at the amino acid level). Despite their similarities, many more pathogenic variants have been documented in *PSEN1* than in *PSEN2*.[8–11] This phenomenon may be explained by differences in cellular localization (for example, PSEN2 plays a selective role in microglia),[31–33] subcellular localization[34] or function (PSEN1 has a greater effect on
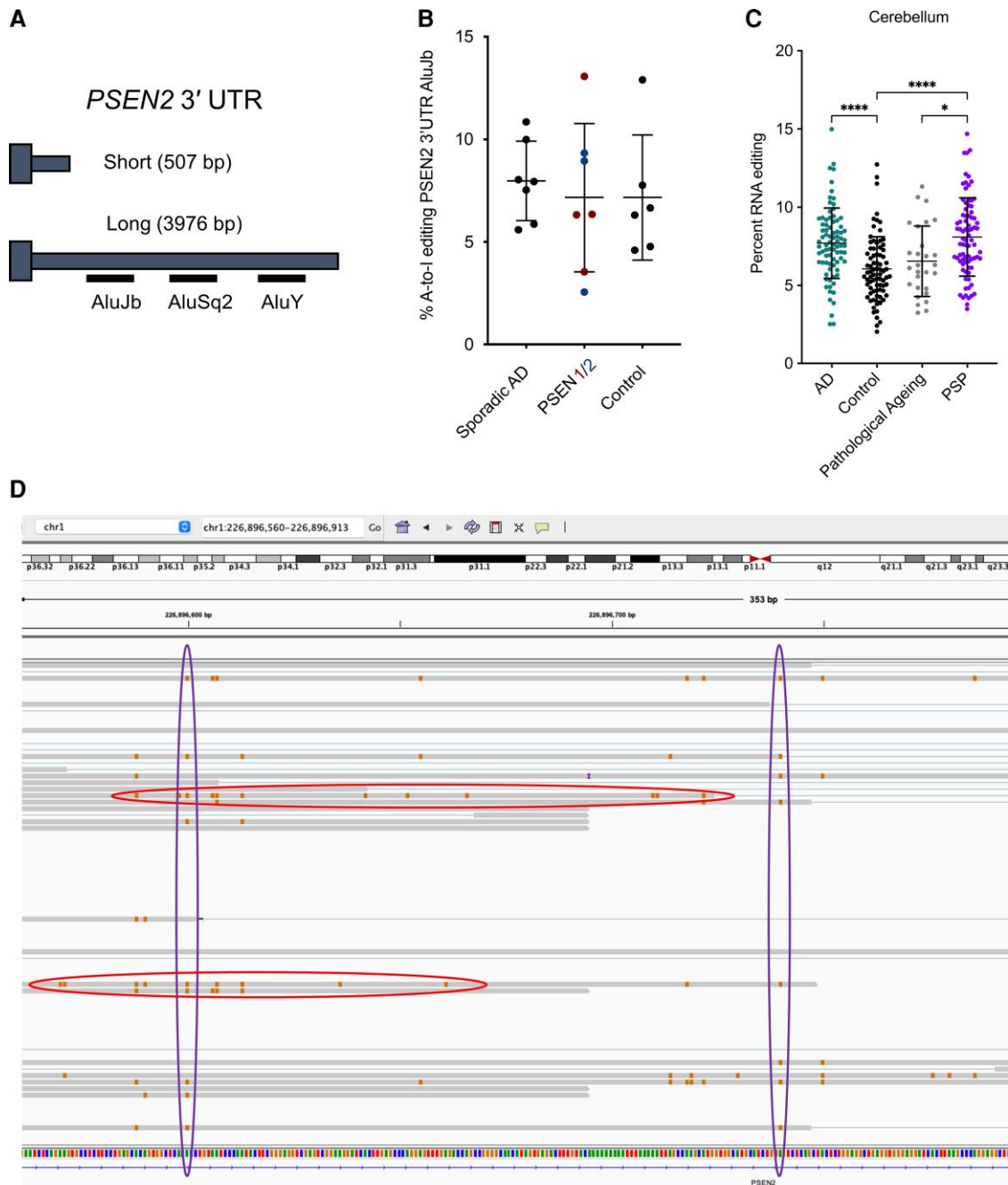
Figure 6 RNA editing in *PSEN2* 3′ UTR. (A) Schematic showing two 3′ UTRs identified for *PSEN2*, short (without Alu elements) and long (with Alu elements). **(B)** Percentage of A-to-I editing of the AluJb and AluY sites, across cohorts. Sporadic Alzheimer's disease (AD) *n* = 7, PSEN variant carrier *n* = 7, control *n* = 6. **(C)** Level of RNA editing in Alu elements in the *PSEN2* 3′ UTR. P-values were determined by a one-way ANOVA, followed by Tukey's multiple comparisons. *P < 0.05, ****P < 0.0001. All bars are mean and standard deviation. Sporadic Alzheimer's disease *n* = 82, control *n* = 76, pathological ageing *n* = 25, progressive supranuclear palsy *n* = 81. **(D)** Examples of individual reads with several edits (circled horizontally) and individual residues with multiple edits (circled vertically).

calcium signalling).[35] It is also possible that pathogenic variants in *PSEN2* are more prevalent than currently thought, but that cases with these variants are considered sporadic due to their later age of onset.[36] A modest shift to *PSEN2* transcripts with premature termination codons emerges both in individuals with *PSEN2* pathogenic variants and in sporadic Alzheimer's disease. Whether these non-productive isoforms have a pathogenic role is currently unclear. However, the commonality is distinct from *PSEN1*, where ample

evidence demonstrates that loss-of-function variants appear unrelated to Alzheimer's disease aetiology and instead predispose individuals to familial acne inversa.[37] The evidence for or against *PSEN2* loss-of-function variants in Alzheimer's disease pathology is less clear—they are not implicated in acne inversa for instance—and our data suggest that further investigation is warranted.

Our confirmation that the *PSEN2* exon 9B splice product is elevated in sporadic Alzheimer's disease cases in an external

RNA-seq dataset (Fig. 5) is consistent with the idea that the exon 9B product plays a role in Alzheimer's disease aetiology, and further experimental evidence of functionality could point to elimination of the exon 9B product as a potential therapeutic target. However, the fact that exon 9B inclusion is highest in the cerebellum is surprising, considering that the cerebellum is relatively spared from Alzheimer's disease pathology until late stages of disease. Even more surprising is the similar inclusion of this splice product in progressive supranuclear palsy cerebellum samples (Fig. 6A). Progressive supranuclear palsy exhibits tau pathology but not Aβ aggregation,[38] which differentiates it from Alzheimer's disease pathology. The significant upregulation of exon 9B isoform in the progressive supranuclear palsy samples thus presents a potential connection between *PSEN2* and tau. Perhaps tau aggregation and mis-localization may promote exon 9B inclusion by sequestering splice factors.[39] However, given that PSEN pathogenic variant carriers also do not harbour increased exon 9B inclusion products, additional genetic and/or environmental risk factors are probably involved in sporadic Alzheimer's disease.

These findings indicate that Iso-Seq of other brain regions that have selective vulnerability in Alzheimer's disease or ageing is warranted to identify the proportion of reads that incorporate *PSEN2* exon 9B. In addition, because our starting tissue was bulk brain tissue, we cannot yet make conclusions about the cell-type specificity of these transcripts, which would be similarly illuminating. These findings also demonstrate the value of examining full-length transcripts, as other databases created using short-read sequencing, such as the Mount Sinai Brain Bank or from the Religious Orders Study and Rush Memory and Aging Project,[40,41] do not exhibit widespread inclusion of the intron 77 bp or exon 9B product. Of note, exon 9B splice junctions are not conserved in other species, including mice and non-human primates. The human-specific nature of exon 9B may help explain why mouse models of sporadic Alzheimer's disease have been difficult to establish.[42]

The discovery of Alu short interspersed nuclear elements and LINEs in a long 3′ UTR of *PSEN2* raises intriguing questions about the cell-specific usage of these isoforms. RNA editing of Alu repeat-containing 3′ UTRs invokes a proposed mechanism to restrict the transcripts to the nucleus: if they are exported to the cytoplasm, they could precipitate a potent immune response.[43] If the edited mRNA is exported to the cytoplasm, reduced levels of polysomes are found on the edited RNA,[44] indicating that this isoform of *PSEN2* may have reduced translation capacity.

Finally, the presence of alternative splice products with non-productive exons deep within traditional introns of both *PSEN1* and *PSEN2* suggests that intronic variants surrounding these sites may promote inclusion of these non-productive exons and act as risk factors for Alzheimer's disease. The increasing number of whole genome sequences available for Alzheimer's disease allows us now to screen for these rare variants and test for cryptic exon inclusion in functional assays, such as minigene reporters.

Using long-read sequencing technology, we have considerably expanded our understanding of *PSEN1* and *PSEN2* alternative splicing—which is consistent with the hypothesis that pathogenic variants in *PSEN1* and *PSEN2* lead to disease not through haploinsufficiency but rather an alteration of function—and identified novel splice isoforms associated with disease. Specifically, we found several transcript differences in *PSEN2*. Future work will help reveal whether they play an important role in the onset of sporadic Alzheimer's disease–the form of which accounts for >95% of Alzheimer's disease cases.[45] Our findings also reveal a direct connection between sporadic and familial Alzheimer's disease,

strengthening the amyloid cascade hypothesis that Alzheimer's disease is caused by disrupted production or clearance of APP cleavage products.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

References

1. Goate A, Chartier-Harlin MC, Mullan M, *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature.* 1991;349:704–706.

2. Levy-Lahad E, Wasco W, Poorkaj P, *et al*. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science*. 1995; 269:973–977.

3. Sherrington R, Rogaev EI, Liang Y, *et al*. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. 1995;375:754–760.

4. Chávez-Gutiérrez L, Bammens L, Benilova I, *et al*. The mechanism of gamma-secretase dysfunction in familial Alzheimer disease. *EMBO J*. 2012;31:2261–2274.

5. Fernandez MA, Klutkowski JA, Freret T, Wolfe MS. Alzheimer presenilin-1 mutations dramatically reduce trimming of long amyloid beta-peptides (abeta) by gamma-secretase to increase 42-to-40-residue abeta. *J Biol Chem*. 2014;289:31043–31052.

6. Veugelen S, Saito T, Saido TC, Chavez-Gutierrez L, De Strooper B. Familial Alzheimer's disease mutations in presenilin generate amyloidogenic abeta peptide seeds. *Neuron*. 2016;90:410–416.

7. Szaruga M, Munteanu B, Lismont S, *et al*. Alzheimer's-causing mutations shift abeta length by destabilizing gamma-secretase-abetan interactions. *Cell*. 2017;170:443–456.e14.

8. Bird TD. Alzheimer disease overview. In: Adam MP, Ardinger HH, Pagon RA, *et al*., editors. *GeneReviews®*. University of Washington; 2018.

9. Schellenberg GD, Montine TJ. The genetics and neuropathology of Alzheimer's disease. *Acta Neuropathol*. 2012;124:305–323.

10. Cacace R, Sleegers K, Van Broeckhoven C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimers Dement*. 2016;12:733–748.

11. Pottier C, Hannequin D, Coutant S, *et al*. High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease. *Mol Psychiatry*. 2012;17:875–879.

12. Cruchaga C, Haller G, Chakraverty S, *et al*. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. *PLoS ONE*. 2012;7:e31039.

13. Lanoiselee H-M, Nicolas G, Wallon D, *et al*. APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med*. 2017;14:e1002270.

14. De Strooper B. Loss-of-function presenilin mutations in Alzheimer disease. Talking point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep*. 2007;8:141–146.

15. Jayadev S, Leverenz JB, Steinbart E, *et al*. Alzheimer's disease phenotypes and genotypes associated with mutations in presenilin 2. *Brain*. 2010;133:1143–1154.

16. Braggin JE, Bucks SA, Course MM, *et al*. Alternative splicing in a presenilin 2 variant associated with Alzheimer disease. *Ann Clin Transl Neurol*. 2019;6:762–777.

17. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci*. 2004;5:347–360.

18. Sato N, Hori O, Yamaguchi A, *et al*. A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue. *J Neurochem*. 1999;72:2498–2505.

19. Moussavi Nik SH, Newman M, Wilson L, *et al*. Alzheimer's disease-related peptide PS2V plays ancient, conserved roles in suppression of the unfolded protein response under hypoxia and stimulation of gamma-secretase activity. *Hum Mol Genet*. 2015;24:3662–3678.

20. Moussavi Nik SH, Newman M, Lardelli M. The response of HMGA1 to changes in oxygen availability is evolutionarily conserved. *Exp Cell Res*. 2011;317:1503–1512.

21. Manabe T, Katayama T, Sato N, *et al*. Induced HMGA1a expression causes aberrant splicing of presenilin-2 pre-mRNA in sporadic Alzheimer's disease. *Cell Death Differ*. 2003;10:698–708.

22. Tseng E, Rowell WJ, Glenn OC, *et al*. The landscape of SNCA transcripts across synucleinopathies: New insights from long reads sequencing analysis. *Front Genet*. 2019;10:584.

23. Kukull WA, Higdon R, Bowen JD, *et al*. Dementia and Alzheimer disease incidence: A prospective cohort study. *Arch Neurol*. 2002; 59:1737–1746.

24. Nicolas G, Wallon D, Charbonnier C, *et al*. Screening of dementia genes by whole-exome sequencing in early-onset Alzheimer disease: Input and lessons. *Eur J Hum Genet*. 2016;24:710–716.

25. Nygaard HB, Lippa CF, Mehdi D, Baehring JM. A novel presenilin 1 mutation in early-onset Alzheimer's disease with prominent frontal features. *Am J Alzheimers Dis Other Demen*. 2014;29:433–435.

26. Hsu S, Pimenova AA, Hayes K, *et al*. Systematic validation of variants of unknown significance in APP, PSEN1 and PSEN2. *Neurobiol Dis*. 2020;139:104817.

27. GTEx Consortium. The GTEx Consortium Atlas of genetic regulatory effects across human tissues. *Science*. 2020;369:1318–1330.

28. Moussavi Nik SH, Porter T, Newman M, *et al*. Relevance of a truncated PRESENILIN 2 transcript to Alzheimer's disease and neurodegeneration. *J Alzheimers Dis*. 2021;80:1479–1489.

29. Smith MJ, Sharples RA, Evin G, *et al*. Expression of truncated presenilin 2 splice variant in Alzheimer's disease, bipolar disorder, and schizophrenia brain cortex. *Brain Res Mol Brain Res*. 2004;127:128–135.

30. Allen M, Carrasquillo MM, Funk C, *et al*. Human whole genome genotype and transcriptome data for Alzheimer's and other neurodegenerative diseases. *Sci Data*. 2016;3:160089.

31. Fung S, Smith CL, Prater KE, *et al*. Early-onset familial Alzheimer disease variant PSEN2 N141I heterozygosity is associated with altered microglia phenotype. *J Alzheimers Dis*. 2020;77:675–688.

32. Jayadev S, Case A, Alajajian B, Eastman AJ, Moller T, Garden GA. Presenilin 2 influences miR146 level and activity in microglia. *J Neurochem*. 2013;127:592–599.

33. Jayadev S, Case A, Eastman AJ, *et al*. Presenilin 2 is the predominant gamma-secretase in microglia and modulates cytokine release. *PLoS ONE*. 2010;5:e15743.

34. Sannerud R, Esselens C, Ejsmont P, *et al*. Restricted location of PSEN2/gamma-secretase determines substrate specificity and generates an intracellular abeta pool. *Cell*. 2016;166:193–208.

35. Payne AJ, Kaja S, Koulen P. Regulation of ryanodine receptor-mediated calcium signaling by presenilins. *Receptors Clin Investig*. 2015;2:e449.

36. Blauwendraat C, Wilke C, Jansen IE, *et al*. Pilot whole-exome sequencing of a German early-onset Alzheimer's disease cohort reveals a substantial frequency of PSEN2 variants. *Neurobiol Aging*. 2016;37:208.e11–208.e17.

37. Wang B, Yang W, Wen W, *et al*. Gamma-secretase gene mutations in familial acne inversa. *Science*. 2010;330:1065.

38. Dickson DW, Ahmed Z, Algom AA, Tsuboi Y, Josephs KA. Neuropathology of variants of progressive supranuclear palsy. *Curr Opin Neurol*. 2010;23:394–400.

39. Lester E, Ooi FK, Bakkar N, *et al*. Tau aggregates are RNA-protein assemblies that mislocalize multiple nuclear speckle components. *Neuron*. 2021;109:1675–1691.e9.

40. Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA. Religious Orders Study and Rush Memory and Aging Project. *J Alzheimers Dis*. 2018;64:S161–S189.

41. Wang M, Beckmann ND, Roussos P, *et al*. The mount Sinai cohort of large-scale genomic, transcriptomic and proteomic data in Alzheimer's disease. *Sci Data*. 2018;5:180185.

42. Zhang L, Chen C, Mak MS, *et al*. Advance of sporadic Alzheimer's disease animal models. *Med Res Rev*. 2020;40: 431–458.

43. Hundley HA, Bass BL. ADAR editing in double-stranded UTRs and other noncoding RNA sequences. *Trends Biochem Sci*. 2010; 35:377–383.

44. Hundley HA, Krauchuk AA, Bass BL. *C. elegans* and *H. sapiens* mRNAs with edited 3' UTRs are present on polysomes. *RNA*. 2008;14:2050–2060.

45. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. *Nat Rev Dis Primers*. 2015;1: 15056.