Identification of a rare *presenilin 1* single amino acid deletion mutation (F175del) with unusual amyloid-β processing effects

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Abstract

We report the novel *presenilin 1* (*PSEN1*) single amino acid deletion mutation F175del. Comprehensive clinical work-up, including cerebral MRI, FDG-PET and CSF analysis, was performed in a male who had developed forgetfulness and personality change at the age of 39. Alzheimer´s disease dementia was diagnosed according to established criteria. The index patient manifested rapid progressive dementia, seizures and myoclonus, and a Pisa syndrome as a side effect of donepezil treatment. The *PSEN1* mutation F175del was found on genetic testing. It was rendered very likely pathogenic as amyloid-β (Aβ) peptide 42 was elevated in a cell culture model compared to presenilin 1 wild type controls. An additional, unusual increase of Aβ39 indicates a rarely observed product line deviation in the generation of the shorter Aβ species. Our observations extend the range of *PSEN1* mutations to be considered in familial dementia. We demonstrate that deletion of a single conserved amino acid, which is very rare compared to missense mutations as the common cause for *PSEN1*-associated AD, can lead to an unusual profile of Aβ species.

Keywords: Alzheimer's disease; autosomal dominant; genetics; *PSEN1*; novel mutation.

1. Introduction

Autosomal dominant Alzheimer's disease (ADAD) is a rare variant of Alzheimer's disease (AD), with an average onset of symptoms at 45 years (Masters, et al., 2015). ADAD is caused by mutations in one of the three genes *PSEN1, PSEN2, or APP,* encoding for presenilin 1 (PS1), presenilin 2 (PS2) or the amyloid precursor protein (APP), or by *APP* duplications (Bateman, et al., 2011). *PSEN1* sequence variants are the most common causes of ADAD (Cacace, et al., 2016). Until now, 247 distinct mutations have been identified, most of them with proven pathogenicity (Cruts, et al., 2012) (www.alzforum.org/mutations). The *PSEN1* gene is located on the long arm of chromosome 14 (q24.3) (Sherrington, et al., 1995). It spans at least 60 kb and has 13 exons (Rogaev, et al., 1997). The *PSEN1* gene encodes PS1, a protein of approximately 50 kDa with 467 amino acids and 9 transmembrane domains (Laudon, et al., 2005,Sherrington, et al., 1995). PS1 and its homolog PS2 are the catalytically active subunits of the γ-secretase complex that mediates the final cleavage of APP to liberate the amyloid-β (Aβ) peptide (De Strooper, et al., 2012,Steiner, et al., 2008,Steiner, et al., 2018). Whereas the majority of *PSEN1* mutations are missense mutations that lead to an exchange of single highly conserved amino acid residues, pathogenic single amino acid deletion mutations - reflected by a number of 4 so far described to our knowledge (www.molgen.ua.ac.be/admutations; www.alzforum.org/mutations) - are very rare. Here we present the index case, a 40 year old male, for a family with ADAD due to a novel *PSEN1* single amino acid deletion mutation.

2. Methods

2.1. Clinical, imaging and CSF analyses

The patient work-up followed established procedures for clinical examination, cognitive testing, EEG, and neuroimaging (cerebral magnetic resonance imaging, cMRI, with a Philips Intera 1.5T, and [^{18}F]fluorodeoxyglucose positron emission tomography, FDG-PET). FDG-PET was acquired on a Siemens ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN, USA) 30 minutes after the injection of 123 MBq [^{18}F]FDG and reconstructed in axial, coronal and sagittal orientation (Fig. 2). Cerebrospinal fluid (CSF) was analyzed with respect to cell count, glucose and protein content, as well as for Aβ40, Aβ42, total tau protein and tau phosphorylated at position 181. For analyses of Aβ40 and Aβ42 assays of IBL International (Hamburg, Germany), and for analyses of total tau protein and phosphorylated tau assays of Fujirebio Europe (Gent, Belgium) were used.

2.2. Genetic testing

Genetic testing for *PSEN1* mutations was performed by CeGaT GmbH (Tübingen, Germany) using a panel based next generation sequencing approach (Custom design Agilent SureSelect enrichment followed by sequencing on Illumina HiSeq2500). Subsequent Sanger sequencing confirmed the identified mutation (Raux, et al., 2005). In addition, in order to sequence the mutation on the affected allele, DNA extracted from blood of the patient was subcloned into a TOPO vector (TOPO® TA Cloning® Kit, Invitrogen) after amplification by polymerase chain

reaction (PCR). For PCR, oligo sequences hPS1_Intron5-6_For (TTAAGGGTTGTGGGACCTGTC) and hPS1_Intron6-7_Rev (ACCAAGTATGACCTATATGTGGAA) were used. Thereafter these plasmids were subjected to Sanger sequencing (GATC Biotech AG, Konstanz, Germany). To establish the novelty of the mutation, the Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB) (www.molgen.ua.ac.be/admutations), the mutation database of Alzforum (www.alzforum.org/mutations), and Pubmed were assessed.

2.3. Biochemical analyses

For *in vitro* analysis of the pathogenicity of the deletion mutation, the PS1 F175del mutant was expressed in human embryonic kidney 293 cells co-expressing the "Swedish" *APP* KM670/671NL mutation (HEK APPswe). This mutation, leading to a substitution of two amino acids in the gene encoding for APP (Citron, et al., 1992), was used because of its feature to strongly increase the amount of APP-carboxyterminal fragment (CTF) β available for amyloidogenic processing without influencing the Aβ42/40 ratio (Suzuki, et al., 1994). Stable single cell clones were selected and the amounts of secreted Aβ38, Aβ39, Aβ40 and Aβ42 in conditioned medium were analyzed by immunoblotting (Kretner, et al., 2016) and/or quantified using the highly specific and sensitive triplex Aβ sandwich immunoassay. Amounts of Aβ38, Aβ40 and Aβ42 were compared to those measured in HEK APPswe transfected with PS1 wild type. Statistical significance of changes in the generation of these Aβ species was assessed using Student's t-tests. To confirm changes in Aβ species, Aβ was additionally analyzed by MALDI-TOF (matrix assisted laser

desorption/ionization-time of flight) mass spectrometry (Page, et al., 2008,Trambauer, et al., 2017). Experimental details are described in the supplement.

The study had been approved by the local ethics committee and written informed consent was obtained from the patient and his companion.

3. Results

3.1. Medical history

A male with neither school graduation (7 years of schooling) nor completed vocational training and weak writing and arithmetic skills presented at the age of 40 years with a ten months history of increasing forgetfulness and personality change. He had shown social withdrawal and had recently developed impairment in activities of daily living, in particular he was incapable to accomplish simple household tasks and frequently got lost. Difficulties with word finding, pronunciation and a decrease in speech output were noted. His previous medical history disclosed no diseases or treatments of relevance and he was on no medication. The patient's mother had shown an onset of cognitive symptoms in her early thirties and dementia had been diagnosed. According to the family members, in the grandmother of the patient a diagnosis of AD had been made, the age of onset of symptoms was unknown. Both his mother and grandmother died at an early age (36 and 50 years, respectively). In the mother of the patient, the finding of cerebral atrophy was reported by his family members. With one affected individual each over three consecutive generations, the family pedigree (Fig. 1A) suggested an autosomal dominant mode of inheritance.

3.2. Clinical and neuropsychological evaluation

On examination at first presentation, the patient was not oriented to time, but to person, place and situation. Due to attention and language problems, his understanding of instructions was reduced. Apart from exaggerated patellar reflexes on both sides and horizontal and vertical saccadic smooth pursuit eye movements,

the general neurological exam was unremarkable. On the Mini Mental State Examination (MMSE) (Folstein, et al., 1975) he scored 15 out of 30 points, failing in orientation, memory, attention and language. While copying figures, visuospatial deficits were obvious. Digit span forward and backward of the Wechsler Memory Scale - Revised Edition (WMS-R) (Wechsler, 1981) were severely impaired (percentile rank < 2 and < 1, respectively). The subtest logical memory of the WMS-R was also significantly affected in the index patient (percentile rank < 1 in both part I and II). Severe impairments (percentile ranks < 1) were also found in confrontation naming, as well as in the semantic and phonemic word fluency tests of the CERAD (Consortium to Establish a Registry for Alzheimer's Disease)-Plus test battery (Schmid, et al., 2014). Tests of attentional performance were not feasible, because the patient repeatedly forgot the instructions. In conclusion, neuropsychological testing disclosed a severe multi-domain cognitive impairment.

3.3. Imaging and CSF analysis

cMRI (Fig. 2A) suggested slight brain atrophy with widened outer CSF spaces, the Sylvian fissure in particular. Medial temporal lobe atrophy was found, with a score of 2-3 on the scale proposed by Scheltens et al. (Scheltens, et al., 1992). FDG-PET showed a pattern of glucose uptake typical for AD, with markedly reduced metabolism in the precuneus/posterior cingulate as well as parietotemporal cortex bilaterally (Fig. 2C), whereas perirolandic metabolism appeared unaffected. On CSF analysis, Aβ42 was decreased to 359 pg/ml (cutoff 620 pg/ml). Aβ40 was 6671 pg/ml (no cutoff provided by the manufacturer). Total tau and phosphorylated tau were increased to 457 pg/ml (cutoff 320 pg/ml) and 76.5 pg/ml (cutoff 50 pg/ml), respectively. Cutoffs were provided by the manufacturers of the assays.

3.4. Genetic testing

A diagnosis of dementia due to Alzheimer´s disease was made on the basis of established criteria of both the International Working Group for New Research Criteria for the Diagnosis of AD (Dubois, et al., 2014) and the National Institute of Aging - Alzheimer's Association workgroups (McKhann, et al., 2011). Genetic testing revealed a rare *PSEN1* deletion mutation, the F175del variant (DNA: NG_007386.2:g.55427_55429del; Protein: NG_007386.2(PSEN1_i001):p.(Phe175del) (den Dunnen, et al., 2016)) (Fig. 1B). This novel, yet unreported trinucleotide deletion leads to the loss of one phenylalanine residue in the third transmembrane domain of PS1. With segregation data from relatives unavailable, however, we sought additional proof, in particular since the known genetic variant F175S at our patient´s deletion site is not regarded as disease-causing (Colacicco, et al., 2002). According to the algorithm for classifications proposed by Guerreiro et al. (Guerreiro, et al., 2010), the mutation can be considered as probable pathogenic. The suggestive family history with early onset dementia in three generations further corroborated the pathogenicity of our patient´s *PSEN1* mutation. For further confirmation Aβ generation was investigated in cultured cells expressing wild type PS1, the novel deletion mutation PS1 F175del as well as, for comparison, the previously described highly pathogenic PS1 L166P mutation (Moehlmann, et al., 2002).

3.5. Biochemical analyses

The PS1 F175del mutant protein allowed normal γ–secretase complex formation as judged from endoproteolysis of PS1 and nicastrin maturation. Both, N- and C-terminal PS1 fragments were readily observed and nicastrin matured to the fully glycosylated variant known to be present in correctly formed γ-secretase complexes (Fig. 3A) (Edbauer, et al., 2002,Leem, et al., 2002). Expression of the PS1 F175del mutant caused replacement (Thinakaran, et al., 1997) of the endogenous PS2 (Fig. 3A) further supporting the conclusion that the mutant assembled normally into the γ-secretase complex. Levels of the APP-CTFs were similar to those in cells expressing wild type PS1 and consequently AICD did not change compared to the controls (Fig. 3A) showing that the mutant does not result in a loss of total γ-secretase activity towards its APP substrate. PS1 F175del expressing cells produced more Aβ42 and less Aβ40 relative to total Aβ, strongly supporting its *in vivo* pathogenicity (Fig. 3B). Interestingly, an Aβ species that migrated at a position between the Aβ38 and Aβ40 standards was observed in conditioned media from the PS1 F175del expressing cells (Fig. 3C). This band was not detected in conditioned media derived from cells expressing PS1 wild type or the well characterized PS1 L166P (Kretner, et al., 2016,Moehlmann, et al., 2002,Page, et al., 2008). This indicates that this mutant induces a change in the cleavage precision of γ-secretase (Fig. 4). Mass-spectrometry identified this species as Aβ39 (Fig. 3D), which is a more rarely generated Aβ species (Morishima-Kawashima, 2014,Page, et al., 2008). As the index patient's family members at risk do not want to know about his or her mutation status, we refrained from further genetic and biochemical analyses in these individuals to safeguard their right of not knowing their genetic status.

3.6. Treatment and clinical course

The patient was treated with 10 mg of donepezil per day, and consecutively 20 mg of memantine per day were added. One year later, the patient showed a Pisa syndrome on examination, tending backwards and to the left while walking. After the reduction of donepezil to 5 mg per day, the Pisa syndrome remitted. The reduced dose of donepezil did not lead to an immediate worsening of cognitive function. Generalized myoclonic twitches appeared after a disease duration of 22 months. Additionally, two generalized epileptic seizures occurred 32 months after onset of the first symptom of ADAD. Treatment with levetiracetam led to seizure freedom. Within 14 months after first presentation, MMSE score dropped from 15 (10 month disease duration) to 5 points (24 month disease duration). The patient was admitted to a nursing home 35 month after the onset of the first cognitive symptom.

4. Discussion

We made a diagnosis of Alzheimer´s disease dementia in a 40 year old male with a family history that suggested an autosomal-dominant inheritance of early onset dementia. On genetic testing we found a novel, very rare *PSEN1* single amino acid deletion mutation, the *PSEN1* F175del mutant. The deleted phenylalanine is encoded in *PSEN1* exon 6 and located in the third transmembrane domain of PS1. On this note, the nomenclature of the mutation at the protein level is a matter of debate. As a result of the mutation, the original base sequence T T C T T T T T T coding for three consecutive phenylalanine residues (F175, F176 and F177; coding triplets TTC and TTT, respectively) is converted to T T T T T T. According to the HVGS nomenclature, this change should be named F177del (http://varnomen.hgvs.org/recommendations/DNA/variant/deletion/). However, to avoid confusion and to reflect the fact that a C base is deleted in the first phenylalanine coding triplet, we decided to name this mutation F175del.

According to *in vitro* analysis, the PS1 F175del mutant can be regarded as causal since a shift in the ratio of Aβ species to Aβ42 strongly hints at the presence of the mechanism shared by disease-causing *PSEN1* mutations (Citron, et al., 1997,Scheuner, et al., 1996). Moreover, the mutation of the index patient not only caused an increased generation of the pathogenic Aβ42 species relative to Aβ total production, but remarkably also an enhanced generation of the scarce species Aβ 39, showing a rarely observed change in the processivity of γ-secretase leading to an altered production of shorter Aβ species (Morishima-Kawashima, 2014). Since Aβ39 is apparently only generated from Aβ42 (Morishima-Kawashima, 2014), the atypically increased levels of this species suggest an increased usage of the Aβ42 producing product line by the mutant (Fig. 4). Mechanistically, these data may

indicate a significant structural alteration in the conformation of the catalytic subunit PS1 that may be associated with distortions in substrate-binding/positioning and/or enzyme-substrate complex stabilities as has been observed for other ADAD-associated *PSEN1* mutations (Fukumori and Steiner, 2016,Okochi, et al., 2013,Szaruga, et al., 2017).

An increase in Aβ39, as observed in the mutation of the index patient, may result in cerebral amyloid angiopathy (CAA), since this peptide was found to contribute especially to vascular amyloid peptide deposition (Reinert, et al., 2016). However, progression of AD in the index patient impedes further investigation, so only the pathohistological analysis will show whether CAA could be a feature of PS1 F175del-associated AD in this case.

Until now, 12 *PSEN1* mutations with deletions of various numbers of base pairs that lead to amino acid loss have been described (Cruts, et al., 2012) (www.alzforum.org/mutations). In a third of these mutations, spastic paraparesis has been reported as clinical manifestation in some individuals who carried the respective mutations (Crook, et al., 1998,Le Guennec, et al., 2017,Smith, et al., 2001,Steiner, et al., 2001). In single cases, parkinsonism, impaired fine coordination of hands, or dysarthria were observed (Ishikawa, et al., 2005,Verkkoniemi, et al., 2000). In the patient with the single amino acid deletion mutation *PSEN1* F175del described here seizures and myoclonus occurred. The exaggerated patellar reflexes may represent a subtle sign of lower limbs spasticity. Of note, to our knowledge only four pathogenic *PSEN1* single amino acid deletion mutations have been described yet (Guo, et al., 2010,Ishikawa, et al., 2005,Knight, et al., 2007,Tiedt, et al., 2013). Another variant that enhances the production of Aβ39 is the *PSEN1* M233V mutation (Page, et al., 2008). This mutation was reported to cause ADAD with a rapid disease

course and seizures, similar to our patient. In addition, the *PSEN1* M233V mutation featured extrapyramidal signs that are common in ADAD (Vöglein, et al., 2019b) and an age of onset between 28 and 34 years (Houlden, et al., 2001). So, based on the patients described so far, the *PSEN1* F175del and M233V mutations share some similarities, but also differ in some clinical aspects.

The *PSEN1* F175del variant is the first reported pathogenic mutation at amino acid position 175 of PS1 (Cruts, et al., 2012) (www.alzforum.org/mutations). The previously described F175S variant was revealed to be not pathogenic (Colacicco, et al., 2002). Interestingly, one of the few reported *PSEN1* deletion mutations is neighboring the deletion mutation of the index patient, the L174del mutant. The latter was observed to be associated with progressive memory loss starting at about 50 years of age (Tiedt, et al., 2013). Furthermore, the novel *PSEN1* F175del mutation is neighbored by the F176L mutation that has been hypothesized to be disease causing in the case of Auguste Deter. However, the pathogenicity of this mutation is still unclear (Muller, et al., 2013,Rupp, et al., 2014).

The index patient showed a Pisa syndrome, also referred to as pleurothotonus, as a side effect of donepezil treatment (Hsu, et al., 2017,Huvent-Grelle, et al., 2009,Kwak, et al., 2000,Vanacore, et al., 2005). Of note, the Pisa syndrome occurred about one year after the implementation of donepezil and fully remitted after dose reduction. In the course of ADAD the patient developed myoclonus and seizures, about 2 and 2.5 years after disease onset, respectively. Seizures and myoclonus are known to affect a subset of individuals with ADAD (Tang, et al., 2016,Vöglein, et al., 2019a). Seizure freedom was achieved with levetiracetam that has been suggested to be a good choice for epilepsy treatment in AD (Giorgi, et al., 2017). Regarding cognitive and functional abilities, the index patient showed a rapid worsening, reflected by a MMSE

score of 5 points 2 years after the onset of the first cognitive symptom and a nursing home admission less than 3 years after disease onset.

In summary, we describe here for the first time a rare single amino acid deletion mutation, *PSEN1* F175del, that causes ADAD with rapidly progressing dementia, uncommon neurological manifestations, and further features exceptional effects on Aβ processing. This broadens the spectrum of mutations that have to be considered in individuals at risk for genetic dementia. In the present case of ADAD inclusion in the Dominantly Inherited Alzheimer Network (DIAN) for observation or treatment studies is the clinical next step of first choice.

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Figures

Figure 1: Title: Pedigree and gene sequence chromatograms. Description: (A) Pedigree of the index patient (arrow). Black colored symbols indicate clinically affected, crossed out deceased. Individuals younger than the index patient at a risk of 50 % to carry a mutation for ADAD are colored gray. (B) Sequence chromatograms derived from DNA extracted from blood of the index patient detected a deletion of three bases including C in the sequence T T C T T T T T T T in exon 6 of *PSEN1*, leading to a loss of phenylalanine. Abbreviation: wt = wild type.

Figure 2: Title: Cerebral imaging of the 40 years old index patient. Description: (A) Axial FLAIR MR images of the index patient. Slight widening of the Sylvian fissures (right image) and the inferior horns of the lateral ventricles (left image) (white arrows), the latter probably due to medial temporal atrophy including hippocampal atrophy. (B) Axial FLAIR images of a 34 years old healthy individual, normal width of the Sylvian fissures and the inferior horns of the lateral ventricles. (C) FDG-PET of the index patient and (D) a 34 years old healthy individual. Axial slices (left) show the signal intensity scaled to the maximum. 3D-stereotactic surface projections (right) depict the difference in cerebral glucose metabolism towards the average of an age-matched healthy population. The index patient indicates a pattern of glucose hypometabolism typical for Alzheimer‘s disease (highly reduced metabolism in the precuneus/posterior cingulate and parietotemporal cortex, well maintained metabolism in the central region), while no relevant hypometabolism is visible in the healthy control. Warmer colors in the 3D projection indicate a higher z-score deviation, i.e. less glucose metabolism compared to the average glucose metabolism of an age-matched healthy population. R: right, L: left, medial: surface projection from medial, lateral: surface projection from lateral.

Figure 3: Title: The PS1 F175del mutant increases Aβ42 and Aβ39 production. Description: (A) A representative clone of HEK (human embryonic kidney) 293 cells co-expressing the “Swedish” APP and the PS1 F175del mutant showed the expected pattern for PS1 and PS2 expression and endoproteolysis, APP expression and nicastrin maturation. The varying PS1-FL levels reflect the different presenilin transfection levels, which typically vary between different cell lines, and are irrelevant for the functional analysis. (B) Conditioned media of these cells were compared for Aβ production using a sandwich immunoassay. This revealed increased Aβ42 and decreased Aβ40 ratios of total produced Ab in three independent PS1 F175del mutant clones compared to cells overexpressing wild type PS1. * p-value < 0.05 (n=3, respectively; Student’s t-test. Error bars indicate standard deviations). (C) In addition to increased Aβ42 ratios, increased Aβ39 levels were detected in conditioned media of the same three individual PS1 F175del clones (PS1 F175del-1; -2; -3) when analyzed on a Tris-Bicine-Urea gel that also separates Aβ42 from Aβ43 (Kretner, et al., 2016,Wiltfang, et al., 1997). For comparison, the PS1 L166P mutation that leads to an excessive overproduction of Aβ42 and Aβ43 is displayed on the very right (Kretner, et al., 2016,Page, et al., 2008). The first three lanes show synthetic Aβ38/Aβ40/Aβ42 peptides. To better visualize the increased Aβ42 generation in the PS1 F175del expressing cell clones, samples were adjusted to Aβ40 levels comparable to the PS1 WT control. (D) The same shift in the spectrum of secreted Aβ species was observed by MALDI-TOF (matrix assisted laser desorption/ionization-time of flight) mass spectrometry analysis of Aβ immunoprecipitated from conditioned media of PS1 F175del expressing cells with relative larger peaks for Aβ42 and Aβ39 compared to the PS1 wild type control. Abbreviations: kDa = kilodalton. Swe = APP KM670/671NL mutation, WT = wild type, APP-FL = full length APP, CTF-β/α = C-terminal fragments of APP, PS1-FL = full

length presenilin 1, PS1-NTF = N-terminal fragment of presenilin 1, PS1-CTF = C-terminal fragment of presenilin 1, PS2-CTF = C-terminal fragment of presenilin 2, AICD = APP intracellular domain, Aβ = amyloid β-peptide.

Figure 4: Title: The PS1 F175del mutant shows a deviation in the Aβ42 product line. Description: Upper panel: Schematic representation of the two product lines including the respective principal γ-secretase cleavage sites in the APP transmembrane domain; the Aβ40 product line (Aβ49 to Aβ37) is shown above the APP sequence and the Aβ42 product line (Aβ48 to Aβ38) below. Bold arrows mark major cleavage sites. Lower panel: Compared to PS1 WT, the PS1 F175del mutant shows a deviation in the Aβ42 product line leading to an enhanced formation of Aβ39.

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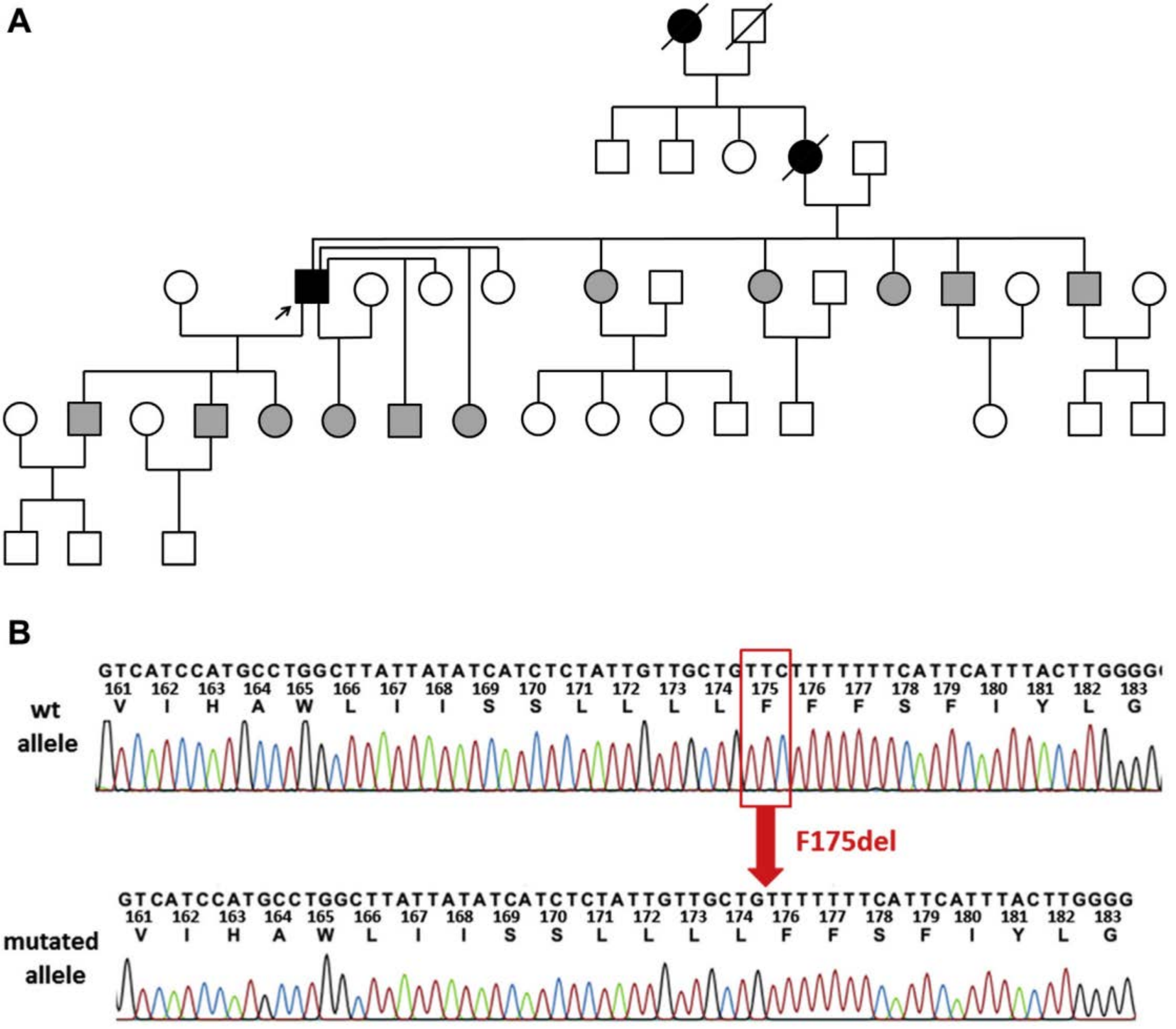
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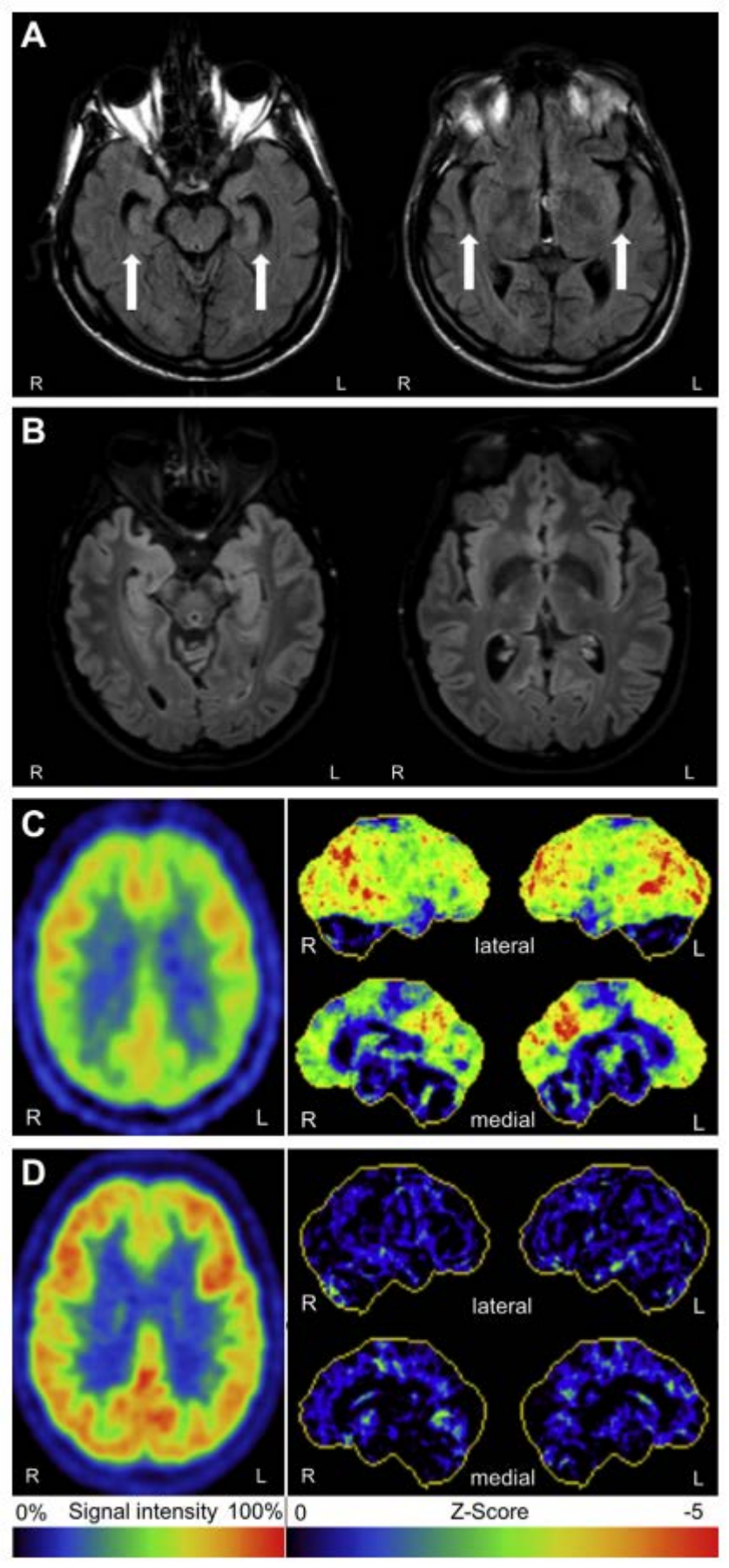
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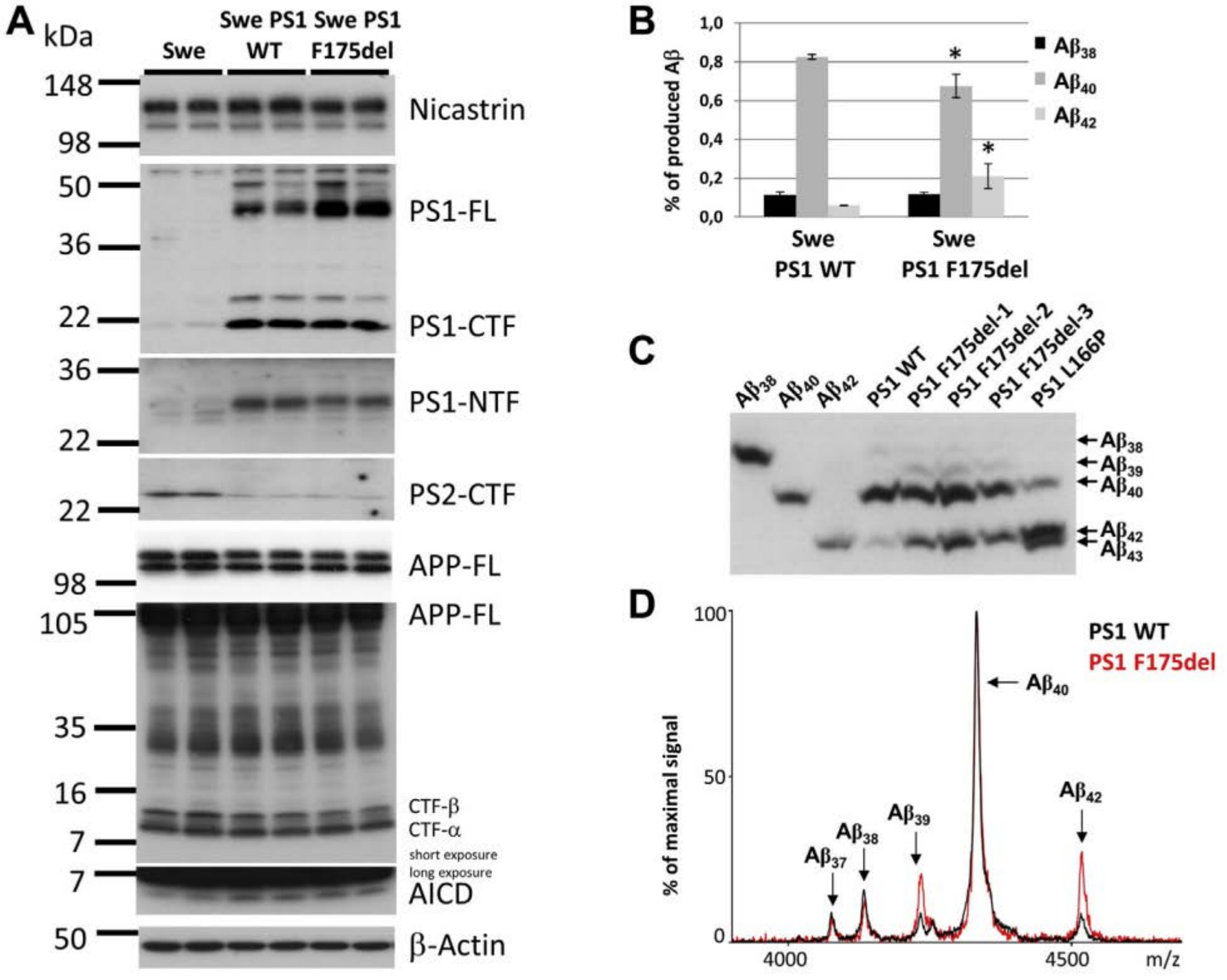
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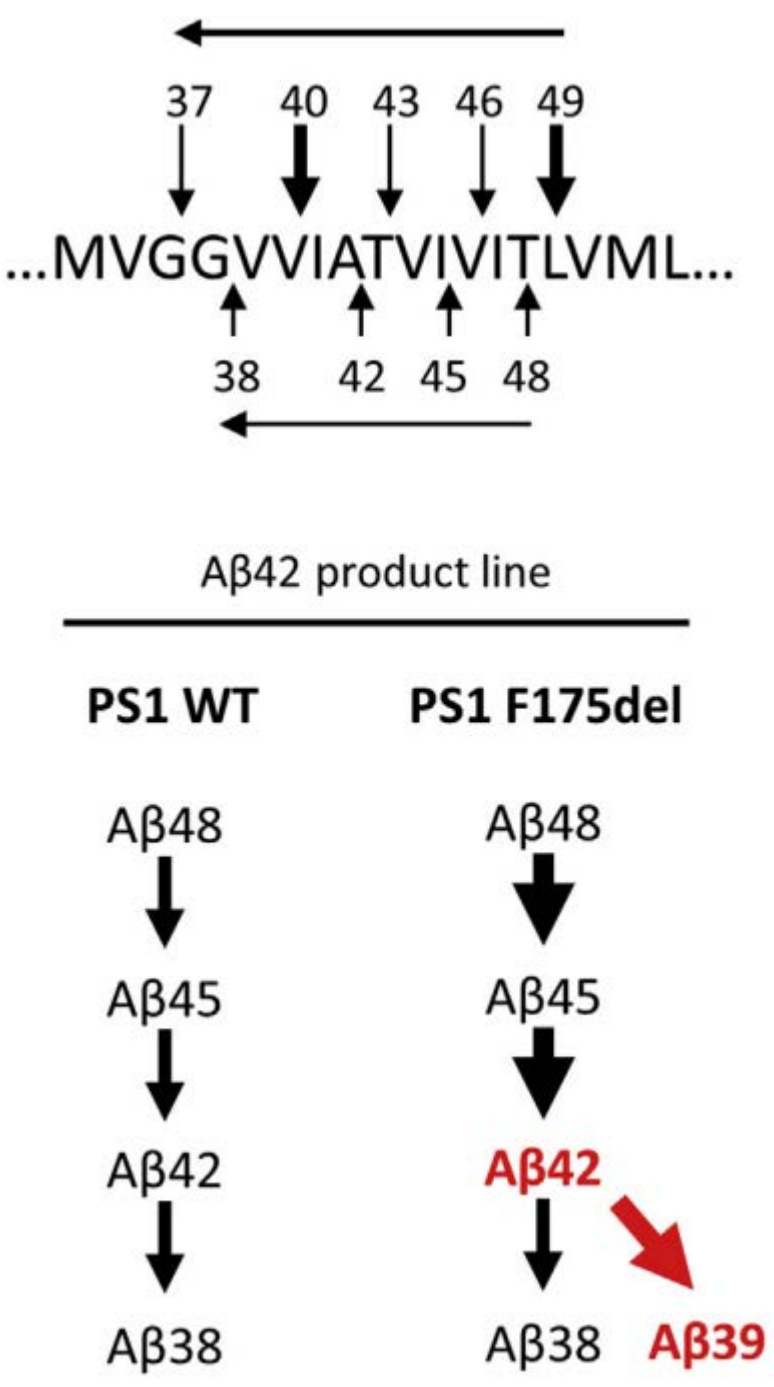
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