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GENETIC SCREENING OF ALZHEIMER'S DISEASE GENES IN IBERIAN AND AFRICAN SAMPLES YIELDS NOVEL MUTATIONS IN PRESENILINES AND APP

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Abstract

Mutations in three genes (*PSEN1*, *PSEN2*, and *APP*) have been identified in patients with early-onset (<65years) Alzheimer's disease (AD). We performed a screening for mutations in the coding regions of presenilins, as well as exons 16 and 17 of the *APP* gene in a total of 231 patients from the Iberian peninsular with a clinical diagnosis of early onset AD (mean age at onset of 52.9 years; range 31–64). We found three novel mutations in *PSEN1*, one novel mutation in *PSEN2*, and a novel mutation in the *APP* gene. Four previously described mutations in *PSEN1* were also found. The same analysis was carried in 121 elderly healthy controls from the Iberian peninsular, and a set of 130 individuals

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from seven African populations belonging to the Centre d'Etude du Polymorphisme Humain-Human Genome Diversity Panel (CEPH-HGDP), in order to determine the extent of normal variability in these genes. Interestingly, in the latter series, we found five new nonsynonymous changes in all three genes and a presenilin 2 variant (R62H) that has been previously related to AD. In some of these mutations, the pathologic consequence is uncertain and needs further investigation. To address this question we propose and use a systematic algorithm to classify the putative pathology of AD mutations.

Keywords

Early-onset Alzheimer's disease; Presenilins; APP; mutations

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia, accounting for more than 50% of all cases in adults. It is a progressive neurodegenerative disorder with an insidious onset, which typically appears in older individuals, but may affect people as early as in the third decade of life (Hardy and Selkoe, 2002). Early onset AD, with symptoms appearing before 65 years of age, represents about 1–2% of all cases. In these patients, the disease commonly aggregates within families, and about 10% of them show an autosomal dominant pattern of inheritance. These cases are linked to mutations in the amyloid precursor protein gene (*APP*, OMIM 104760), in the presenilin 1 gene (*PSEN1*, OMIM 104311) and in the presenilin 2 gene (*PSEN2*, OMIM 600759) (Tanzi and Bertram, 2001; Pastor and Goate, 2004).

A general problem in clinical genetics, is that when a locus for a disease is found by positional cloning, and subsequent point mutations are discovered, the gene is sequenced in others with the disease and novel DNA changes are described whose pathogenicity is then not assessed by either linkage or association (Hardy and Singleton, 2007). Usually, these mutations are simply screened for in a number of controls and pathogenicity is assumed if they are not found. In addition, most sequencing is done in highly studied populations, such as Caucasians or East Asians and little is done in other populations. The result of this strategy is that variants are reported and assumed to be pathogenic. This is damaging both from a basic scientific perspective, because it misleads research on basic mechanisms and from a clinical genetic perspective, because it could lead to incorrect information being given, especially to those from under studied communities. Examples of the precedent of misassignment of pathogenicity are the mutations E318G and InsR352 within *PSEN1*. Both variants were initially reported to be pathogenic and were later found to either be normal coding variants (E318G) or a rare non-pathogenic mutation (InsR352) (Mattila et al., 1998; Boeve et al., 2006). With this background, we have embarked on a systematic reassessment of pathogenicity of the genes involved in AD. First, we sequenced the presenilins and *APP* genes in a large series of early onset AD patients from Iberia. Then, in order to have a better knowledge of genetic variation within these genes, we have performed the same sequencing analysis in a series of unrelated African individuals from seven different populations obtained from CEPH-HGDP, as well as in Iberian controls. We chose to study this African series because genetic diversity is greatest in Africans and this population has been little studied. Therefore our chance of finding variants was highest in this population.

MATERIAL AND METHODS

Alzheimer series

A total of 231 unrelated patients (61.4% women) were recruited from 9 Iberian centers. All individuals included in this study were Caucasian with apparent Spanish or Portuguese

ancestry. Mean age at onset was 52.9 years, ranging from 31 to 64. Seventy-four patients (32%) showed a family history of dementia (defined as at least one affected first degree relative) and 99 (43%) did not report any familial aggregation of disease. In 25% of individuals, no information was available. For all patients, the diagnosis of probable AD was established according to the standard Diagnostic and Statistical Manual, revision 4 (DSM IV) (American Psychiatric Association, 1994) criteria and the National Institute of Neurological Disorders and Stroke, and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) protocol guidelines (McKhann et al., 1984). Written informed consent was obtained from all participants or surrogates.

Control series

Written informed consent was obtained from 121 neurologically normal and age-matched control subjects from the Iberian peninsular (mean age at collection 67.4). These were either patient's spouses or unrelated caregivers. Coding exons 3 to 12 of *PSEN1* and *PSEN2*, and exons 16 and 17 of the *APP* gene were sequenced in this series and in 130 African samples obtained from the CEPH-HGDP (Cann et al., 2002). These samples originated from 7 different African populations: 29 Biaka Pygmy, 13 Mbuti Pygmy, 23 Mandenka, 22 Yoruba, 2 San, 17 Bantu, and 24 Mozabite.

DNA Sequencing

Genomic DNA was isolated by standard procedures. The exonic regions of *APP* (exons 16 and 17), *PSEN1* (exons 3–12) and *PSEN2* (exons 3–12) genes, as well as the flanking intronic sequences, were PCR amplified using the respective primers (Goate et al., 1991; Cruts et al., 1998) and Roche FastStart PCR Master Mix polymerase (Roche Diagnostics Corp., IN). Each PCR product was sequenced using the same forward and reverse primers with Applied Biosystems BigDye terminator v3.1 sequencing chemistry and run on an ABI3730xl (Applied Biosystems) genetic analyzer as per manufacturer's instructions. The sequences were analyzed with Sequencher software, version 4.2 (Genecodes, VA).

RESULTS

Mutation screening in AD patients

A total of five novel non synonymous mutations were found. Three of them (H214D, c. 640C>G; L248R, c.743T>G; and S365A, c.1093T>G) were present in *PSEN1*, one (M174V, c.520A>G) in *PSEN2*, and a change in residue 716 of the *APP* gene (I716F, c.2146A>T). Four previously described mutations were also detected in *PSEN1*: T116N, c.347C>T; M233T, c. 697A>C; V272A, c.815T>C; and A260V, c.779C>T.

Additionally, another new mutation in *PSEN2* (R71W, c.211T>C) was detected in a 77 year-old woman with disease onset at 75 years. This patient was not included in the early onset Alzheimer's disease series, but was studied at a clinician request.

Screening in Iberian controls

In the 121 controls screened for mutations in *PSEN1*, *PSEN2* and *APP* we found no non-synonymous changes.

Screening in African individuals

Two non-synonymous changes were found in the *PSEN1* gene in the African series: the previously reported mutation R35Q (c.104G>A) (Rogaeva et al., 2001; Raux et al., 2005) which was present in one Mozabite individual and a new variant (V191A, c.572T>C), found in a San subject.

In the *PSEN2* gene we found three new non-synonymous changes (R29H, c.86C>T; L143H, c.428T>A; and A252T, c.754G>A). Strikingly, twenty individuals presented the R62H (c. 185G>A) variation, which had been previously described as a variant with an unclear pathogenic role in AD (Cruts et al., 1998).

In the *APP* gene, a new non-synonymous variation (H733P, c.2198A>C) was found in an individual from the Mandenka population.

DISCUSSION

Mutations found in Alzheimer cases and their interpretation

APPI716F: exon 17; transmembrane (TM) domain of the protein. APOE 3/3—The novel *APP* I716F is associated with the earliest age at onset described for this locus. Clinical symptoms of the proband started at the age of 31, and the patient died two years later. Neuropathological examination revealed the presence of neurofibrillary degeneration (stage VI of Braak and Braak) (Braak and Braak, 1991) and amyloid deposits (stage C), thus confirming the clinical diagnosis. The patient's father died at age 41 with clinically diagnosed Alzheimer's disease. Although we were not able to demonstrate segregation of the mutation with the disease in this family, it is likely to be a pathogenic variant, since other mutations have been described in the same residue (I716V and I716T) (Eckman et al., 1997; De Jonghe et al., 2001; Terreni L et al., 2002).

PSEN1 T116N: Exon 5, hydrophilic loop I (HL-I), conserved domain in PSEN2 T122, where pathogenic variants (T122P and T122R) have been described. APOE 3/4—This mutation was found in a man with onset of symptoms at age 37. It has previously been reported as a pathogenic mutation on three occasions (Romero et al., 1999; Rogaeva et al., 2001; Raux et al., 2005), with some segregation information and with an onset age between 30 and 40 years, being associated with an aggressive familial type of AD with a rapid progression. In the same residue, the T116I mutation was also reported (La Bella et al., 2004; Raux, Guyant-Marechal, Martin, Bou, Penet, Brice, Hannequin, Frebourg and Campion, 2005). According to the algorithm we propose (Figure 2) this is a Definitely Pathogenic mutation.

PSEN1 H214D: Exon 7, HL-IV, conserved in H220 PSEN2 domain where no mutations are described. APOE 3/3—This mutation has not been previously reported. It was found in a woman with first clinical symptoms at the age of 55, including atypical signs such as bradykinesia and mild bilateral action tremor. Family history was positive: the father and grandmother of the proband presented late onset dementia. This mutation alters a conserved residue between the two presenilins where the H214Y mutation was previously described in one family (Raux et al., 2005). According to our algorithm (Figure 2), this mutation is considered to be Possibly Pathogenic.

PSEN1 M233T: Exon 7, TM-V, residue conserved in PSEN2 M239, where M239V and M239I are described. APOE 3/3—This mutation was found in a man who presented the first clinical symptoms at age 35 and died at the age of 42. The proband was first observed in a psychiatric hospital presenting atypical first symptoms with frontal profile (dysexecutive syndrome) and behavioural symptoms (depression/apathy and aggressiveness). He also presented extrapyramidal signs such as dysarthria, left hand apraxia, face and foot dystonia and pyramidal signs (Babinski). Other late signs included myoclonus and tonic-clonic seizures. Neuroimaging studies revealed hippocampal and bi-parietotemporal atrophy.

The family history was unclear. This mutation has been reported several times before (Kwok et al., 1997; Campion et al., 1999; Raux et al., 2005), as other mutations in the same residue

(M233L, M233V and M233I) (Aldudo et al., 1999; Houlden et al., 2001; Rogaeva et al., 2001; Mendez and McMurtray, 2006). It alters a conserved residue between *PSEN1* and *PSEN2* located in the fifth transmembrane domain. Pathogenic mutations have also been reported in the homologous *PSEN2* residue. It produces a greater proportion of Aβ42 (Scheuner et al., 1996; Qi et al., 2003; Qi-Takahara et al., 2005) and fits the helix rule (Hardy and Crook, 2001), aligning with other mutations in transmembrane domain 5 (Figure 1). This mutation is Definitely Pathogenic (Figure 2).

PSEN1 L248R: Exon 7, TM-VI, residue not conserved in PSEN2 V254, no mutations described. APOE 3/3—This mutation has not been reported before. It was found in a man presenting an age at onset of 54 years that died at the age of 65. Neuroimaging studies revealed a prominent atrophy in the lateral fissure together with a less prominent atrophy in parietofrontal regions. Family history was reported as negative. The mutation L248R does not alter a conserved residue between presenilins (V254 in PSEN2) and is located in the sixth transmembrane domain of PSEN1. Although it aligns with other mutations found in the same transmembrane domain (Figure 1), the paucity of the genetic data means that we cannot be certain of the pathogenicity of this mutation. We would assign this mutation as Possibly Pathogenic (Figure 2).

PSEN1 A260V: Exon 8, TM-VI, residue conserved in PSEN2 A266 where no mutations are described. APOE 3/3—This mutation was found in a woman who presented the first clinical symptoms at the age of 30. Imaging studies revealed hippocampal and parietotemporal atrophy. SPECT revealed bilateral temporoparietal hypoperfusion. Family history was positive: the mother, one aunt and one cousin developed early onset dementia (<40 years). This mutation has been reported several times before (Rogaev et al., 1995; Ikeda et al., 1996). It alters a conserved residue between presenilins (PSEN2 A266) and is located in the sixth transmembrane domain of PSEN1. It aligns with other mutations in this domain (Figure 1) and increases the Aβ 42/Aβ 40 ratio (Kametani et al., 2001). This mutation is Definitely Pathogenic (Figure 2).

PSEN1 V272A: Exon 8, HL-VIa, residue conserved in PSEN2 V278 where no mutations are described. APOE 3/3—This mutation was found in a man who at the age of 34 years presented myoclonus and dementia. The patient died at age 42 and had a positive familial history of dementia: the father and three siblings also presented with dementia. These three siblings also carried the V272A mutation. This mutation alters a residue conserved between PSEN1 and PSEN2 but is not in a transmembrane domain (HL-VIa). It was previously reported in a three generations family with four affected subjects, two of which carried the V272A mutation, and associated with an increased Aβ42 levels in plasma (Jimenez-Escrig et al., 2004). Interpretation of these data as in the scheme presented in Figure 2 indicates that this mutation is Definitely Pathogenic.

PSEN1 S365A: Exon 10, HL-VIb, residue not conserved in PSEN2 it does not align with any aminoacid in PSEN2. APOE 4/4—The *PSEN1* S365A mutation is a novel variation. It was found in a woman with clinical symptoms beginning at age 55, and was not present in her two healthy siblings. Although the proband's father suffered from dementia, in him, the disease started at the age of 70. Biological specimens were not available and no further segregation studies were possible. A mutation at this residue (S365Y) has been previously reported in a patient, who also carried the *PSEN1* M146V mutation, which is almost certainly pathogenic (Rogaeva et al., 2001). This residue is not conserved between *PSEN1* and *PSEN2* and is not in a transmembrane domain (HL-VIb). Clearly, we cannot be certain whether this variant is pathogenic. We suggest this mutation is Possibly Pathogenic (Figure 2).

PSEN2 R71W: Exon 4, N-Terminal, residue not conserved in PSEN1 Q65. APOE 2/4—This novel variant was found in an elderly sporadic Alzheimer case which was not part of the central screening in this paper. Neuroimaging features included chronic periventricular microangiopathic leukoencephalopathy with multiple nucleo-capsular and periventricular white matter lacunar infarctions. This mutation is Possibly Pathogenic.

PSEN2 M174V: Exon 6, TM-III, residue not conserved in PSEN1 I168. APOE 3/3 —This novel variant was harbored by a woman who developed Alzheimer's disease at 54 years of age. Neuroimaging features included atrophy in both parietal regions (R>L) and SPECT revealed hypoperfusion in temporoparietal regions (R>L). This variant alters a non conserved residue between presenilins (I168 in PSEN1) located in the third transmembrane domain of PSEN2. Due to the lack of family history and the fact that this is a not-conserved residue between PSEN1 and PSEN2 we assign this mutation as Possibly Pathogenic.

Variations found in African population

APP H733P: found in a Mandenka sample (Exon 17, C-Terminal)—This variant was found in exon 17 of the *APP* gene. It alters a residue located in the C-terminal of the protein. No variants have been reported at this residue or close to this residue before.

PSEN1 R35Q: found in a Mozabite sample (Exon 4, N-Terminal), residue not conserved in PSEN2 G37—This variant has been described before in 2 families with AD, but in neither case did it segregate with disease and it was interpreted as non-pathogenic (Rogaeva et al., 2005). The data we present here suggests this interpretation is correct. The residue is not conserved in PSEN2 and no studies of the effect of this mutation on Aβ metabolism have been reported.

PSEN1 V191A: found in a San sample (Exon 7, HL-III, residue conserved in PSEN2 V197)—This variant has not been reported before. It was found in exon 7 of *PSEN1* and the residue where this mutation occurs is conserved between PSEN1 and PSEN2.

PSEN2 R29H: found in a Mandenka Sample (Exon 3, N-Terminal), residue conserved in PSEN1 R27—This variation has not been previously reported. It is located in exon 3 of the *PSEN2* gene and alters a residue that is conserved between PSEN1 and PSEN2.

PSEN2 R62H found in 20 African samples (allele frequency 9%) (Exon 4, N-Terminal), residue conserved in PSEN1 R60—This variant had previously been reported in a sporadic Alzheimer case. These data indicate it is a relatively common polymorphism in African populations. This residue, located in the N-terminal of the protein, is conserved between PSEN1 and PSEN2 (R60 in PSEN1) and studies of the effect of the variant on Aβ metabolism showed no alteration in the production of Aβ42 (Walker et al., 2005).

PSEN2 L143H found in a Bantu sample (Exon 5, TM-II, residue not conserved in PSEN1 A137)—This variant occurs in exon 5 of *PSEN2*. It alters a residue that is not conserved between PSEN1 and PSEN2 (A137 in PSEN1), located in the second transmembrane domain of the protein. No mutations have been reported in the correspondent residue of *PSEN1* and it does not fit with the helix rule for pathogenic mutations.

PSEN2 A252T: found in a Mandenka and in a Yoruba sample (Exon 7, TM-VI, residue conserved in PSEN1 A246)—This variant, found in exon 7 of the *PSEN2* gene modifies a residue conserved between PSEN1 and PSEN2 (A246 of PSEN1) that localizes in

the sixth transmembrane domain of PSEN2. Two different pathogenic mutations have been reported in the corresponding *PSEN1* residue.

With this background, we herein propose a scale for grading mutations as not pathogenic, possibly pathogenic, probably pathogenic and definitely pathogenic (Figure 2). We recognize that this scale is merely pragmatic and subject to improvement, not least because it is possible that some variants may increase the risk of disease rather than being truly pathogenic.

1. Segregation: Has the mutation been shown to segregate with the disease? This is clearly the strongest evidence. We would suggest that if a mutation has been shown to segregate with the disease in three or more cases in a family it should be regarded as Definitely Pathogenic and if it has shown segregation in two cases, Probably Pathogenic.
2. Association: Has the mutation been found in one case and not in controls? Evidence of association with the disease is fundamentally weaker than segregation because it is not clear how many controls from different populations have been sequenced. Thus, one does not know, for any individual mutation, what the denominator is. However, we suggest that if a mutation has been found in at least three early onset non related Alzheimer cases and in no controls; and more than 100 controls have been sequenced, that it be regarded as Probably Pathogenic. If less than three have been found, then we would suggest the designation as Possibly Pathogenic.
3. Residue and Aβ levels: Have other pathogenic mutations been described in that residue before? If it is a presenilin mutation, does the mutation alter a residue conserved between PSEN1 and PSEN2 and, if the residue is in a transmembrane domain does it follow the helix rule? Does the mutation alter APP processing such that a greater proportion of Aβ42/Aβ40 ratio is produced? We would suggest that if two of the answers to any of these questions is “yes”, then this should allow the “promotion” of a mutation.
4. Obviously, finding the mutation in controls is strong evidence that it is not simply pathogenic (although small changes in risk will remain a possibility).

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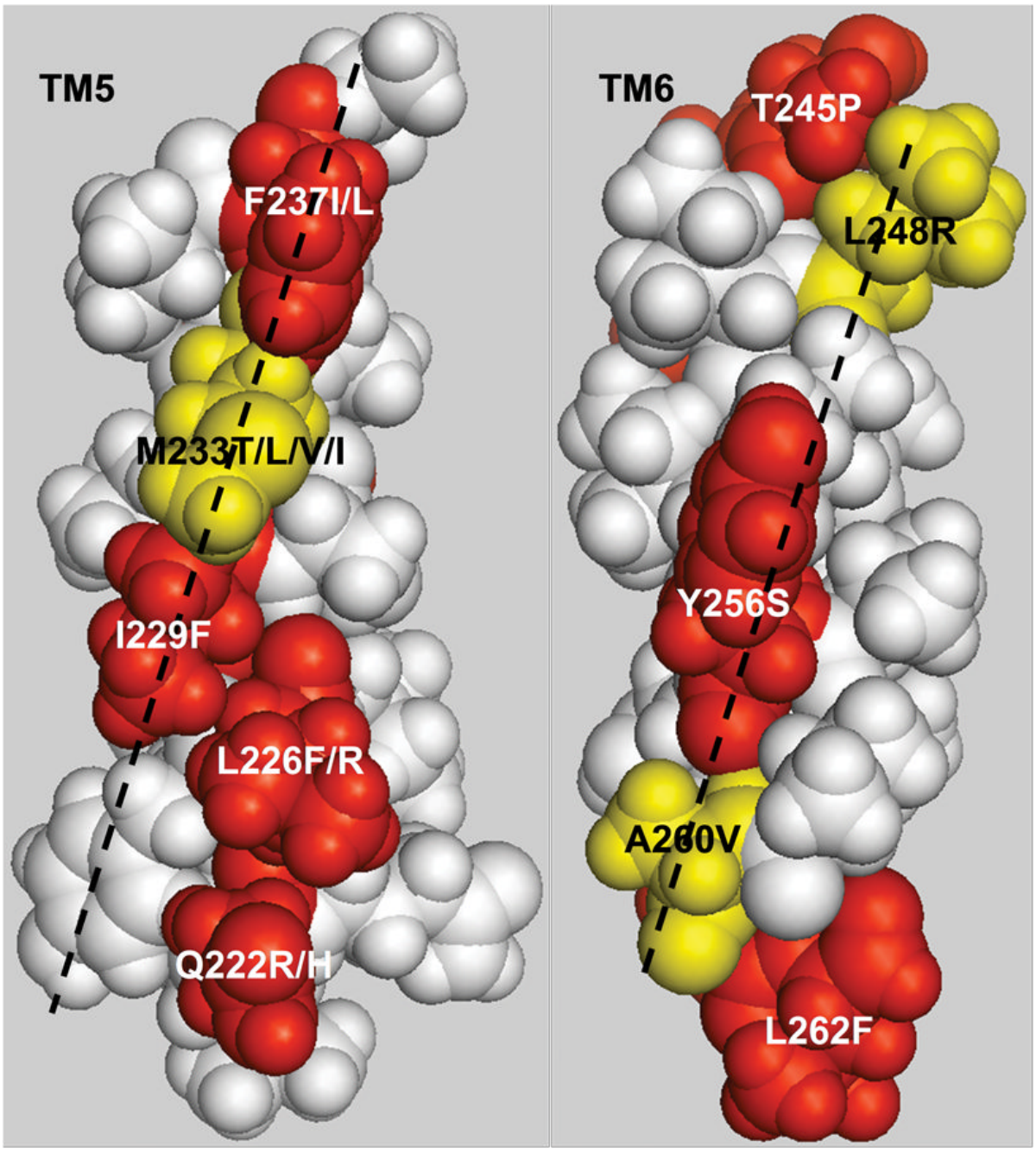

Figure 1.
Example of the alignment of the mutations found in this study (in yellow) and the mutations previously described (red) in transmembrane domains 5 and 6.

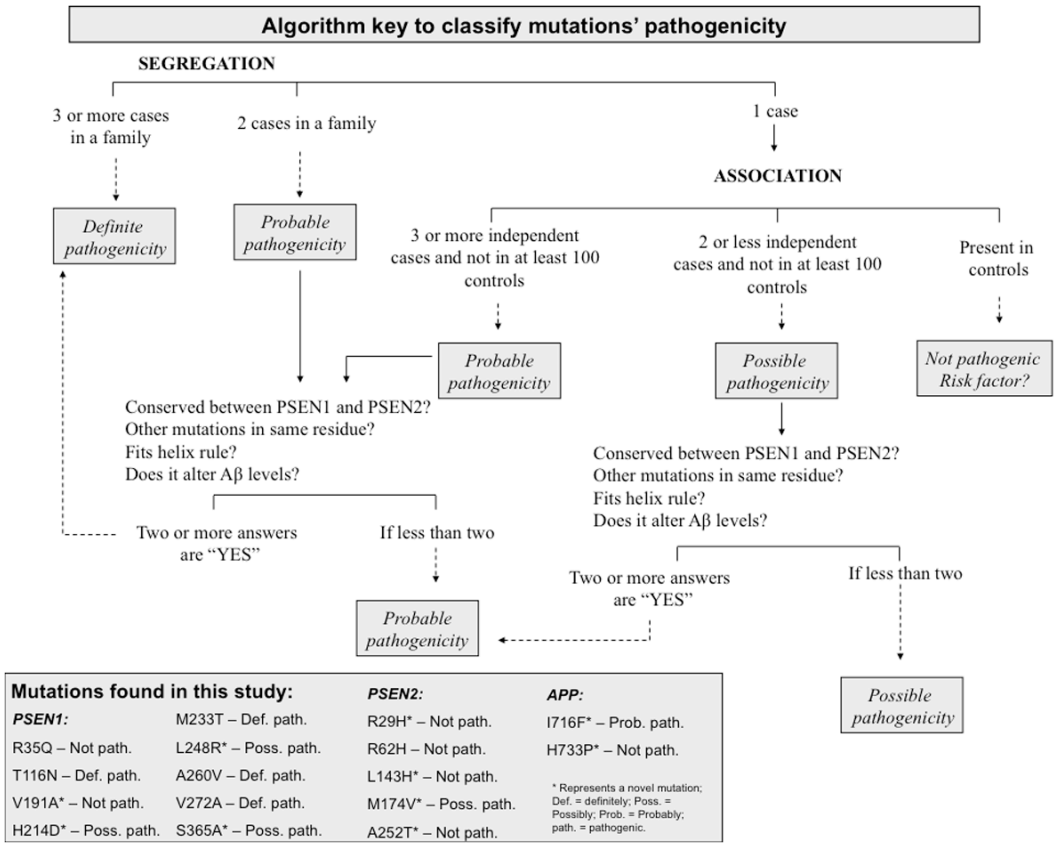

Figure 2.
Algorithm to classify mutations as possibly pathogenic, probably pathogenic, and definitely pathogenic.