No association of spastic paraparesis genes in *PSEN1* Alzheimer's disease with spastic paraparesis

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Familial Alzheimer's disease due to presenilin 1 (*PSEN1*) mutations shows considerable phenotypic variability with differences in neuropathology and neurological symptoms. Spastic paraparesis is a common neurological phenotype associated with Alzheimer's disease arising from *PSEN1* mutations. To investigate whether known genes that cause spastic paraparesis could act as Alzheimer's disease-modifier genes, we sequenced nine spastic paraparesis genes in three Alzheimer's disease families with *PSEN1* exon 9 deletions. We did not observe any correlation of polymorphisms or mutations in the nine spastic paraparesis genes with the variable phenotype seen in families with Alzheimer's disease and spastic paraparesis. These results suggest a need for a continuing search for genes that cause the phenotypic variation in Alzheimer's disease and spastic paraparesis. *NeuroReport* 18:1267–1269 © 2007 Lippincott Williams & Wilkins.

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Introduction

The understanding of Alzheimer's disease has been greatly aided by the identification of causal mutations in three genes in rare families with early-onset (before age 65 years) autosomal-dominant inheritance: amyloid precursor protein, presenilin1 and 2 (*PSEN1*, *PSEN2*) [1]. The majority of *PSEN1* mutations are associated with classic Alzheimer's disease and the neuropathology is characterized by the presence of intraneuronal neurofibrillary tangles consisting of hyperphosphorylated-tau and extracellular amyloid plaques mainly consisting of amyloid β-peptide (Aβ) [1]. In 1997, a key observation was made that *PSEN1* Alzheimer's disease mutations were associated with the presence of the neurological condition, spastic paraparesis, suggesting that certain abnormalities in the *PSEN1* protein give rise to both spastic paraparesis and Alzheimer's disease [2]. In addition, in 1998, it was shown that *PSEN1* Alzheimer's disease pedigrees frequently have a variant neuropathology, namely large, noncored plaques without neuritic dystrophy called cotton wool plaques (CWP) [3]. Some individuals with *PSEN1* mutations present initially with spastic paraparesis and have remained dementia-free for up to 10 years [4,5]. The existence of a protective modifying factor is one explanation for this phenomenon. We have investigated sequence variations in nine spastic paraparesis genes based on the inheritance pattern in our three pedigrees. All three pedigrees have a mutation in the *PSEN1* gene that results in the loss of exon 9, a mutation that is overrepresented in families with Alzheimer's disease plus spastic paraparesis [4].

Description of the families

The family reported by Smith *et al*. [5] is an Australian early onset Alzheimer's disease kindred, EOFAD1, which has a 5.9 kb genomic deletion spanning exon 9. Members of two generations developed early onset Alzheimer's disease, confirmed by autopsy in two cases. Members of one sibship in the third generation, at 50% risk, remained asymptomatic for Alzheimer's disease until their mid-40s, an age by which affected family members usually had well-established cognitive decline, although four siblings subsequently developed spastic paraparesis (Fig. 1). One died of breast cancer without apparent dementia. At autopsy, there was pyramidal tract degeneration in the medulla and spinal cord with pyramidal cell loss in the motor cortex and significant numbers of CWP but very few neurofibrillary tangles and no significant neuronal loss elsewhere in the brain. Another sibling became cognitively impaired in her 50s at about the same time as she developed spastic paraparesis and at autopsy had pyramidal-tract degeneration and Alzheimer's disease with CWP together with cerebral-amyloid angiopathy. Her brain MRI had previously shown severe white matter disease, though this was not found in her brother, who remained without significant dementia until he was admitted to a nursing home at the age of 59 years, because of disabling spasticity in the lower limbs and subsequent bulbar involvement with impaired speech and swallowing. In this family, it is notable that the mean age at onset of symptoms in individuals with spastic paraparesis at presentation is later than that for individuals

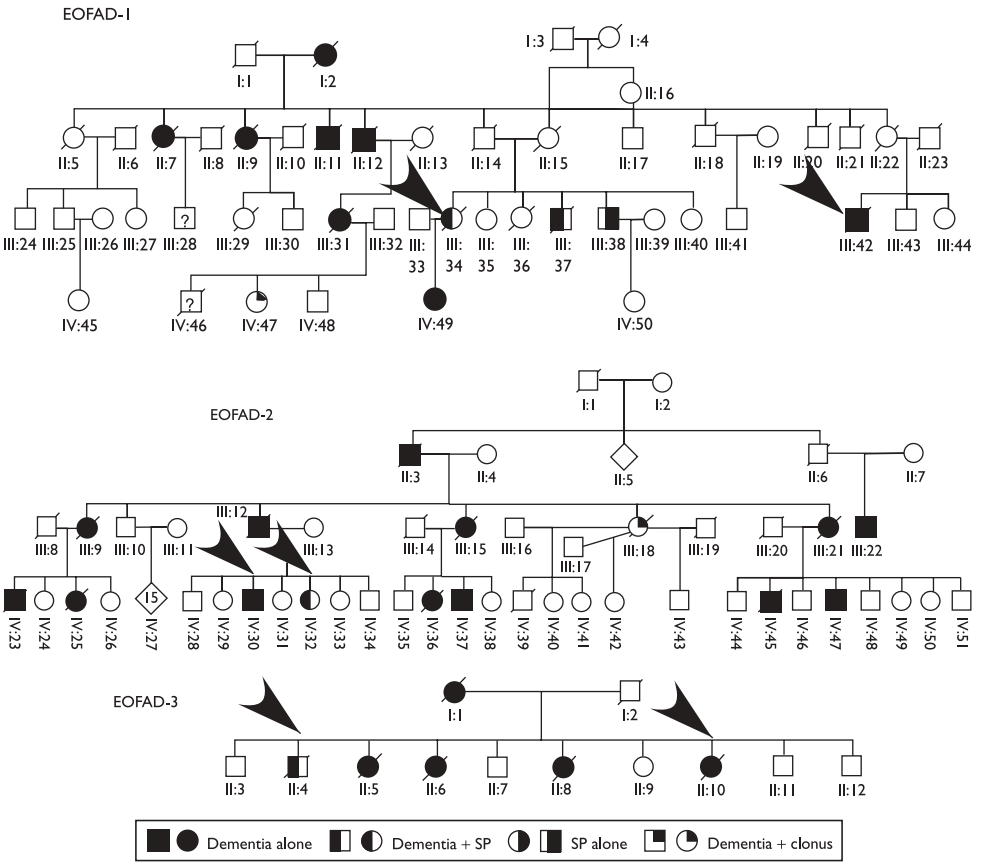

Fig. 1 Three Australian early-onset Alzheimer's disease pedigrees with *PSEN1* exon 9 deletion mutations. Black symbols indicate early-onset dementia. Left-half-black symbols indicate spastic paraparesis at presentation and unfilled symbols are unaffected or unknown. Arrows indicate family members on whom DNA sequencing was performed.

with dementia as the initial presentation. For individuals with spastic paraparesis at presentation, either alone or with cognitive decline, cortical CWP were a feature, compared with patients with only dementia who developed the characteristic neuritic Alzheimer's disease plaques [5].

The second family, EOFAD2, described by Brooks *et al*. [6], had the presence of spastic paraparesis in some members (Fig. 1). The family has a deletion of exon 9, because of the G/A splice acceptor mutation. Affected individuals had early onset Alzheimer's disease with age at symptom onset ranging from the late 30s to the early 50s. Four affected members examined had typical Alzheimer's disease without spastic paraparesis; one, with a relatively late onset age of 48 years, developed spastic paraparesis concurrently with cognitive impairment [6].

The third family, EOFAD3, carries the exon 9 G/T splice acceptor mutation. Most developed cognitive impairment as a presenting feature in their early 40s and died in their late 40s or early 50s, but one mutation-positive individual presented in his late forties with spasticity, surviving until 58 years (Fig. 1). A sibling who presented with dementia developed spasticity subsequently and died at 51 years. Neuropathological examination of the cervical spinal cord showed bilateral degeneration of the corticospinal tracts [6].

Methods

Genomic DNA and complementary DNA were prepared from either brain tissues or blood samples from individuals affected with spastic paraparesis in the three families shown in Fig. 1. PCR was performed using oligonucleotides that span the exons and approximately 200 base pairs of flanking intronic sequence of nine genes that, when mutated, can cause spastic paraparesis: *maspardin*, *spastin*, *atlastin*, *paraplegin*, *spartin*, *sacsin*, *hsp60*, *Nipa1* and *KI5FA* (Table 1). All oligonucleotide sequences are available upon request. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) and analysed on the ABI 3100 Genetic

Table 1 Identified SP genes and their genetic loci

	Inheritance	Locus name	Chromosome location	Gene
Complicated SP	AR	SPG7	16q24.3	*Paraplegin*
Complicated SP	AR	SPG20	13q12.3	*Spartin*
Complicated SP	AR	Mast syndrome	15q21-q22	*Maspardin* (*ACP33*)
Complicated SP	AR	ARSACS	13q12	*Sacsin*
Complicated SP	X-linked	SPG1	Xq28	*LICAM*[a]
Complicated SP	X-linked	SPG2	Xq22	*PLP1*[a]
Uncomplicated SP	AD	SPG3A	14q11-q21	*Atlastin*
Uncomplicated SP	AD	SPG4	2p22–p21	*Spastin*
Uncomplicated SP	AD	SPG6	15q11.1	*NIPA1*
Uncomplicated SP	AD	SPG10	12q13	*KIF5A*
Uncomplicated SP	AD	SPG13	2q24	*Hsp60*

AD, autosomal dominant; AR, autosomal recessive; SP, spastic paraparesis.
[a]The X-linked genes have not been analysed.

Analyzer (Applied Biosystems) using the software Sequencing Analysis 5.1 (Applied Biosystems) according to the manufacturer's description.

Results

Sequencing of the nine spastic paraparesis genes did not reveal any coding sequence mutations or polymorphic variations in the coding exons or their intronic flanking sequences in any of the probands. We detected a polymorphism in the coding sequence in exon 2 of the *hsp60* gene, where the C in position +27 was changed to a G in two out of three Alzheimer's disease patients but not in patients with Alzheimer's disease plus spastic paraparesis. This, however, did not change the amino-acid sequence (Arg −> Arg) and this polymorphism has been reported in the National Centre for Biotechnology Information Single Nucleotide Polymorphism (NCBI SNP) database to be a frequent SNP (refSNP ID rs11551349).

Discussion

Approximately 25 families have been reported to display features of Alzheimer's disease and spastic paraparesis, one-third of whom have a *PSEN1* exon 9 deletion [4]. Mutations affecting exon 9 are markedly overrepresented in early onset Alzheimer's disease patients with spastic paraparesis (5–6% of Alzheimer's disease plus spastic paraparesis cases versus 1.4% of *PSEN1* mutations leading to Alzheimer's disease) [4]. As this *PSEN1* deletion/mutation (and other *PSEN1* mutations) have been reported in families with both classic and variant Alzheimer's disease, we have previously proposed that there is a protective modifying factor inherited with the spastic paraparesis phenotype in Alzheimer's disease families, which is unlinked to *PSEN1*, but is acting in concert with a *PSEN1* mutation [4,5]. This encouraged us to investigate the sequences of nine spastic paraparesis genes in three families with *PSEN1* exon 9 deletion mutations. We did not examine two spastic paraparesis genes, *L1CAM* and *PLP1*, which are X-linked as this mode of inheritance was not seen in the three pedigrees. Rogaeva *et al.* [7] previously examined the sequence of three spastic paraparesis genes, *spastin*, *atlastin* and *paraplegin*, in patients with Alzheimer's disease and spastic paraparesis, and also failed to identify any mutations or polymorphisms in these three genes.

Other explanations for the pathogenesis of the spastic paraparesis variant have been proposed, namely an increased amount of Aβ1−42 or a dosage effect [8]. Most spastic paraparesis-causing mutations selectively increase Aβ1−42 production compared with those mutations that lead to the more typical dementia phenotype. Whereas this proposal may explain the presence of CWP in Alzheimer's disease and spastic paraparesis pedigrees, it does not provide an explanation for the altered sites of neuropathology such as the degeneration of the motor cortex, which is normally spared in Alzheimer's disease, and the degeneration of the corticospinal tracts. In addition, the variable presentation of spastic paraparesis and dementia within large sibships carrying identical *PSEN1* mutations, who presumably have many common environmental exposures, suggests that environmental factors may not be the primary determinant, although this cannot be excluded.

Although the exact mechanism by which the Alzheimer's disease plus spastic paraparesis phenotype arises remains to be determined, the most likely explanation is the presence of modifier genes. In the case of individuals in Alzheimer's disease pedigrees with spastic paraparesis, the putative modifier gene is proposed to delay the onset of dementia in carrier individuals, perhaps by preventing pyramidal cell loss in nonmotor cortical regions, despite the deposition of Aβ in CWP. Whereas this study failed to find any evidence for genetic modifiers among nine known spastic paraparesis genes, there is a need for a continuing search for modifier genes. The examination of other candidate genes involved in Aβ degradation and clearance, as well as tau-phosphorylation, would also be of great interest. If such modifier genes can be identified, new disease therapeutics could be based on mimicking or enhancing the effects of these naturally occurring genetic modifiers.

Conclusion

Spastic paraparesis is a common neurological phenotype associated with Alzheimer's disease arising from *PSEN1* mutations, and its presence leads to an apparent delay in the onset of dementia, suggesting that this effect is due to modifier genes. We sequenced nine spastic paraparesis genes in patients from pedigrees with Alzheimer's disease and spastic paraparesis but did not identify any mutations or polymorphisms that were associated with the disorder.

Acknowledgements

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