Somatic and germline mosaicism in sporadic early-onset Alzheimer’s disease

Jonathan A. Beck1, Mark Poulter1, Tracy A. Campbell1, James B. Uphill1, Gary Adamson1, Jennian F. Geddes3, Tamas Revesz2, Mary B. Davis2, Nicholas W. Wood2, John Collinge1,* and Sarah J. Tabrizi1

1MRC Prion Unit and Department of Neurodegenerative Disease and 2Department of Molecular Neuroscience, Institute of Neurology UCL, Queen Square, London WC1N 3BG, UK and 3Department of Histopathology and Morbid Anatomy, Royal London Hospital, Whitechapel, London, UK

Received January 21, 2004; Revised April 6, 2004; Accepted April 19, 2004

Alzheimer’s disease (AD) is the commonest neurodegenerative disease worldwide. Rare familial cases may be caused by mutations in one of three genes—amyloid precursor protein, presenilin-1 and presenilin-2; however, the molecular basis of >99% of AD cases is unknown. Somatic mutation has been considered to be a mechanism that may account for a proportion of sporadic cases of AD, but to date there has been no evidence for this. We now report a sporadic early-onset patient with AD, and show that this individual is a somatic mosaic for a mutation in the presenilin-1 gene, suggesting a novel molecular mechanism for AD. Quantification of the mosaicism demonstrated the degree of mosaicism at 8% in peripheral lymphocytes and 14% in cerebral cortex in the index patient; a clear gene dosage effect on age of presentation and clinical phenotypic presentation is demonstrated. This finding has important implications for the aetiology of sporadic AD, and for other apparently sporadic neurodegenerative diseases such as Parkinson’s disease, motor neuron disease and Creutzfeldt–Jakob disease.

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disease characterized clinically by memory impairment, language and visuo-spatial problems and personality changes, followed by global cognitive decline. It accounts for up to 70% of all late-onset cases of dementia, with a prevalence of 4% at 75 years. With the prediction of 1 billion people in the world over 60 years old by 2025, AD represents a major public health concern. Neuropathologically it is characterized by the accumulation in the brain of extracellular β-amyloid plaques and neurofibrillary tangles with neurons of the cerebral cortex and hippocampus. The molecular basis of >99% of AD is unknown, but mutations in three genes have been found associated with ~50% of autosomal dominant, early-onset (<65 years) familial AD—the amyloid precursor protein (APP), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) (1–3). These genes have provided important mechanistic insights into the pathophysiology of AD. The presenilins are components of novel enzyme complexes involved in the physiological γ-secretase proteolytic cleavage of APP; mutations in PSEN1 and PSEN2 affect the precision of γ-secretase cleavage with resultant increased formation of the neurotoxic isof orm of Aβ (Aβ42), the major component of β-amyloid plaques (4). Inheritance of the APOE*E4 allele has been shown to confer increased risk of AD (5,6), but the search for other genes causing sporadic and late-onset AD has been difficult (7).

Germline and somatic mosaicism have emerged as important factors in the aetiology of cancer, ageing and mitochondrial disorders; they are also recognized causes of the marked phenotypic variation seen in a number of monogenic diseases (8,9). Mosaicism for trisomy of chromosome 21 has been proposed as a cause of late-onset AD (10), and somatic mosaicism, with the development of specific disease-associated mutations in neuronal precursor cells, has been postulated as a possible cause of sporadic AD and other neurodegenerative disorders, but to date there has been no evidence for this in vivo.

Here we report the first description of somatic and germline mosaicism in a patient with sporadic early-onset AD; this finding also has important implications for the aetiology of other apparently sporadic neurodegenerative diseases.

*To whom correspondence should be addressed. Tel: +44 2078374888; Fax: +44 2078378047; Email: j.collinge@prion.ucl.ac.uk

RESULTS

The index patient (II.1, Fig. 1) presented at the age of 52 with a 10-year history of progressive parkinsonian syndrome, with additional features of mild spastic paraparesis and dementia. She died at the age of 58 after a 16-year illness. A definitive diagnosis of early-onset (defined as onset <65 years) AD was only made post-mortem after neuropathological investigation of her brain revealed Aβ immunoreactive senile plaques (classified as CERAD frequent), many of which were of the cotton wool type (Fig. 2A), together with severe neurofibrillary tangle pathology (Braak and Braak stage VI) (11) (Fig. 2B). Her daughter (III.2, Fig. 1) presented at the age of 27 with a very different phenotype consisting of a progressive cerebellar syndrome, with spastic paraparesis and dementia, which suggested a different aetiology to her mother’s disease; she died 12 years after diagnosis. Autopsy was not performed on patient III.2.

During the clinical investigation of the daughter III.2, sequencing of the PSEN1 gene was undertaken and revealed an exon 12 P436Q mutation (g. 71111C > A) in peripheral lymphocyte DNA (12). No other sequence variation was detected in any of the other AD-associated genes (1–3), and prion protein (PRNP) genotyping was negative. Testing of both mother (II.1) and daughter (III.2) by direct sequencing and allele-specific oligonucleotide hybridization (ASOH) demonstrated an unexpected disproportionate ratio of mutant to wild-type signals. We were unable to detect the mutant allele by direct sequencing using peripheral lymphocyte DNA extracted from whole blood, from II.1, the mother and our index patient (Fig. 3C); ASOH using the same DNA revealed a markedly reduced signal for the mutant allele (Fig. 4). Similar tests for the daughter III.2 demonstrated the expected heterozygous patterns of wild-type to mutant signal (Figs 3D and 4). We then investigated, by haplotype analysis using allele-specific PCR (asPCR) of peripheral lymphocyte DNA and DNA isolated from cerebral cortex, whether the disproportionate signals from individual II.1, in conjunction with the distinct clinical presentation in her daughter (III.2), were consistent with a somatic mutation. We performed pre-symptomatic genetic screening for individuals III.1 and III.4, as part of our molecular diagnostic service, which demonstrated that they were negative for the P436Q mutation (Fig. 3A and B). Individual III.3 did not seek pre-symptomatic genetic testing.

Heterozygosity at a single nucleotide polymorphism (SNP) position flanking the mutation in II.1 (see Materials and Methods and Fig. 5A) allowed us to perform PCR using all combinations of two allele-specific forward primers, defining mutant and wild-type alleles, and two allele-specific reverse primers to allow identification of all four potential haplotypes (double asPCR) (Fig. 5A). The demonstration of two wild-type alleles and a third mutant allele in II.1 in different tissues indicates somatic mosaicism in this individual (Fig. 5B). The unaffected siblings inherited their mother’s CT progenitor allele, whereas individual III.2 inherited the mutant AT allele. Extended haplotype analysis including data from six microsatellite markers, in addition to the allele-specific PCR data, was performed (Fig. 5C). All offspring, both affected (III.2) and unaffected (III.1, III.4), inherit the same microsatellite haplotype from individual II.1. These data, combined with the SNP haplotypes, are consistent with mosaicism in individual II.1, with the P436Q mutation on the progenitor A haplotype background generating haplotype A1. Although sensitivity of DNA sequencing, compared with ASOH, did not allow for the detection of the mutant allele in DNA from peripheral lymphocytes, it was demonstrable using DNA extracted from cerebral cortex of II.1 (Fig. 3E) at the limits of detection suggesting differing degrees of mosaicism between these two tissues.

In addition to quantitative phosphorimaging of ASOH products, a new application for allele-specific minor groove binder (MGB) probes (13) was employed. An increase in the cycle threshold difference [delta (Δ)] between a Q436 specific probe and a reference probe specific for M129 of the human PRNP gene was detected in DNA from both tissues of individual II.1 (cerebral cortex and peripheral lymphocytes) compared with the heterozygote offspring III.2. Using the comparative Ct method (14) (ΔΔCt), the estimated degrees of mosaicism in peripheral lymphocytes and cerebral cortex were 7.7% (ASOH 10.5%) and 14.4% (ASOH 16.5%) of the locus, respectively (Fig. 6).

DISCUSSION

This is the first description of somatic and germline mosaicism in AD; our index patient II.1 was negative for mutations in the
PSEN1 gene when DNA extracted from her peripheral blood sample was sequenced; however, the P436Q mutation was detected after direct sequencing of DNA extracted from her cerebral cortex, indicating that the mother was a somatic mosaic for this mutation. This was confirmed by detailed haplotype analysis; in particular, combined microsatellite and SNP haplotype data demonstrate that the affected daughter (III.2) inherited the mutant haplotype designated A/C3, whereas her two unaffected siblings (III.1, III.4) inherited the progenitor haplotype A from the mother, which differs only by the presence of the P436Q missense mutation on haplotype A/C3.

This is an important finding as it may be that many patients with AD harbour causative pathogenic somatic mutations expressed in neural tissue, that are undetectable on conventional laboratory examination of DNA extracted from peripheral blood lymphocytes. Our findings also demonstrate that the mother (II.1) is likely to be both a germline and somatic mosaic, and thus we were able to detect the P436Q mutation in DNA extracted from her daughter’s blood as the latter was a heterozygote. Germ cell lineage is established during gastrulation, as is the trilaminar embryo. The central nervous system is derived from the ectoderm whereas hemopoietic stem cells are derived from the mesoderm. Therefore the mutation must have occurred before gastrulation, perhaps within the inner cell mass of the blastocyst or at some time prior to the second week of embryogenesis (15). Tissue derived from the endodermal germ layer was unavailable for analysis.

The P436Q mutation was originally described in an Australian family (16). Despite clinical similarities observed in the affected individual in their pedigree and III.2, the genealogical data available for our pedigree suggests two separate mutation events and implicates a de novo somatic and germline mutation in individual II.1 (17). The parents of individual II.1 died at the age of 73 (I.1) and 37 (I.2), without any reported signs of dementia; the latter from myocardial infarction. I.2 had six siblings, none of whom had dementia. In addition, the four living siblings of II.1 are as yet unaffected in their sixth decade, one sibling died at birth (II.7) and the other at the age of 35 in an accident (II.6).

More than 100 pathogenic mutations of PSEN1 have been reported in early-onset AD [PSEN1 mutations database (2004): http://molgen-www.uia.ac.be/ADmutations], and the majority of these exist around putative transmembrane domains of the presenilin 1 protein suggesting functional mutation hotspots (18). Evidence of mechanistic mutational hotspots have also been reported for other genes, including the E200K mutation in PRNP, where this variation has been shown to have occurred in at least four separate occasions (19). Utilizing the presence of such hotspots for demonstrating mosaicism in the diagnosis of apparently sporadic disease would be greatly facilitated by the use of specific assays such as 5' nuclease MGB probe allelic discrimination, as described here. However, screening of families with no family history of neurological disease, or those with a history that does not follow a Mendelian pattern, would be best approached by the sequencing of putative genes using DNA isolated from post-mortem brain tissue rather than peripheral lymphocytes. This is unlikely to be feasible in routine clinical genetic diagnostics, but will be important to ascertain on a
research basis. Determining the frequency of cases of sporadic early- or late-onset AD that may be accounted for by somatic mosaicism will inevitably depend upon the ability to detect such mutations, the availability of suitable post-mortem tissue and the patient selection criteria employed. In addition, apparently autosomal recessive AD may also be due to germline mosaicism in either parent and recurrence risk will be dependent on the level of this mosaicism.

In individual II.1 we describe degrees of mosaicism of 8% in peripheral lymphocytes and 14% in cerebral cortex, significantly delaying onset of disease in the index patient, and resulting in a quite different phenotype from her daughter III.2. Assuming that the mosaicism of the P436Q mutation in PSEN1 accounts for this marked phenotypic variation and disease severity (8,9), extrapolation of our data suggests that very low levels of mosaicism could result in manifestation of clinical symptoms of disease within the expected lifetime of a patient. Therefore a proportion of late-onset AD, and other age-related neurodegenerative conditions, could be accounted for by this mechanism. The demonstration of a clear PSEN1 gene dosage effect in disease pathogenesis and phenotypic expression may also have relevance to therapeutic strategies in AD.

**MATERIALS AND METHODS**

This work has the approval of the Institute of Neurology/National Hospital for Neurology and Neurosurgery Research Ethics Committee and the family of the index patient.

To avoid cross-contamination from the index patient II.1, DNA was extracted from two separate blood samples taken at different times, and a single cerebral cortex sample, in different laboratories. The P436Q mutation has been described previously in II.1 (12). Replicates from both tissues of II.1 tested implicate somatic mosaicism as described, while all control individuals and unaffected family members tested, using various assays, never demonstrated the P436Q mutation.
DNA sequencing and SNP characterization

DNA was extracted from peripheral blood lymphocytes. Sequencing was performed in both directions by the dideoxy chain terminator method using an Applied Biosystems (ABI) Prism 377 DNA sequencer. Specifically, for the mosaic individual, oligonucleotide primers were designed to amplify sequence flanking the P436Q mutation. Amplicons were sequenced to identify positions of heterozygosity.

Haplotype analysis

Oligonucleotide primers were designed to perform asPCR (20) between the P436Q (CCA→CAA) transversion in exon 12 of PSEN1 (g. 71111C>A) and the SNP 1244 bp 3' (dbSNP 165935). Allele frequencies for the SNP for 114 CEPH chromosomes were C = 0.53, T = 0.47. The integrity of all allele-specific amplifications were confirmed by duplexing with control oligonucleotide primers specifically targeted at intron 11 of the PSEN1 gene. Primer sequences and amplification conditions are available on request. Extended haplotype analysis was performed using previously characterized microsatellite markers D14S268, D14S620, D14S1028, D14S77, D14S1004 and D14S1025. Details of PCR fragment size range, number of alleles, primer sequences and degree of heterozygosity are available at http://www.genlink.wustl.edu/genethon_frame/chr14.html. Fluorescently labelled PCR products were electrophoresed on an Applied Biosystems (ABI) Prism 377 DNA sequencer, and data analysis performed using Genescan software.

Quantification by allelic discrimination using the 5′ nuclease assay in conjunction with MGB probes

MGB probes (ABI) were designed to detect the M129 allele of the PRNP gene and the mutant Q436 allele of the PSEN1 gene. An ABI Prism 7000 Sequence Detection System was used to perform relative quantification of the Q436 allele in the mosaic individual using the ΔΔCt method. This assumed that the affected offspring (III.2) of our mosaic individual (II.1) is heterozygous for the Q436 allele. Both the mosaic individual (II.1) and the affected offspring (III.2) were M129V PRNP genotype and therefore no correction for M129 allele copy number was necessary. Standard curves (data not shown) for individual III.2 showed that both probes had gradients within 0.1 of each other with least squares fit correlation coefficients greater than 0.99, demonstrating that ΔCt was constant relative to DNA quantity over the dynamic range of the experiment and satisfying criteria for the comparative Ct method of quantification. The probes used showed >100-fold specificity, and dilution experiments (data not shown) indicated that amplification of the P436 allele did not prove to be limiting on Q436 probe fluorescence.

Quantification using allele-specific oligonucleotide hybridization

ASOH was performed as previously described (21). Quantification was performed using a STORM 840 phosphorimager (Molecular Dynamics). Q436 and P436 probe signals of the mosaic individual (II.1) were normalized against signals of affected (III.2) and unaffected offspring (III.1, III.4).

Figure 6. (A–C) Real-time PCR replicate results using an internal control probe (M129 PRNP, blues) and Q436 PSEN1 (reds) for individuals II.1 and III.2, showing cycle number against change in fluorescence (ΔRn). ΔCt values are as follows: (A) ΔCtIII.2 = 1.82, (B) ΔCtII.1CC = 3.62, (C) ΔCtIII.1 = 4.52. Increase in ΔCt when compared with heterozygote (Q436 = 50% of locus) individual II.1 are: ΔΔCtIII.2 = 4.52 – 1.82 = 2.7 (1/2)2.7 = 0.154; therefore, Q436 = 7.7% of locus. ΔΔCtII.1 = 3.62 – 1.82 = 1.8 (1/2)1.8 = 0.287; therefore, Q436 = 14.4% of locus (PL = peripheral lymphocytes, CC = cerebral cortex).
ACKNOWLEDGEMENTS

We would like to thank Dr Sarah Lloyd, Professor Elizabeth Fisher and Dr Simon Mead for useful comments on the manuscript. We also thank the family for allowing us to undertake this study. We are grateful to Professor Martin Rossor who studied the family via an MRC Programme Grant No. G9626876, and referred the patients to the Neurogenetics Clinic. This work was funded by the UK Medical Research Council. S.J.T. is a UK Department of Health National Clinician Scientist.

REFERENCES


14. ABI Prism 7700 sequence detection system user bulletin #2 relative quantitation of gene expression P/N 4303859B stock no.777802-002 http://docs.appliedbiosystems.com/search.taf


