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Clinical and radiographic features of a family with hypochondroplasia owing to a novel Asn540Ser mutation in the fibroblast growth factor receptor 3 gene

EDITOR—Hypochondroplasia is a mild, autosomal dominant skeletal dysplasia. The relative dearth of specific clinical manifestations and the absence of pathognomonic radiographic features often make the diagnosis of hypochondroplasia difficult.[1–4] Short limbed dwarfism is rarely recognised before the age of 2 years and is usually mild with heights up to the low normal range. Muscular body build, macrocephaly with mild frontal bossing, and lumbar hyperlordosis are frequently reported. The radiographic features are variable and can be almost normal in mildly affected subjects.[4] They most typically include no change or decrease in the interpedicular distance from the first to the fifth lumbar vertebral bodies, anteroposterior shortening of the lumbar pedicles, short iliac bones with flat acetabular roof, small sacrosciatic notches, short tubular bones, short and broad femoral necks, and relative elongation of the distal or proximal portion of the fibula.[2 4] Proof that achondroplasia and hypochondroplasia are allelic disorders came with the discovery that both conditions map to the distal short arm of chromosome 4.[5 6] Subsequently,

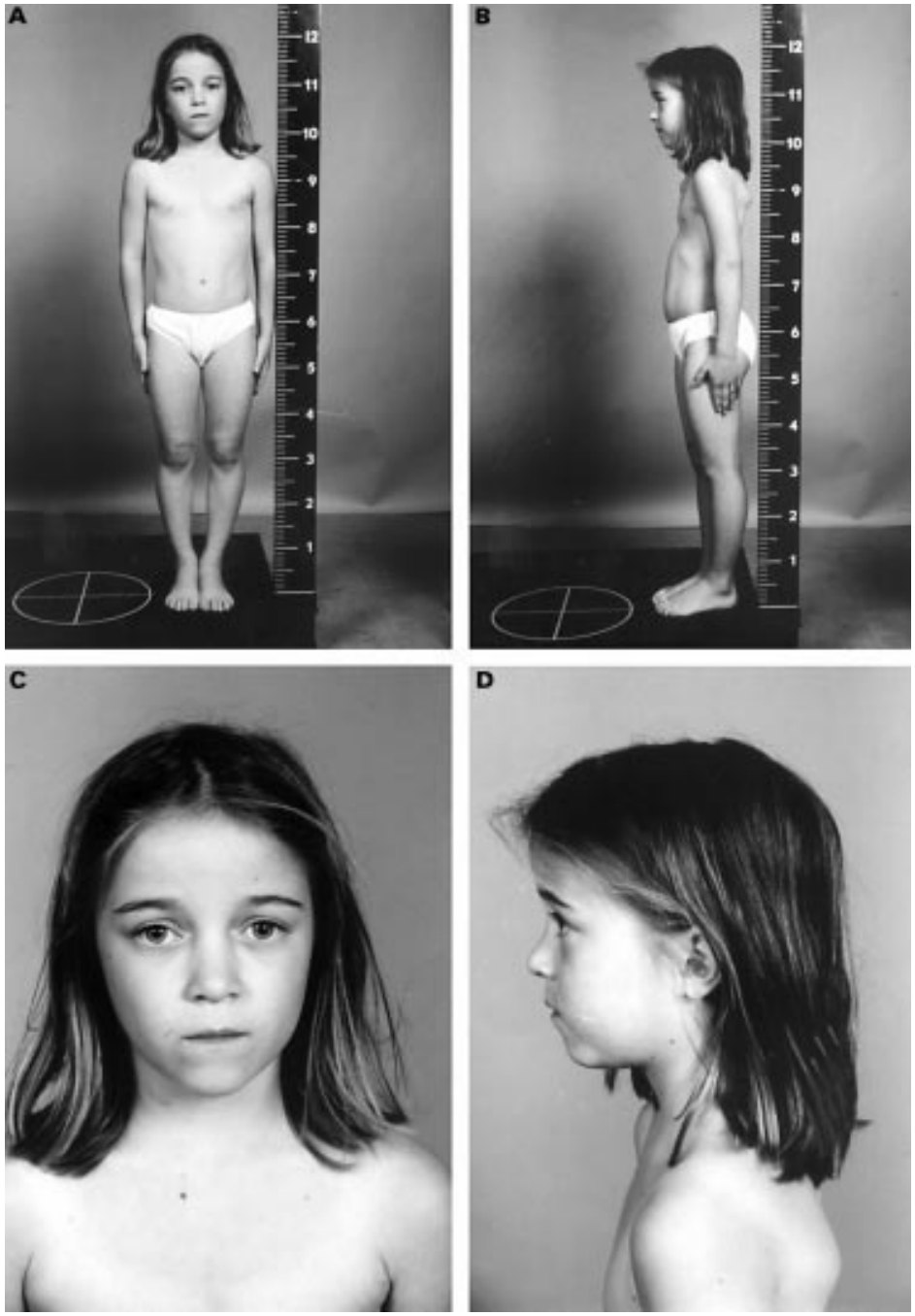


Figure 1 The proband at 8 years 9 months of age. Front and side views illustrate prominent forehead, low nasal bridge, anteroposteriorly flattened thorax, and lumbar hyperlordosis.

mutation analysis of the *FGFR3* gene, located in the 4p16.3 region, showed a recurrent mutation (N540K) in several unrelated hypochondroplasia patients.[7 8] Recently, two novel mutations in the same region of the *FGFR3* gene causing hypochondroplasia have been identified: N540T in a Dutch family and I538V in a Swedish kindred.[9 10] In some sporadic patients and families with clinical or radiographic features of hypochondroplasia, a causal involvement of the *FGFR3* gene has been ruled out, suggesting locus heterogeneity.[11–14]

We report here a novel N540S mutation in the *FGFR3* gene and provide evidence that this mutation causes hypochondroplasia in a Belgian family. The proband is an 8 year old girl referred to the paediatric endocrinologist because of short stature. She was born after an uncomplicated pregnancy to young and non-consanguineous Belgian parents. Birth weight was 3350 g (50th centile) and length 49 cm (25th-50th centile). Psychomotor development was normal. She presented at the age of 8 years 9 months with mild disproportionate short stature. Anthropometric mea-

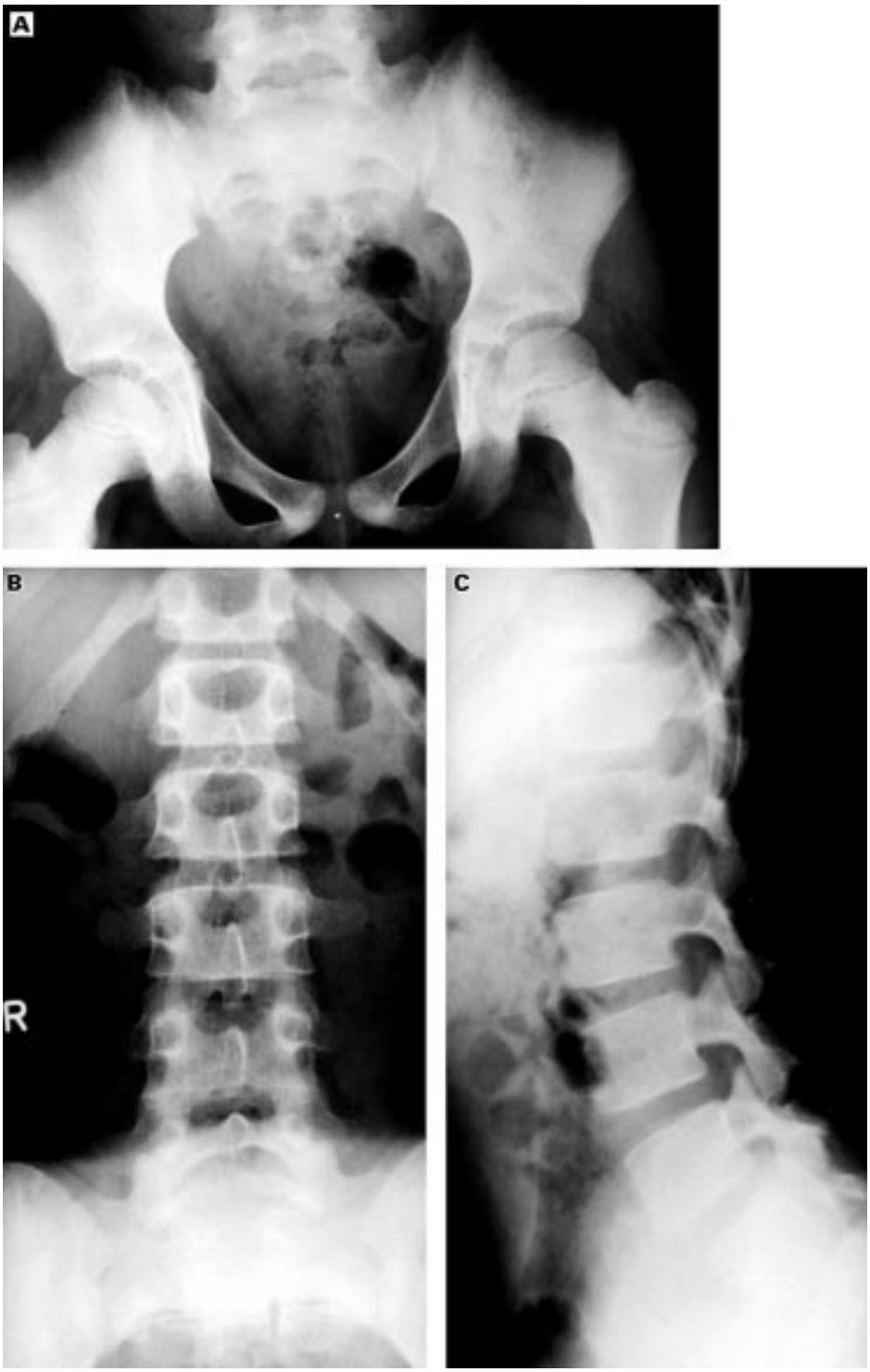


Figure 2 Radiographs of the spine and pelvis in the proband at the age of 8 years 9 months. (A) The pelvis is normal. (B) Anteroposterior view of the lumbar spine shows minimal increase in interpedicular distance from the first to fifth lumbar vertebra. (C) Lateral view of the lumbosacral spine illustrates mild anteroposterior shortening of the lumbar pedicles and accentuated lumbar lordosis with the sacrum tilted more horizontally.

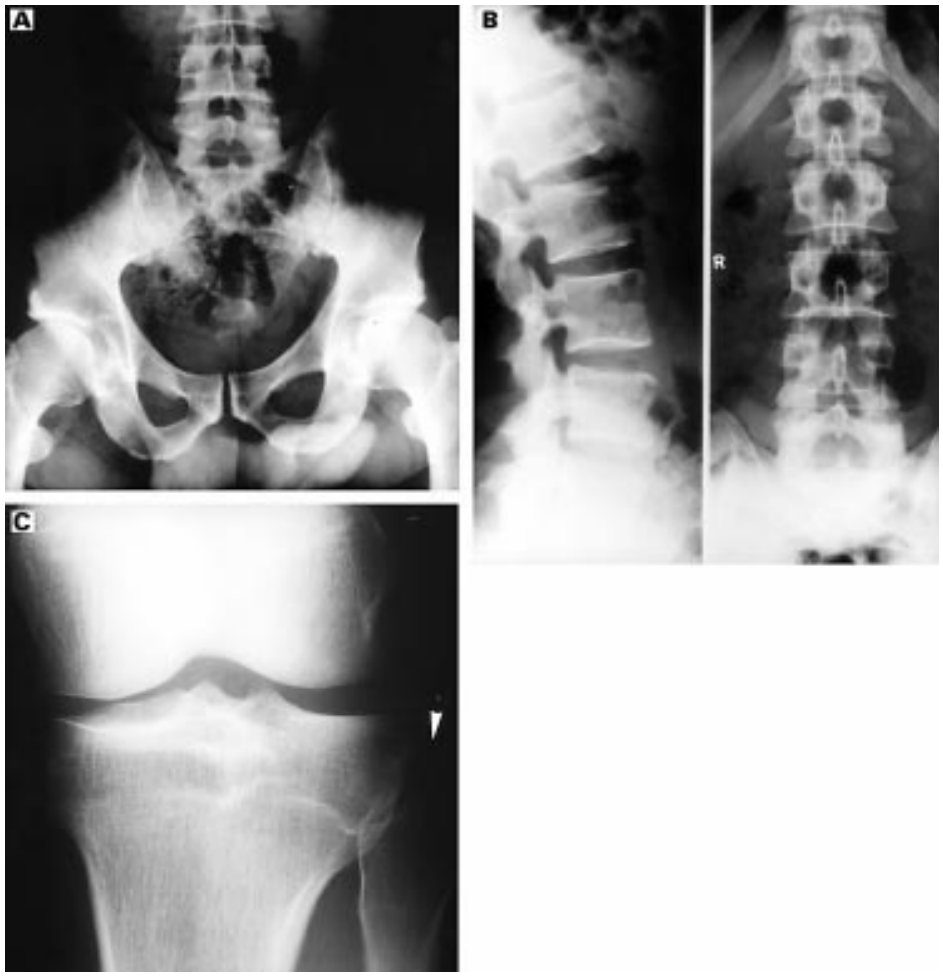
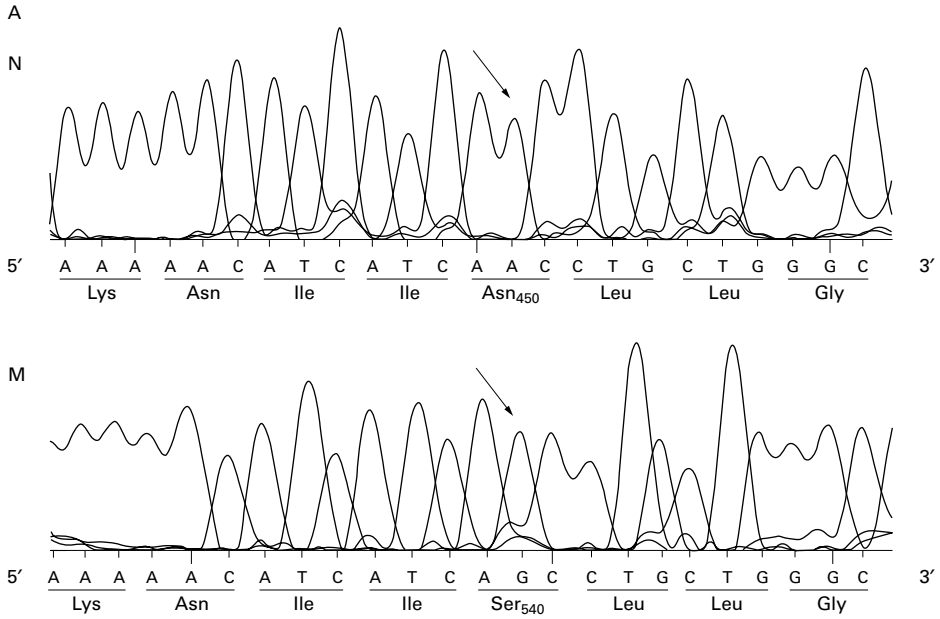


Figure 3 Radiographs of the spine, pelvis, and knees in the father aged 37 years. (A) Short femoral necks on the frontal radiographs of the pelvis. (hips rotated outwards are shown) (B) Normal increase of interpedicular distances in lumbar spine. Anteroposterior shortening of the lumbar pedicles and vertebral bodies. (C) Elongation of the proximal end of the left fibula (top shown by arrow).

surements showed a height of 120.1 cm (3rd centile=123 cm), weight 23.8 kg (3rd centile), head circumference 52.5 cm (50th-75th centile), span 120 cm, lower segment 58 cm (upper to lower segment ratio 1.07), hand length 13.2 cm (3rd centile), and foot length 18 cm (3rd centile=18.7 cm). In addition, a prominent forehead, low nasal bridge, anteroposteriorly flattened thorax, and lumbar hyperlordosis were found on physical examination (fig 1). Radiographic study of the skeleton showed mild shortening of the tubular bones, minimal increase in lumbar interpedicular distance, anteroposterior shortening of the lumbar pedicles and vertebral bodies, and accentuated lumbar lordosis with horizontally tilted sacrum (fig 2). The 37 year old father is also short with a height of 167.9 cm (3rd-25th centile), head circumference 60.3 cm (98th centile=58 cm), span 175 cm, and lower segment 82 cm (upper to lower segment ratio 1.05). His clinical phenotype is characterised by macrocephaly with a prominent forehead, low nasal bridge, muscular build, and broad thorax. Radiographs of the skeleton showed mild shortening of the tubular bones, increase in lumbar interpedicular distance, anteroposterior shortening of the lumbar pedicles and vertebral bodies, long proximal portion of the fibula, and remarkably short femoral necks (fig 3).

Because the clinical and radiographic features in both father and daughter suggested the diagnosis of hypochondroplasia, sequence analysis of the tyrosine kinase I domain of the *FGFR3* gene was performed. Genomic DNA was extracted from peripheral blood leucocytes by the Qiagen-Blood miniprep kit (Qiagen Inc, Chatworth, CA). Oligonucleotide primers and PCR conditions for amplification of exon 11 and part of exon 12 were used as previously described.[8] The amplified DNA fragments were cloned using the TA cloning kit (Invitrogen) and sequenced. This analysis showed heterozygosity for an A to G transition in both patients, resulting in substitution of serine for asparagine at position 540 (N540S) of the *FGFR3* protein (fig 4). This nucleotide sequence change creates a cleavage site for the restriction endonuclease *Mwo*I. Restriction analysis of amplified genomic DNA fragments confirmed that both patients were heterozygous for the mutation and that neither unaffected family members nor any of a panel of 100 unrelated, healthy controls carried the nucleotide change (data not shown).

Both subjects are mildly but variably affected. The radiographic changes in the daughter are subtle whereas the father, with a height in the low normal range, shows convincing radiological features of hypochondroplasia. It is highly likely that this mutation is responsible for hypochondroplasia based on the following strong arguments. First, the mutation is not present in the unaffected family members or in 100 unrelated, healthy controls. Second, the mutation resides in a highly conserved region when comparing all four human *FGFR*s.[15] Third, the nucleotide change implies the replacement of the same amino acid as in the common N540K mutation, which has been clearly established to cause hypochondroplasia. Fourth, substitution of the same asparagine by threonine, a neutral and



B

	538	540	
5′ AAA AAC ATC ATC AAC CTG CTG GGC 3′			
Lys Asn Ile Ile Asn Leu Leu Gly			
		AAG	Asn540Lys (N540K)
		AAA	Asn540Lys (N540K)
		ACC	Asn540Thr (N540T)
	GTC		Ile538Val (I538V)
		AGC	Asn540Ser (N540S)

Figure 4 (A) Adenine to guanine transition in codon 540 of the FGFR3 gene in the proband. Partial nucleotide sequence of the normal (N) and mutant (M) allele is shown. The nucleotide change was found in three of seven clones. (B) Partial nucleotide sequence of the tyrosine kinase I domain of the FGFR3 gene related to the Asn540Ser and other mutations known to cause hypochondroplasia.

polar amino acid similar to serine, has been reported in hypochondroplasia.[9]

The identification of yet another novel mutation, resulting in the substitution of asparagine in position 540 of the FGFR3 protein and with hypochondroplasia as the phenotype, emphasises the important role of this specific site of the tyrosine kinase I domain in the pathogenesis of the disorder. Therefore, in patients with clinical/radiographic features of hypochondroplasia in whom restriction analysis or mutation detection methods do not show the presence of the common N540K mutation, sequence analysis of the tyrosine kinase I domain of the *FGFR3* gene should be performed to exclude other nucleotide changes in that region.

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Screening British CADASIL families for mutations in the *NOTCH3* gene

EDITOR—CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy) is a hereditary form of multi-infarct vascular dementia.[1] Clinical symptoms often present in middle adult life (30-50 years of age) and include recurrent subcortical ischaemic strokes, migraine with or without aura, major psychiatric symptoms, and dementia. Magnetic resonance imaging (MRI) shows high intensity signal lesions, often confluent, and areas of cystic degeneration of the subcortical white matter and basal ganglia. Pathological examination shows multiple, small, deep cerebral infarcts, leucoencephalopathy, and a non-atherosclerotic, non-amyloid angiopathy involving mainly the small, deep, perforating cerebral arterioles. Severe alterations of vascular smooth muscle cells are evident on ultrastructural analysis.

The term CADASIL was adopted after linkage of French families with these symptoms to chromosome 19,[2 3] but families with many of the features of CADASIL had been described by Worster-Drought *et al*[4 5] in the 1930s as familial presenile dementia with spastic paralysis, by Sourander and Walinder[6] as hereditary multi-infarct dementia, and by Stevens *et al*[7] as chronic familial vascular encephalopathy. In 1996, the responsible gene was identified as *NOTCH3*,[8] a member of the Notch family of signalling proteins originally identified in *Drosophila*.[9]

Notch and Notch homologues control the ability of non-terminally differentiated cells to respond to differentiation/proliferation signals through local cell interactions.[10] They are transmembrane proteins with distinct extracellular and intracellular domains. Notch is activated by binding of a ligand to the extracellular so-called epidermal growth factor (EGF) repeats.[11] This is thought to release the intracellular domain which translocates to the nucleus to regulate the transcription of genes that ultimately determine cell fate.[12 13]

To date, 26 separate mutations have been found in *NOTCH3*, 24 as described by Joutel *et al*[14] in the French families and two additional mutations from American families, as reported by Meeks *et al*.[15] Twelve of these mutations are clustered in exon 4. All of these mutations predict the introduction or replacement of cysteine residues in the extracellular EGF repeat domain. CADASIL has also been reported in Dutch,[16] German,[17] Swiss,[18] Italian,[19 20] American,[21 22] and Japanese families.[23] We report here the results of linkage analysis and screening for mutations in British families with a diagnosis of CADASIL.

DNA was available from four multiplex families from the central belt of Scotland, all of Scottish ancestry, a family from south west England of English ancestry, and one further isolated subject from Scotland with suspected CADASIL. We classified the diagnosis of CADASIL into definite, when there was neuropathological confirmation or evidence of genetic linkage to chromosome 19p12 or both, and probable, where there were clear clinical symptoms and MRI findings typical of CADASIL plus a positive family history. The clinical-demographic details and results described in the text below are summarised in table 1. In spite of extensive genealogical investigations we were unable to find a common ancestor for any of the families described.

Families 1 and 2, both Scottish, had sufficient meioses available to perform analysis of genetic linkage to chromosome 19p12. We genotyped the families on a Perkin Elmer Applied Biosystems (PE ABI) 377 automated genotyper with 5' FAM labelled primers for six polymorphic microsatellite markers, D19S226, D19S411, D19S885, D19S199, D19S923, and D19S841 flanking the *NOTCH3* gene (GDB accession numbers 188569, 199752, 608544, 182271, 611676, and 593357, respectively), and analysed the results using Perkin Elmer Genescan software (version 2.1).

Clear genetic linkage to chromosome 19p12 was found in both families. Family 2 was originally reported not to be linked to chromosome 19.[24] However, further characterisation of the phenotypes in these families by MRI scanning, which had not been performed at the time of the initial reporting, required us to reclassify several cases showing key recombinants. We also determined haplotypes at *NOTCH3* in the other multiplex families and found the same patterns of microsatellite allele sizes for families 3 and 4.

We then sequenced exon 4 of the *NOTCH3* gene (GDB accession number AF058883) in all our families, by automated sequencing of PCR products. After checking size and yield on 1% agarose gel electrophoresis, PCR products were purified using Centrikon™ columns. Purified PCR products were sequenced by automated cycle sequencing using PE ABI BigDye™ chemistry. The products were run on polyacrylamide gels on a PE ABI 377 automated sequencer and analysed using PE ABI Factura (2.0.1) and Sequence Navigator (1.1) software.

Table 1 Diagnostic details for families studied

	Diagnosis	No of cases	Neuropathology	Clinical symptoms	MRI	Family history	Linkage analysis	Mutations	
								Nucleotide	Amino acid
Family 1	Definite	19	Definite	1, 2, 3, 4	Typical	Yes	Pos	C583T	R171C
Family 2	Definite	21	Definite	1, 2, 3, 4	Typical	Yes	Pos	C475T	R135C
Family 3	Probable	2	NA	1, 3, 4	Typical	Yes	NA	C499T	R143C
Family 4	Probable	4	NA	1, 2, 3	Typical	Yes	NA	C499T	R143C
Family 5	Probable	3	NA	1, 3, 4	Typical	Yes	NA	C622T	R184C
Proband family 6	Probable	1	NA	1, 2	Typical	Yes	NA	C622T	R184C

NA: not available.
Clinical symptoms: (1) strokes, (2) psychiatric symptoms, (3) migraine with or without aura, (4) dementia.