A novel *Frabin* (FGD4) nonsense mutation p.R275X associated with phenotypic variability in CMT4H

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ABSTRACT

Background: Charcot Marie Tooth (CMT) disease is a heterogeneous group of inherited peripheral motor and sensory neuropathies. CMT4H is an early onset autosomal recessive demyelinating neuropathy. The locus responsible for CMT4H was assigned to chromosome 12p11.21-q13.11 by homozygosity mapping and mutations in the *Frabin* gene (*FGD4 Rho GDP/GTP* exchange factor) were subsequently identified in six families.

Methods: We sequenced the *Frabin* gene in a cohort of 12 UK CMT families with clinically defined autosomal recessive demyelinating neuropathy.

Results: We identified a novel homozygous *Frabin* p.R275X mutation in a family from Northern Ireland. The two affected cases in this family had a very slowly progressive neuropathy with both cases remaining ambulant into middle age. Examination of mRNA from lymphoblasts showed that this stop mutation caused very little nonsense mediated mRNA decay and the predominant mRNA species was the mutant form that is likely to be translated into a truncated protein.

Conclusions: This work extends the understanding of the pathogenesis of *Frabin* mutation-associated Charcot Marie Tooth (CMT) 4H and suggests that mutations in *Frabin* should also be considered in ambulant adults with CMT1. ***Neurology*® 2009;72:617–620**

GLOSSARY

AR = autosomal recessive; **CMT** = Charcot Marie Tooth; **MCV** = motor conduction velocity; **MRC** = Medical Research Council; **NMD** = nonsense mediated mRNA decay.

Charcot Marie Tooth (CMT) is clinically and genetically heterogeneous and forms the most common group of inherited neuromuscular disorders, with an estimated overall prevalence of 1 in 2,500 individuals.[1] The autosomal recessive (AR) CMT phenotype is usually more severe and has an earlier onset than dominant CMT.[2,3] The AR form may have additional clinical features such as scoliosis and cranial neuropathies that can give clinical clues to the genetic cause.[4]

There are at least 13 causative genes for ARCMT1 and this group is classified as CMT4.[5,6] The CMT4H gene was recently identified as the *Frabin* gene on chromosome 12[7] in six families with early onset demyelinating CMT.[8,9] *Frabin* is a GDP/GTP nucleotide exchange factor, a member of the Rho family of small GTP binding proteins, and plays a role in Cdc42-mediated cell shape changes.[8,9] The expression of mutant *Frabin* (M298R)[8] induced fewer microspikes in rat primary motoneurons and Schwann cells.[10] These data along with mRNA expression studies suggest a loss of function disease mechanism for this particular mutation.

The reported *Frabin* mutations cause a childhood onset moderately severe, progressive AR demyelinating CMT. There is associated scoliosis in two families and focally folded myelin on sural nerve biopsy.

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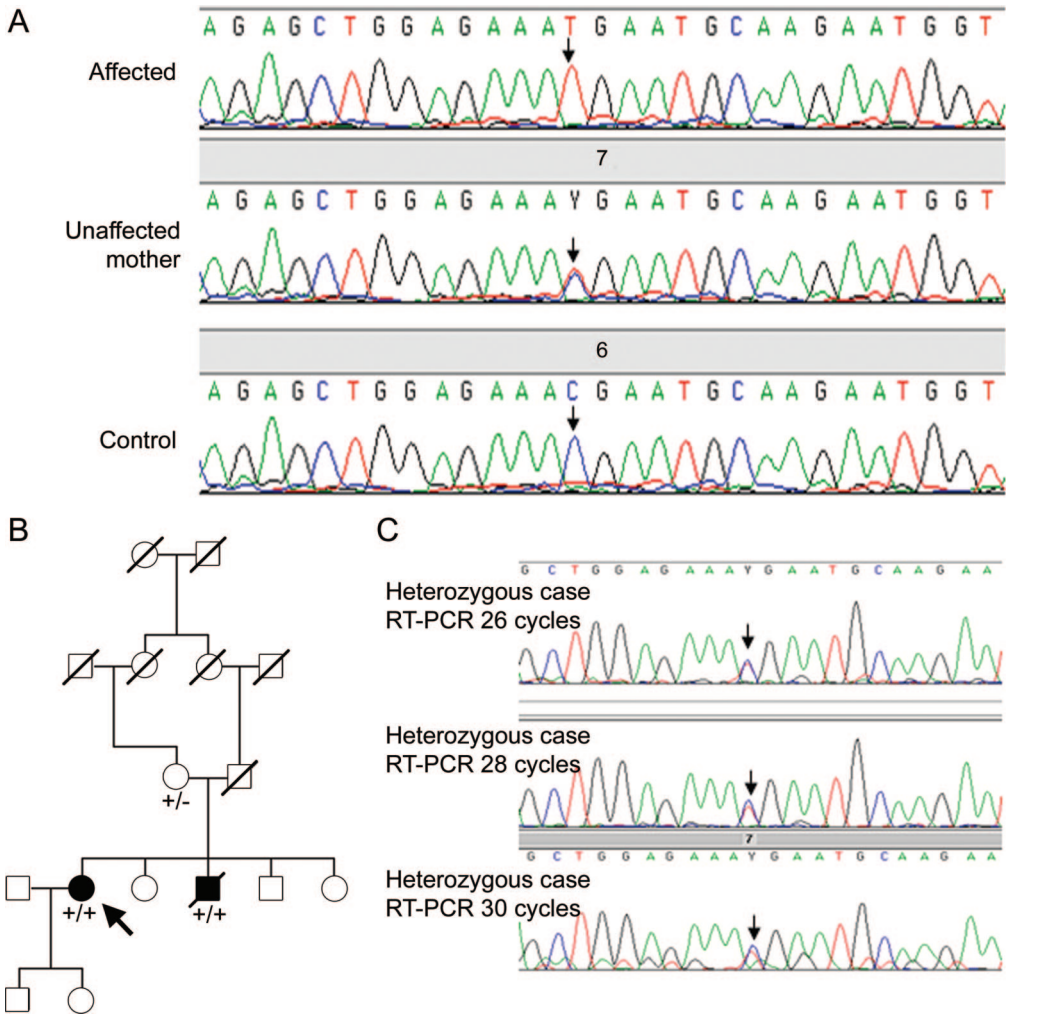
Figure 1 A homozygous p.R275X, c.723C>T defect in the *Frabin* gene



(A) Chromatogram of the *Frabin* homozygous affected patient, unaffected heterozygous mother, and control. The R275X (CGA to TGA) mutation is indicated by an arrow. (B) Family tree. (C) Sequencing of *Frabin* exon 6 to 8 RT-PCR of the heterozygous case at 26, 28, and 30 cycles. Mutation is indicated by an arrow. The mutation is clearly present and not subject to significant nonsense decay and there is very little difference among 26, 28, and 30 cycles.

To assess the frequency of *Frabin* mutations in our CMT cohort, we analyzed 12 families, identifying one mildly affected Belfast kindred with a homozygous mutation. The effect of this mutation on lymphoblast mRNA was analyzed to assess the mechanism and possible genotype-phenotype associations.

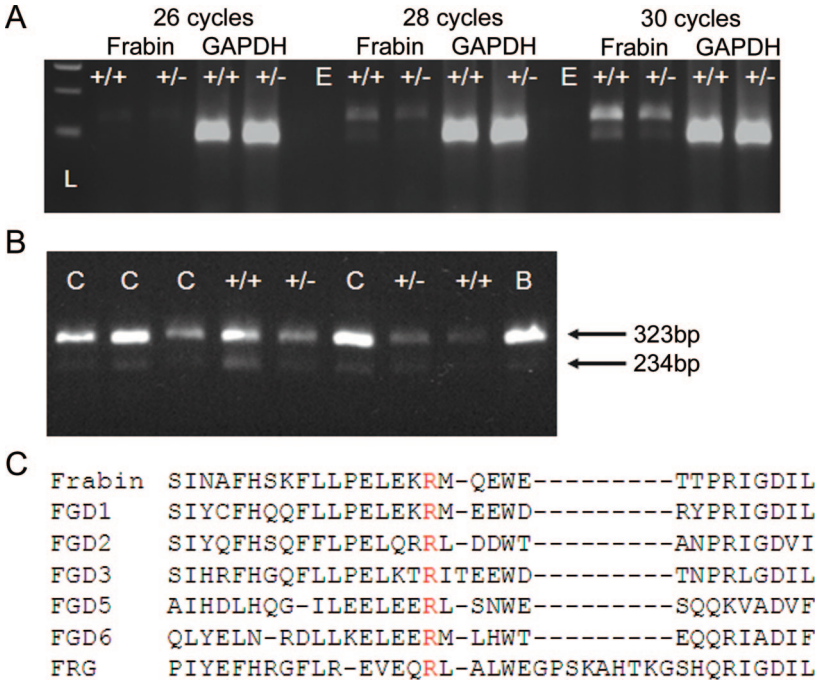
METHODS All patients gave informed consent and ethics approval was obtained from the ethics committee at The National Hospital for Neurology and Neurosurgery.

Molecular genetics. The *Frabin* gene was amplified and sequenced by standard methods, primers available on request. Mutations were confirmed by repeat sequencing where the p.R275X mutation was not identified in 190 control chromosomes.

Total RNA was isolated from blood lymphoblasts from a homozygous affected and a heterozygous unaffected individual by the Trizol procedure (Invitrogen) according to the manufacturer's instructions. Primers for *Frabin* were designed to span the introns around the mutation (introns 6 and 7); RT-PCR was amplified for 26, 28, and 30 cycles. Products were sequenced to determine the mutation and the ratio of the mutant base to the wild type base in the heterozygous carrier. RT-PCR reactions were carried out using GAPDH-primers as a housekeeper control gene. Equal volumes of these RT-PCR products were resolved on metaphor agarose gels and stained with SYBR green-1 and densitometry analysis of RT-PCR products was carried out.

RESULTS **Genetic analyses.** We identified a homozygous p.R275X, c.723C>T defect in the *Frabin* gene (figure 1, A and B). This stop mutation was present in the two affected individuals in the family (figure 1A) and heterozygous in the unaffected mother. The arginine at codon 275 was highly conserved (figure 2C). Analysis of lymphoblast mRNA from one affected patient and the unaffected mother showed that the mutation was present in mRNA and not decayed. In the heterozygous mother, the mutant

Figure 2 Genetic analysis of the *Frabin* gene



(A) *Frabin* RT-PCR 6F to 8R shows that there is no loss of density of the homozygous affected vs the unaffected heterozygous RNA expression of the *Frabin* gene. Paradoxically there is probably slightly greater total expression in the affected case. The *GAPDH* expression is not significantly different between the two cases. (B) *Frabin* 6F to 8R RT-PCR in affected, carrier, and controls. The top band is the *Frabin* transcript with exon 6, 7, and 8 and the lower band is exon 6 (5′ region spliced out), exon 7, and exon 8. This splicing was confirmed with sequencing and the same in affected and controls. C = control; +/+ = affected; +/− unaffected carrier; E = empty well; B = Brain cDNA. (C) Conservation of the mutated amino acid within other members of the *Rho* gene family as indicated in red.

T and the wild type C were both clearly seen at 26, 28, and 30 RT-PCR cycles (figure 1C). The T was not significantly below the C as compared to genomic sequencing. RT-PCR between exons 6 and 8 showed that the 5′ region of exon 6 and the first base of exon 7 (89 bp) was spliced in or out in controls and the family and this was confirmed by sequencing (figure 2A). Products were analyzed on an agarose gel and by peak areas ratios with *GAPDH* and no difference was observed between the PCR product of the affected patient, unaffected mother, and controls (figure 2A).

Clinical features and electrophysiology. The proband in the family (figure 1) was assessed at age 58. She first had problems obtaining comfortable shoes as a young child and she found it difficult to run and had poor balance. She worked in a public house and her CMT progressed very slowly with occasional cramps and paraesthesia in her feet in her 30s. In her 50s she developed greater weakness distally and worsening sensory symptoms in her limbs and she remains ambulant with one stick. Examination at the age of 58 shows pupil size asymmetry, pes cavus, and wasted small hand and foot muscles. Power was decreased in the hands (Medical Research Council [MRC] grade 4/5) and feet (ankle dorsiflexion and plantar flexion, MRC 4/5). She was areflexic with a glove and stocking sensory loss to the elbows and knees for pinprick and touch. Joint position sense was absent in the toes. Vibration sense was absent to the hips.

Motor conduction velocities (MCVs) (age 49) were slow in the upper limbs with left ulnar MCV 13.0 m/s (distal motor latency 9.0 m/s, amplitude 0.4 mV) and left median MCV 8 m/s (latency 8.4 m/s, amplitude 2.3 mV). There were no recordable sensory responses.

The patient's brother had a similar phenotype. As a child he was clumsy and tended to drop things. He stopped work as a television technician at age 36 years due to lack of fine coordination in his hands. By age 50 he used two crutches or a wheelchair to mobilize. At 40 years, his gait was clumsy and he was unable to stand on his heels. He had pes cavus, claw feet, wasting, and weakness distally in the limbs. He was areflexic with distal reduction in all modalities of sensation. Limited nerve conduction study carried out at age 22 of the right median nerve showed a velocity of 6 m/s and latency 12.7 m/s (compound muscle action potential not documented). EMG of tibialis anterior showed a striking neurogenic pattern.

DISCUSSION We report a further mutation in the *Frabin* gene that is associated with CMT. The mutation is a stop codon change (p.R275X, c.723C>T) segregating with the disease. The arginine 275 is highly conserved in species and other members of the Rho family.

The previous two reports on mutations in the *Frabin* gene[8,9] identified families with missense, premature stop as well as splice site mutations. The *Frabin* M298R mutation was shown to have an interesting mechanism; it is a splicing rather than a missense mutation and is predicted to result in a frameshift mutation (p.Met298fsX8), which explains the nonsense mediated mRNA decay (NMD) of the *Frabin* gene with this change.[8] Interestingly, there was still some residual mutant mRNA leftover (40% of control), suggesting some abnormal mutated protein was still present. Analysis of mRNA extracted from lymphoblasts in our family showed the mutation was clearly present in the cDNA on sequencing of the proband and the unaffected heterozygous mother; affected mRNA was not degraded at 26, 28, and 30 cycles as would be expected with NMD. These data indicate that a truncated protein may be translated; however, a rapid protein degradation and resulting haploinsufficiency cannot be excluded at this stage of the investigation.

The phenotype in our family was less severe than the previously published families.[8,9] This may be due to a genotype-phenotype effect although the position and type of the mutation is similar and in close proximity to three other reported stop mutations. Two other mutations causing a premature stop site (E543fs and G586X) are located toward the C-terminal end of the *Frabin* gene and these have a more severe phenotype.[9] This indicates that the length of the Frabin protein truncation has little effect on predicting the phenotype and suggesting that multiple domains of this protein are important for Cdc42-mediated cell shape changes. This report extends the understanding of the pathogenesis of *Frabin* mutation-associated CMT4H and suggests that mutations in *Frabin* should also be considered in ambulant adults with CMT1.

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