

SHORT REPORT

Missense mutation (R15W) of the connexin32 gene in a family with X chromosomal Charcot-Marie-Tooth neuropathy with only female family members affected

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Abstract

A small family with sensorimotor neuropathy of dominant inheritance was examined. All three affected members were female. They had unusually severe symptoms and pronounced reduction of motor nerve conduction velocities with absent sensory nerve action potentials. Molecular genetic analysis disclosed a missense mutation in the connexin32 gene in codon 15 (Arg15Trp) which predicts the replacement of a basic amino acid to a non-polar amino acid in the first cytoplasmic loop of the protein. This report illustrates that in small pedigrees in which only women are affected, and which show a severe clinical phenotype, X chromosomal Charcot-Marie-Tooth neuropathy should be considered as differential diagnosis.

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Charcot-Marie-Tooth syndrome is a clinically and genetically heterogeneous hereditary disorder of the peripheral nerves.¹ Slow motor nerve conduction velocities (NCVs) are characteristic for the autosomal dominant Charcot-Marie-Tooth disease type I. One of the genes mutated in Charcot-Marie-Tooth disease type I is located on chromosome 17 (17p11.2; CMT1A), a second one on chromosome 1 (1q22-q23; CMT1B). The X linked dominant form of Charcot-Marie-Tooth syndrome (CMTX) is caused by mutations in the connexin32 (Cx32) gene, mapped to Xq13, that encodes a gap junction protein.² Clinically CMTX is characterised by distal sensorimotor deficit with muscle atrophy. Male CMTX patients are usually more severely affected than CMT1 or female CMTX patients. Typical features of X linked dominant inheritance consist of the absence of male to male transmission of the trait and predominance of females among

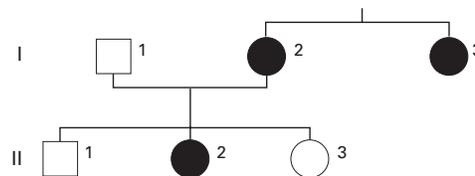


Figure 1 Pedigree of the proband.

affected members for all daughters of CMTX male patients carry the mutation. Since the identification of the Cx32 gene several mutations have been reported.³⁻⁸ We describe here a missense mutation in a Charcot-Marie-Tooth pedigree which was not initially suggestive for an X linked inheritance of the trait.

Case report

The index patient (II.2 in fig 1) presented at age 55 years. She developed pes cavus as a child and was bad at running and jumping at school. No progression of symptoms was noted, until at 33 years of age cramps occurred in her gastrocnemius muscles. She subsequently experienced burning pain in her forearms and feet which continued to be a major incapacitating factor. From about 35 years on, locking doors and opening bottles with her hands started to become difficult. Five years later tremor was noted. Progressive muscle wasting and increasing walking difficulties have been noticed since then. At the age of 37 she quit her job as an anaesthetist and took a position as a public health officer. By 47 she was substantially disabled and went on preterm pension.

Clinical examination disclosed severe distal muscle atrophy and paresis (small hand muscles: MRC grade 3, foot extensors: MRC grade 2), areflexia, and distal sensory loss for all modalities. Motor NCVs were profoundly slowed (median motor NCV 33 m/s), sensory nerve action potentials were absent. EMG showed axonal involvement. Sural nerve biopsy showed signs of chronic neuropathy with axon loss and demyelination degeneration.

The 90 year old mother has never complained of neuropathy symptoms. On examination, muscle strength of the small hand

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muscles and foot extensors was slightly reduced, vibration perception was impaired; only ankle reflexes were absent. Median motor NCV was 42 m/s, muscle action potentials after tibial and peroneal nerve stimulation were absent, sensory nerve action potentials could not be obtained.

The sister of the patient's mother has had pes cavus since childhood. She developed walking difficulty at age 57 and has been confined to a wheelchair since the age of 70. She lives in South Africa and was therefore not examined by us.

Both the brother and the sister of the patient are unaffected.

DNA ANALYSIS

Polymerase chain reaction, single strand conformation polymorphism, and direct sequencing of DNA were performed as reported previously.⁴ After the mutation was detected in the index patient, screening of family members was performed by restriction enzyme cleavage, as the mutation destroys a restriction site.

Results

Molecular genetic analysis by polymerase chain reaction amplification and direct sequencing of the only exon of the Cx32 gene identified a heterozygous C to T transition of the first nucleotide of codon 15 (fig 2) predicting an amino acid replacement (Arg15Trp). The mutation eliminates an Msp I restriction site present at this position in the wild type Cx32 allele. Fragment sizes in the wild type on digestion were 254 and 52 base pair (bp), whereas the corresponding fragment of the mutated gene was 306 bp large (fig 3; the 52 bp fragment is not visible). Restriction digest confirmed cosegregation of the mutation with the disease phenotype.

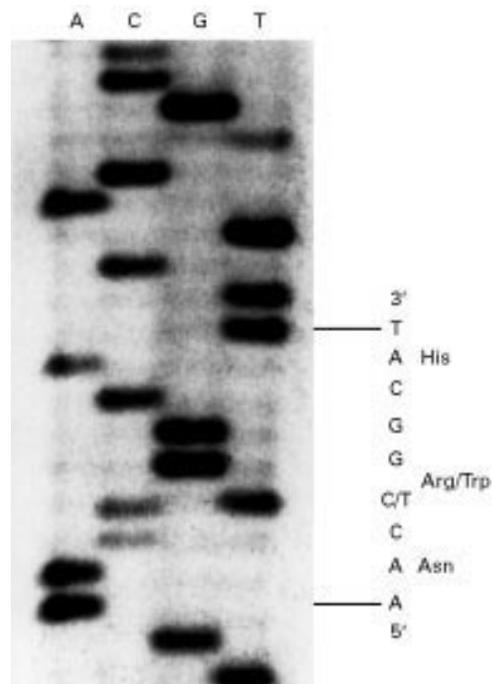


Figure 2 Direct sequencing of the human connexin32 gene of the index case (cut out).



Figure 3 Detection of the Arg15Trp mutation by Msp I digestion in the family members of the index case. Lane 1: I.1; lane 2: II.1; lane 3: II.2; lane 4: II.3. M: DNA molecular weight marker (ϕ X HaeIII digest). Numbers on the left indicate the size of digestion products in base pairs (bp). For details see text.

Discussion

Clinical features in CMTX are usually less severe in female patients than in affected males.^{9,10} Motor NCVs are reduced less in CMTX than in patients with CMT1, and affected females in CMTX show significantly less reduction in median motor NCV compared with related male patients.^{10,11} Yet, in the family presented here, although subject to intrafamilial variability, the disorder led to loss of independent ambulation and independency in performing everyday tasks in one case. Also, median motor NCVs were unusually slow. Non-random X chromosome inactivation, resulting in inactivity of the normal allele in a higher percentage of cells, might be the mechanism implicated in the determination of such atypically severe phenotypes. Ionasescu *et al* found similarly severe clinical deficit in an affected girl from a family with a 29 bp deletion in the 3'-end of the Cx32 gene.⁷ Therefore the family described here justified mutation screening in the Cx32 gene. The Cx32 point mutation detected in this family predicts the replacement of Arg15 in the first cytoplasmic loop (fig 4). In the mutant protein, a basic amino acid is substituted by a non-polar one, thus leading to a decrease in hydrophilicity of the protein surface. As both the same mutation and a different mutation of the same codon have already been identified in unrelated CMTX cases,⁸ the pathogenic nature of Arg15Trp seems to be very likely. It has been suggested that mutations located in this part of the protein may alter the channel function or interfere with gating stimulus control. Detecting further mutations and correlating them with the associated phenotype will be crucial to elucidate the underlying pathomechanism.

For the clinician it is of note that, although females with neuromuscular disease of X linked inheritance usually present, if at all, with minor symptoms and signs, in female patients with severe hereditary sensorimotor neuropathy, the X linked dominant form of Charcot-Marie-Tooth disease should be considered. If the most frequent cause of Charcot-Marie-Tooth disease, a large duplication on chromosome 17, is excluded by pedigree analysis (no dominant inheritance) or negative mutation screening result, also in pedigrees with affected women only search for mutations in the Cx32 gene may be worthwhile.

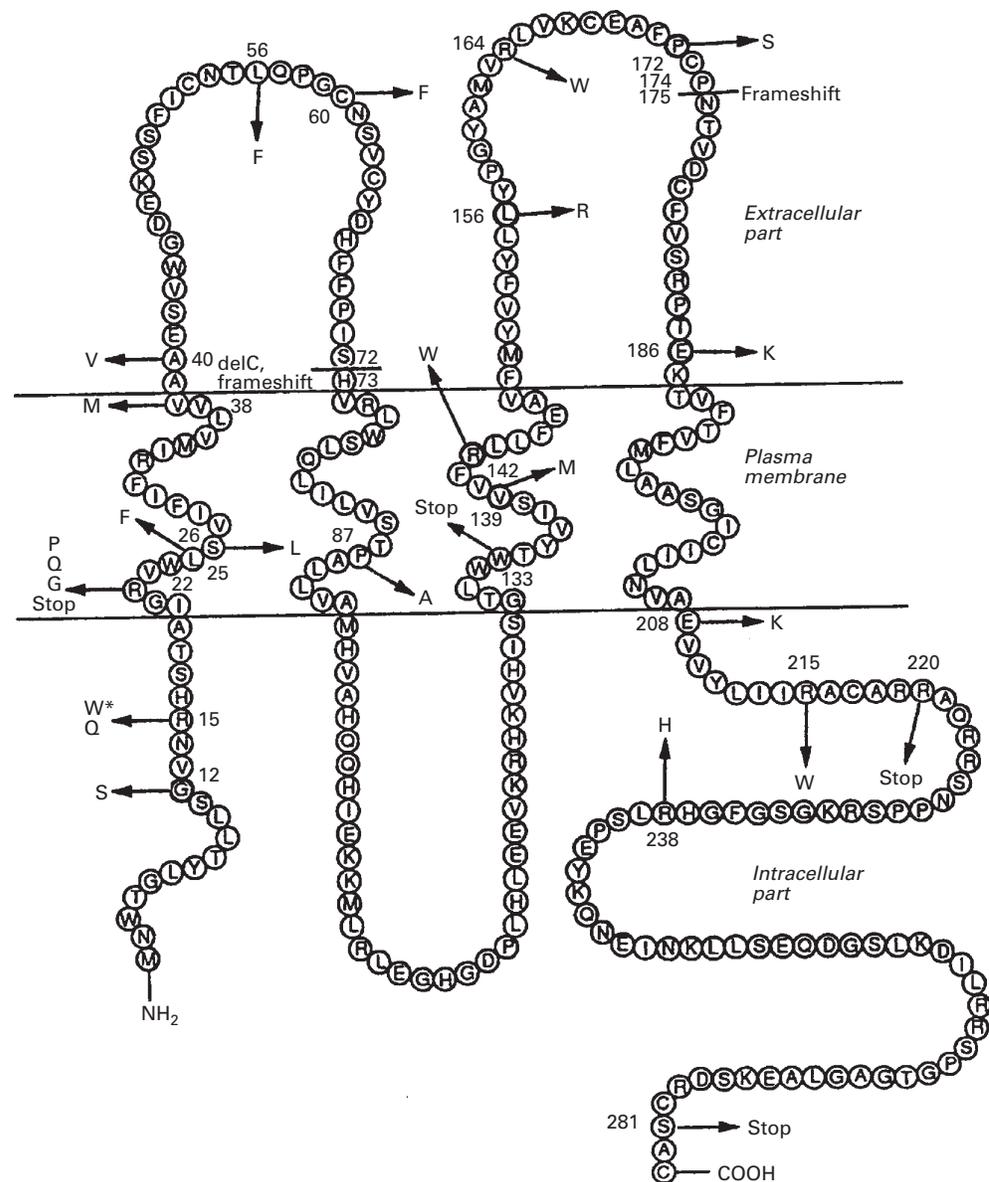


Figure 4 Model of the connexin32 protein with location and nature of mutations reported so far. The mutation present in the family described here is marked with *. A=alanine; R=arginine; N=asparagine; C=cysteine; Q=glutamine; G=glycine; H=histidine; I=isoleucine; L=leucine; K=lysine; M=methionine; F=phenylalanine; P=proline; S=serine; T=threonine; W=tryptophan; Y=tyrosine; V=valine. stop=nonsense mutation; ins=insertion; del=deletion.

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