The first de novo mutation of the connexin 32 gene associated with X linked Charcot-Marie-Tooth disease

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Abstract

X linked Charcot-Marie-Tooth disease (CMTX) is a hereditary motor and sensory neuropathy caused by mutations in the connexin 32 gene (Cx32). Using the SSCP technique and direct sequencing of PCR amplified genomic DNA fragments of the Cx32 gene from a Moroccan patient and her relatives, we identified the first de novo mutation of the Cx32 gene, consisting of a deletion of a G residue at position 499 in the Cx32 open reading frame. This previously unreported mutation produces a frameshift at position 147 in the protein and introduces a premature stop codon (TAG) at nucleotide 643, which results in the production of a truncated Cx32 molecule. This mutation illustrates the risk of an erroneous diagnosis of autosomal recessive CMT, especially in populations where consanguineous unions are frequent, and its consequences for genetic counselling, which can be avoided by molecular analysis.

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Charcot-Marie-Tooth disease (CMT) is the most common inherited disorder of the peripheral nervous system with an estimated frequency of 1/2500.[1] CMT is characterised by distal muscle weakness and amyotrophy, descreased or absent tendon reflexes, and highly arched feet.[2] CMT exhibits both clinical and genetic heterogeneity, with all forms of mendelian inheritance, autosomal dominant, autosomal recessive, or X linked. Sporadic cases of CMT have also been reported.[3] In X linked dominant CMT (CMTX), males are clinically more severely affected than females. Median nerve conduction velocities (MNCV) range between 30 and 40 m/s in affected males, while they are often above 40 m/s in heterozygous females.[4–7] These features and the absence of male to male transmission leads to the diagnosis of CMTX which is caused by mutations in the connexin 32 (Cx32) gene which maps to Xq13.[8–10]

We performed a molecular analysis of DNA from a female CMT patient from Morocco, presenting with intermediate motor nerve conduction velocities (MNCV), and her unaffected parents and sibs. A G499 deletion in the Cx32 gene was detected in the patient by non-radioactive single strand conformation polymorphism (SSCP) analysis, but was not present in the parents or sibs. This is the first described de novo mutation in the Cx32 gene.

The index case, a 17 year old girl, was referred to the Service of Neurology, Hôpital des Spécialités, Rabat, Morocco. From the age of 13, she suffered from progressive distal muscle weakness and wasting, predominantly in the lower limbs, with areflexia, pes cavus, and kyphoscoliosis. Motor nerve conduction velocities (MNCV) were slightly reduced at 37 and 38 m/s for the right and the left median nerve and 52 and 39 m/s for the ulnar and peroneal nerves. Compound muscle action potentials (CMAP) and sensory nerve action potentials (SNAP) were reduced. Her parents were not related. Clinical and electrophysiological examination of both parents and of her two brothers and two sisters were normal. After informed consent, a blood sample was taken from the seven family members and DNA was isolated using standard procedures.

The 5' portion of the Cx32 gene, corresponding to the 62 bases from the end of intron 1 to nucleotide 297, was amplified with primers Cx0 (CAC CCC AGC TTT CTG ACA GC) and Cx2 (TTG CTG GTG AGC CAC CTG CAT GGC), producing a 359 bp fragment. An intermediate (287 bp) and the 3' (303 bp) fragments of the Cx32 coding sequence were amplified by PCR using primers Cx4 (GGA GGT GAA GAG GCA CAA)/Cx5 (GAT GAT GAG GTA CAC CAC CT), and CxA1 (TGG CAG GTT GCC TGG TAT GT)/CxS1 (CGT CTT CAT GCT AGC TGC CTC TGG), respectively.[8] These PCR fragments were analysed by non-isotopic SSCP, according to Rouger *et al.*[11] The 287 bp Cx4/Cx5 PCR fragments were purified and directly sequenced, using *Taq* polymerase. The 5'3' and 3'5' strands were both analysed using the sense and antisense primers. The sequence was analysed using Sequence analysis 2.11, Factura, and Sequence Navigator 1.0.1 software.[11] To avoid errors through misincorporation of bases by *Taq* polymerase, all experiments were performed twice. The 499delG mutation leads to the loss of a *Hae*III restriction site in the 287 bp Cx4/Cx5 PCR fragment. *Hae*III cuts twice in the wild type sequence resulting in three bands of 180, 86, and 21 bp (not visible on gels), and only once in the mutated sequence (two bands of 265 and 21 bp). In order to detect false paternity,

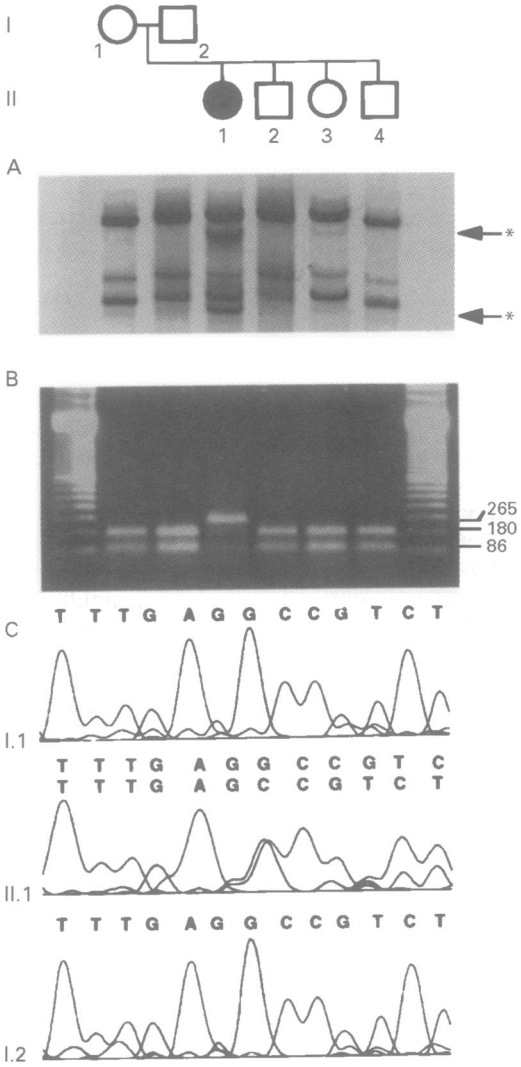

Figure 1 Connexin 32 gene analysis. (A) SSCP analysis of the 287 bp PCR fragment showed an abnormal profile for the proband II.1, but not for her parents or for her two brothers and two sisters. The abnormal bands are indicated by an arrow. (B) HaeIII restriction analysis of the 287 bp PCR fragment. (C) Automated sequence analysis of the 287 bp PCR product of patient II.1 and of her parents I.1 and I.2.

genotyping of family members was performed with the following microsatellites, as previously described[12]: D13S171, D13S173, D13S175, D17S122, D17S839, D17S921, DXS983, DXS986, and DXS1216.

Intermediate MNCV in a female Moroccan patient presenting with isolated CMT suggested that an alteration in the connexin 32 gene might be responsible for the phenotype, as reported in families with CMTX.[4-7 11] SSCP analysis of a 287 bp Cx4/Cx5 PCR fragment detected the presence of two additional bands in the patient (fig 1A). Direct sequencing showed that the patient was heterozygous for a point mutation (499delG) (fig 1C) that caused a frameshift, introducing a premature stop codon (TAG) at nucleotide 643. The resulting transcript encoded a Cx32 protein of 194 amino acids, truncated in the fourth transmembrane region, and lacking the intracellular COOH terminal domain, which seems to be of importance, since many point mutations and deletions in this sequence are associated with CMTX.[9-11] This previously unreported mutation is therefore very probably the cause of the CMTX phenotype in this patient. The mutation was not detected in the patient's parents or sibs by single strand conformation polymorphism (SSCP) or by PCR/restriction techniques (fig 1A, B). Automated sequence analysis of the 287 bp fragment confirmed its absence in the parent's DNA (fig 1C). False paternity was excluded by analysis of allele segregation with nine highly polymorphic markers located on chromosomes 13, 17, and X (data not shown). The deletion in the patient's Cx32 gene was therefore a de novo mutation.

This case is interesting for two reasons. (1) It is, to our knowledge, the first reported de novo mutation of the Cx32 gene in X linked Charcot-Marie-Tooth disease. Unfortunately, it could not be determined, for this female patient, whether the chromosome on which the mutation occurred was of paternal or maternal origin, since the patient had no offspring and the haplotype segregating with the deletion could not be reconstructed. (2) This case clearly shows that when the parents of a patient presenting with intermediate MNCV are unaffected, an erroneous diagnosis of autosomal recessive CMT is possible, particularly in countries like Morocco where consanguineous unions are frequent. Since this has important consequences for genetic counselling, molecular analysis of the Cx32 gene should be undertaken in such cases.

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