

Deletion of P2 promoter of GJB1 gene a cause of Charcot-Marie-Tooth disease

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Abstract

X-linked Charcot-Marie-Tooth disease (CMT) is the second most common cause of CMT, and is usually caused by mutations in the gap junction protein beta 1 (*GJB1*) gene. This gene has nerve specific P2 promoter that work synergistically with *SOX10* and *EGR2* genes to initiate transcription. Mutation in this region is known to cause Schwann cell dysfunction. A single large family of X linked peripheral neuropathy was identified in our practice. Next generation sequencing for targeted panel assay identified an upstream exon-splicing deletion identified extending from nucleotide c.-5413 to approximately – c.-49. This matches the sequence of 32 nucleotides at positions c.*218-*249 in the 3'UTR downstream of the *GJB1* gene. The deleted fragment included the entire P2 promoter region. The deletion segregated with the disease. To our knowledge a deletion of the P2 promoter alone as a cause of CMT has not been reported previously.

Highlights:

- Report of novel mutation as a cause of CMT.
- Complete deletion of P2 promoter region of *GJB1* gene.
- Supporting the observation that P2 promoter is central for Schwann cell function.

Key words: Charcot Marie Tooth disease; X-linked; *GJB1*; connexin 32; deletion; P2 promoter; Schwann cell dysfunction.

Abbreviations: CMT: Charcot–Marie–Tooth; *GJB1*: gap junction beta 1; CX32: connexin 32; *SOX10*: SRY-Box 10; *EGR2*: early growth response 2; IPN: inherited peripheral neuropathy; NGS: next generation sequencing;

1. Introduction

X linked Charcot–Marie–Tooth (CMT) disease is second most common cause of CMT caused by mutation in gap junction beta 1 (*GJB1*) gene which code for connexin 32 (CX32).[1] Clinically patients present with distal muscle weakness, wasting and sensory loss.[2] The connexin 32 messenger RNA and protein are expressed in Schwann cells in peripheral nervous system,[3] confined to Schmidt-Lantermann incisures and paranodal segment of nodes of Ranvier.[1] *GJB1* gene has nerve tissue specific P2 promoter [4,5] that is crucial in initiating transcription.[6] Mutations in the P2 promoter have been reported to cause X linked CMT suggesting its impairment leading to Schwann cell dysfunction.[7,8] The loss of connexin 32 expression is secondary to failed activation of its promoter by *SOX10* and *EGR2* which work synergistically for *GJB1* gene transcription.[9] In this case series we report a novel mutation of P2 promoter region of *GJB1* gene segregating with the disease.

2. Patients and Method

In our practice we identified a large family of X linked inherited peripheral neuropathy with unconfirmed molecular genetics. Genomic DNA was extracted from peripheral blood according to standard methods. Analysis of 56 genes associated with inherited peripheral neuropathy (IPN) was achieved using a custom-designed next generation sequencing (NGS) targeted panel assay. Raw data analysis and filtering was performed using a bespoke open-source pipeline with UCSC hg19 human genome as the reference. Basic copy number enumeration was performed using the CONTRA tool as a component of the analysis pipeline.[10] Clinical details including onset of symptoms, signs, neurophysiology and histology features of affected family members were obtained from the case records with the consent of participants for this publication.

3. Results

3.1. Genetics

In the female index patient no pathogenic sequence variants were detected on testing for panel genes including *SOX10* and *EGR2*. However on review, target coverage analysis of the gene panel data indicated a possible heterozygous partial deletion in the *GJB1* gene region. This deletion was evident on MLPA dosage analysis¹³ using MRC Holland probe mix P405-A1, which showed abnormal dosage of a single probe, indicating a heterozygous deletion within or including the exon 1B (non-coding) region of the *GJB1* gene at Xq13.1, and corresponding to the P2 promoter. Direct PCR (using primers flanking the deletion break points) and Sanger sequence analysis was subsequently used to confirm and characterise this deletion further. The identified deletion in the proband removed 5365 nucleotides and extends from position c.-5413 (5769 bases upstream of the ATG start codon) to nucleotide c.-49 (405 bases upstream of the ATG) inclusive. Additionally, a 32 base pair sequence is inserted between these break points; a BLAST search [<http://blast.ncbi.nlm.nih.gov/Blast.cgi>] indicated that this matches the sequence of 32 nucleotides at positions c.*218_*249 in the 3' untranslated region, downstream of the *GJB1* gene. Sanger sequence analysis has revealed that it is likely this sequence is duplicated and inserted between the break points. The deleted fragment in this patient (c.-5413_-49) includes the entire P2 promoter (figure 1). This deletion is confirmed to be present in all affected family members.

3.2. Clinical features

Affected individuals showed clinical evidence of a length dependent peripheral neuropathy. Clinical features of affected individuals were variable. They had clawing of toes, feet deformity (cavovarus and plano valgus), wasting of small muscles of hand, (figure 2) absent reflexes and a glove and stocking distribution of sensory involvement. Clinical features are

summarized in table 1. Males had earlier onset of symptoms than females. Neuropathy was inherited in X linked dominant fashion with no male-to-male transmission (figure 3). Five affected family members had neurophysiology assessment. They had sensorimotor demyelinating (four patients) and axonal (one patient) neuropathy. Nerve conduction velocities were considerably slowed (< 38 m/sec), suggesting serious demyelinating component in four patients (table 2). They did not have central nervous system features hence magnetic resonance imaging of brain and spine was not performed.

3.3. Histology

The sural nerve biopsy was assessed in one patient by light microscopy and ultrastructurally by electron microscopy (figure 4). Immunohistochemical (IHC) shows prominent loss of large myelinated fibres (IHC for myelin basic protein, SMI94 antibody), whilst unmyelinated and small myelinated fibres are better preserved (immunostaining for neurofilaments, SMI31 antibody). Ultrastructural examination highlights the regeneration clusters but shows no signs of active macrophage-associated demyelination or chronic demyelinating/re-myelinating process. The nerve biopsy excluded inflammation, showed chronic axonal neuropathy with no evidence of inflammatory vasculitic process.

4. Discussion

GJB1 gene, a common cause of X linked CMT maps to chromosome Xq13.1 and is 10 kb in length. This gene encodes 238- aminoacid gap junction, beta 1/connexin 32 protein that is involved to form hemichannel connexons as diffusion pathway between Schwann cell nucleus and axon.[11] The gene has nerve specific P2 promoter,[4,5] coding region (exon 2) and three non-coding exons (1,1A and 1B). Pathogenic mutations are described in both coding and non-coding region of the gene (<http://www.molgen.ua.ac.be/CMTMutations/>). Similarly pathogenic variants in P2 promoter region have been reported.[7,8] In our family

the deleted fragment (c.-5413_-49) includes the entire P2 promoter region of the gene and segregated with the disease. All affected family members have typical features of peripheral neuropathy with varying severity suggesting absence of P2 in causing pathogenic nerve dysfunction. Male members reported early onset of symptoms. None of the affected individuals had hip dysplasia which is an association described with CMT.[11] They did not have central nervous involvement as reported with some X linked CMT families.[13,16] Electrophysiology was consistent with peripheral nerve dysfunction and nerve biopsy in one individual excluded inflammatory cause of neuropathy.

P2 promoter region is important for gene function as reduced or absent (as in our family) activity will cause a reduction/ loss of messenger RNA transcript, hence reduced *GJB1* protein.[9] Functional analysis of promoter region by Houlden et al. (2004) identified its role to cause Schwann cell dysfunction by interacting with *SOX10* and *EGR2* genes.[9] Interaction between them is complex. *SOX10* and *EGR2* bind to the P2 promoter region of *GJB1* gene at S1-S2 and E2-E3 sites respectively for transcription.[17,18] This interaction between *GJB1* P2 promoter, *SOX10* and *EGR2* can be interrupted by either mutations in *SOX10*[19] and *EGR2*[20] genes or mutation in P2 promoter region of *GJB1*.[9] Our patients did not have mutations in *SOX10* and *EGR2* genes supporting restriction of transcription by latter mechanism. This observation strengthens the hypothesis by Houlden et al. that P2 promoter is central in function of Schwann cell in peripheral nerve.

5. Conclusion

We have described a novel mutation deleting entire P2 promoter of *GJB1* gene in a single large family with CMT. Inheritance and phenotype of affected individuals had classical features of X linked peripheral neuropathy. This study affirms the role of P2 promoter being vital for Schwann cell function.

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