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Case report

# *PMP22*-related disease: A novel splice site acceptor variant and intrafamilial phenotype variability

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**Abstract**

*PMP22* is the most frequent mutated gene in Charcot-Marie-Tooth disease (CMT) type 1A. Another phenotype, hereditary neuropathy with pressure palsies (HNPP), could be caused by *PMP22* mutations. *PMP22* encodes a peripheral myelin protein with molecular weight 22-kDa. Various pathomechanisms have been postulated in *PMP22*-related disease, including dysfunction due to missense mutations, and alteration of a gene dose due to duplication/deletion mutations. We identified a novel *PMP22* splice site acceptor variant, c.179–1G>A, in a patient with adult-onset chronic generalized polyneuropathy and two asymptomatic family members. Pathophysiological features of the members mainly overlapped with those reported in HNPP, but broad intrafamilial clinical variations were observed. *PMP22* transcripts lacking of exon 4 were produced by the variant, presumably leading to in-frame deletion of 47 amino acids. The variant was also shown to exert effect on dosage of *PMP22* mRNA. The complex molecular pathology would lead to the unique clinical and pathophysiological conditions.


*Keywords:* *PMP22*-related disease; Splice site acceptor variant; Gene dose effect; *PMP22* protein with in-frame deletion; Overlapped pathophysiology.

## 1. Introduction

Demyelinating form or Charcot-Marie-Tooth disease (CMT) type 1A is caused by either 1.5-Mb tandem duplication containing *PMP22* or missense mutations. On the other hand, hereditary neuropathy with pressure palsies (HNPP) is caused by either 1.5-Mb deletion of *PMP22* or missense mutations [1,2]. *PMP22* encodes peripheral myelin protein 22, component of myelin, which wrap around the nerves and enhance saltatory conduction. Depending on the nature of *PMP22* mutation, broad phenotypic variations have been reported, including CMT types 1A and 1E, HNPP, Dejerine-Sottas hypertrophic disease, and Roussy–Levy syndrome [1]. Different molecular pathogenesis has been proposed, loss-of-function or altered distribution of PMP22 protein by missense mutations and gene dose effect by deletion/duplication mutation. Previous studies using animal models for CMT type 1A, overexpressing *Pmp22* mRNA, showed amelioration of the phenotype using ascorbic acid [3] or antiprogesterone [4]. Investigation of dosage of *PMP22* mRNA is a prerequisite for designing effective therapeutic approach for CMT type 1A. We describe here the aberrant splicing pattern caused by a novel splice site acceptor variant identified in one *PMP22*-related disease family with phenotype variability.

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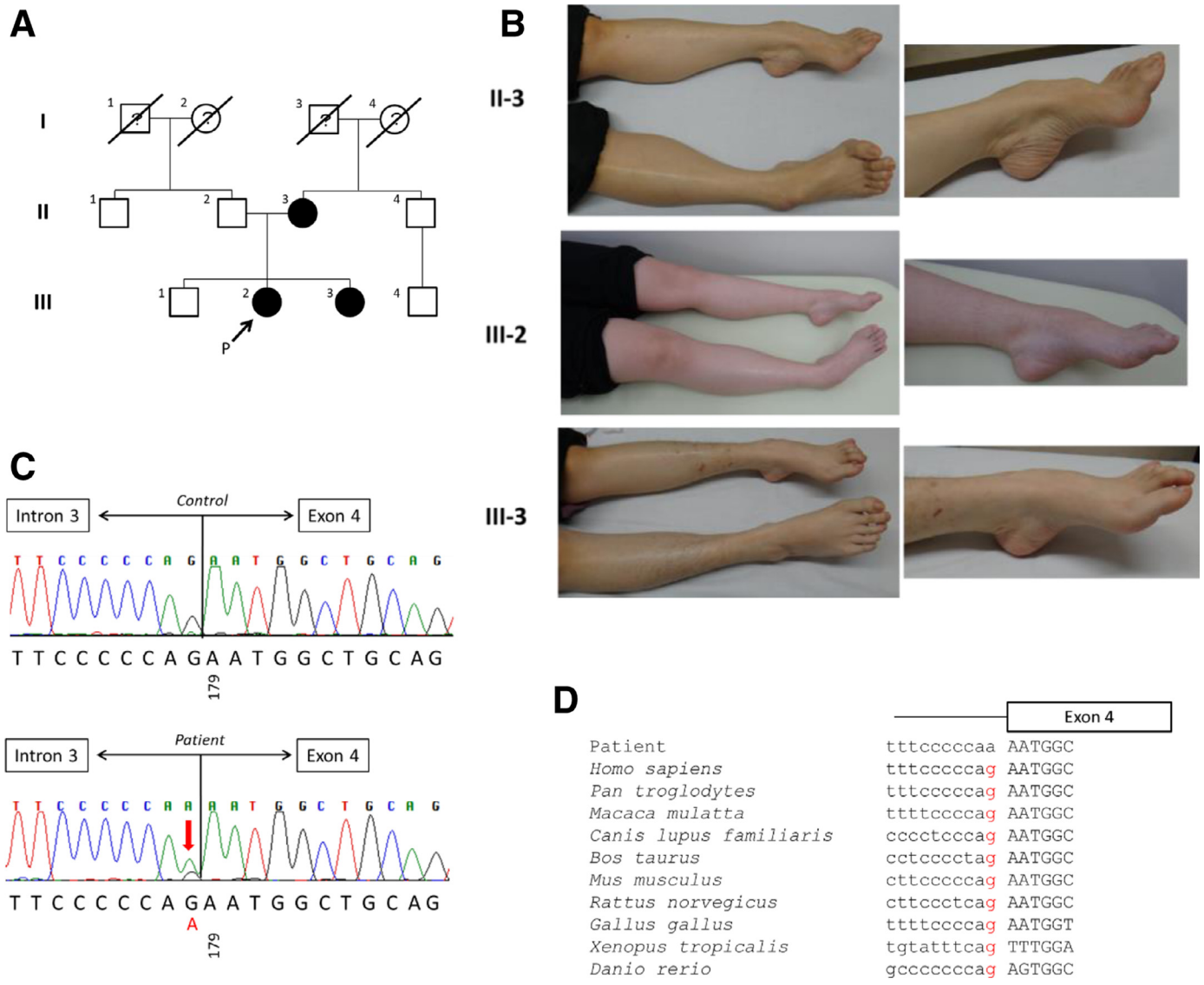


Fig. 1. (A) Pedigree of the family with novel *PMP22* splice site acceptor variant. Circle, female; Square, male; filled symbols, affected individuals; open symbols, unaffected individuals; slashed, deceased individual. Slanting arrow, the proband. (B) Foot pictures of the mutation carriers. One family member (III-2) is symptomatic, while others (III-3 and II-3) are asymptomatic. Foot deformity is observed in the three members, *cavovarus* deformity with hammer toes in II-3, *cavovarus* deformity in III-2 and *pes cavus* and calf atrophy in III-3. (C) Sequence fluorescent chromatograms of the splice site acceptor of exon 4. Control (upper panel) and the proband (lower panel), demonstrating heterozygous carriers of the c.179–1G>A variant in the proband (red arrow). The nucleotide number is based on the longest PMP22 transcript (NM_000304.3). (D) Schematic representation and sequence of boundary between *PMP22* intron 3 and exon 4 in various species. The first nucleotide at 3′-splice-site, guanine, in human as well as other species is shown in red colour, which is substituted by adenine in the patient. Information of mRNA and genomic sequence is described in Table S3, Supplementary Methods.

## 2. Case report

### *2.1. Clinical, electrophysiological and neurosonographical findings*

This study was approved by the Institutional Review Boards (IRBs) of the Tokushima University Hospital. Written informed consent was obtained from all participating individuals according to the Declaration of Helsinki. The family of Japanese ethnicity included 3 affected and 9 unaffected members over three generations with presumed autosomal dominant inheritance (see Fig. 1-A). The proband (III-2 in Fig. 1-A) is a Japanese woman born to non-consanguineous parents after uneventful pregnancy. Developmental milestone was normal, except mild intellectual disability. She was slow of foot, but able to walk and run without any aid in her school days. She began to experience stumbling or faltering at age of 27. Later, she could not climb upstairs or go down the stairs without holding on to the railing. Walking difficulties slowly progressed over time, but she could walk alone without any support. Neurological examination at age of 31 revealed reduced limb tone, mildly reduced/absent tendon reflexes at upper/lower extremities, proximal and distal weakness prevalent in lower limbs, and

Table 1
Electrophysiological and neurosonographical findings of the three members.

| Motor nerve conduction velocities | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Right Median nerve | | | Right Ulnar nerve | | | | |
| Subjects | **DL (ms)** (3.49±0.34) | **CMAP (mV)** (7.0±3.0) | **MCV (m/s)** Elbow to wrist (57.7±4.9) | **DL (ms)** (2.59±0.39) | **CMAP (mV)** (5.7±2.0) | **MCV (m/s)** Wrist to below elbow (58.7±5.1) | **MCV (m/s)** below elbow to above elbow (61.0±5.5) | **MCV (m/s)** Above elbow to axilla (66.5±6.3) |
| III-2 | 7.5 | 5.9 | 38.8 | 4.2 | 5.9 | 40.0 | 24.3 | 50.0 |
| III-3 | 6.4 | 4.6 | 34.2 | 4.0 | 9.1 | 37.2 | 23.3 | 33.3 |
| II-3 | 6.6 | 4.5 | 41.3 | 3.8 | 8.2 | 46.1 | 26.3 | 41.0 |
| **Neurosonography** | | | | | | | | |
| | **Right Median nerve** | | | | **Right Ulnar nerve** | | | |
| Site of examination | Wrist (8.9±1.7) | Forearm (6.6±1.1) | Elbow (6.9±1.4) | | Wrist (5.5±1.1) | below elbow (6.7±1.5) | Elbow (7.2±1.6) | above elbow (6.3±1.7) |
| Cross-sectional area ($mm^3$) In-house standard | | | | | | | | |
| III-2 | 11.6 | 5.2 | 6.6 | | 5.8 | 4.6 | 9.4 | 6.7 |
| III-3 | 11.1 | 5.9 | 7.5 | | 4.3 | 4.5 | 12.1 | 5.9 |
| II-3 | 19.7 | 8 | 6.9 | | 4.7 | 7.0 | 17.1 | 9.1 |

(Upper table) DL: distal latency, CMAP: compound muscle action potential, MCV: motor nerve conduction velocity.
(Lower table) Cross-sectional area measured at median and ulnar nerves.
Standard values are determined based on the in-house data obtained from 30 Japanese (female 66%, 52.7±10.7 years (range 30 – 63)) control samples. Values out of the standard range are described in bold style.

*cavovarus* deformity (see Fig. 1-B). Muscle atrophy was not evident. No palpable nerves were noted. Laboratory analyses excluded paraneoplastic polyneuropathy, impaired glucose tolerance neuropathy, polyneuropathy associated with vitamin deficiency, and polyneuropathy associated with anti-MAG IgM antibodies. The proband's mother (II-3) was asymptomatic and had no walking difficulty, however, neurological evaluation showed mildly reduced/absent tendon reflexes at limbs and *cavovarus* deformity with hammer toes (see Fig. 1-B). The proband's young sister (III-3) was also asymptomatic, however, tendon reflexes at limbs were mildly reduced. Foot deformity, *pes cavus*, and calf atrophy were observed (see Fig. 1-B). Nerve conduction studies in the three family members showed predominantly demyelinating features rather than axonal dysfunction in the legs. Conduction delay was evident within the cubital tunnel. Conduction block was not evident (Table 1). Electromyography showed no active denervation, but rather a chronic denervation in the tibialis anterior and posterior muscles in the proband. Neurosonography showed focal nerve enlargement at wrist in median nerve and site of above elbow in ulnar nerve (Table 1, Fig. S1 in Supplementary Material).

### 2.2. Genetic analysis

After we obtained written informed consent, genomic DNA of the five family members (II-2, II-3, III-1, III-2, and III-3) was extracted from peripheral blood. Diagnostic clinical resequencing of 64 known CMT-related genes were conducted using a combination of DNA microarray and whole exome sequencing, as previously described [5]. Total RNA was prepared from lymphocytes, which was transcribed into a complementary DNA (cDNA) using reverse transcriptase for subsequent analysis, including reverse transcription PCR (RT-PCR) and quantitative PCR (RT-qPCR). Oligonucleotides used in this study are described in Table S1 in Supplemental Material. Effects by nonsense-mediated mRNA decay (NMD) were evaluated in cultured T cells under treatment with or without NMD inhibitor, emetin. Potential effect(s) by the intronic splice acceptor variant was also evaluated bioinformatically. Details are described in Supplemental Material.

A novel heterozygous splice site acceptor variant, c.179–1G>A, was demonstrated in PMP22 gene (NM_000304.3), which is highly conserved across species (Fig. 1-C, D). The intronic variant was found in all the three members (III-2, III-3 and II-3). The variant was absent in our control datasets, and was not registered in public databases, including the dbSNP Build 141 database, the 1000 Genomes Project, ESP5400 exomes, and ExAC (accessed May 15, 2018). The intronic variation is predicted to have drastic consequences on the maturation processes of *PMP22* mRNA. The secondary structure of the wild-type RNA sequence contains a stem-loop structure, which would be altered by the intronic variant, c.179–1G>A, accompanying by reduction of minimum free energy (see Fig. S2 in Supplementary Material).

RT-PCR experiments showed *PMP22* transcripts lacking of exon 4 in the patient, leading to in-frame deletion of 47 amino acids (Fig. 2-A, B). Basic expression level of

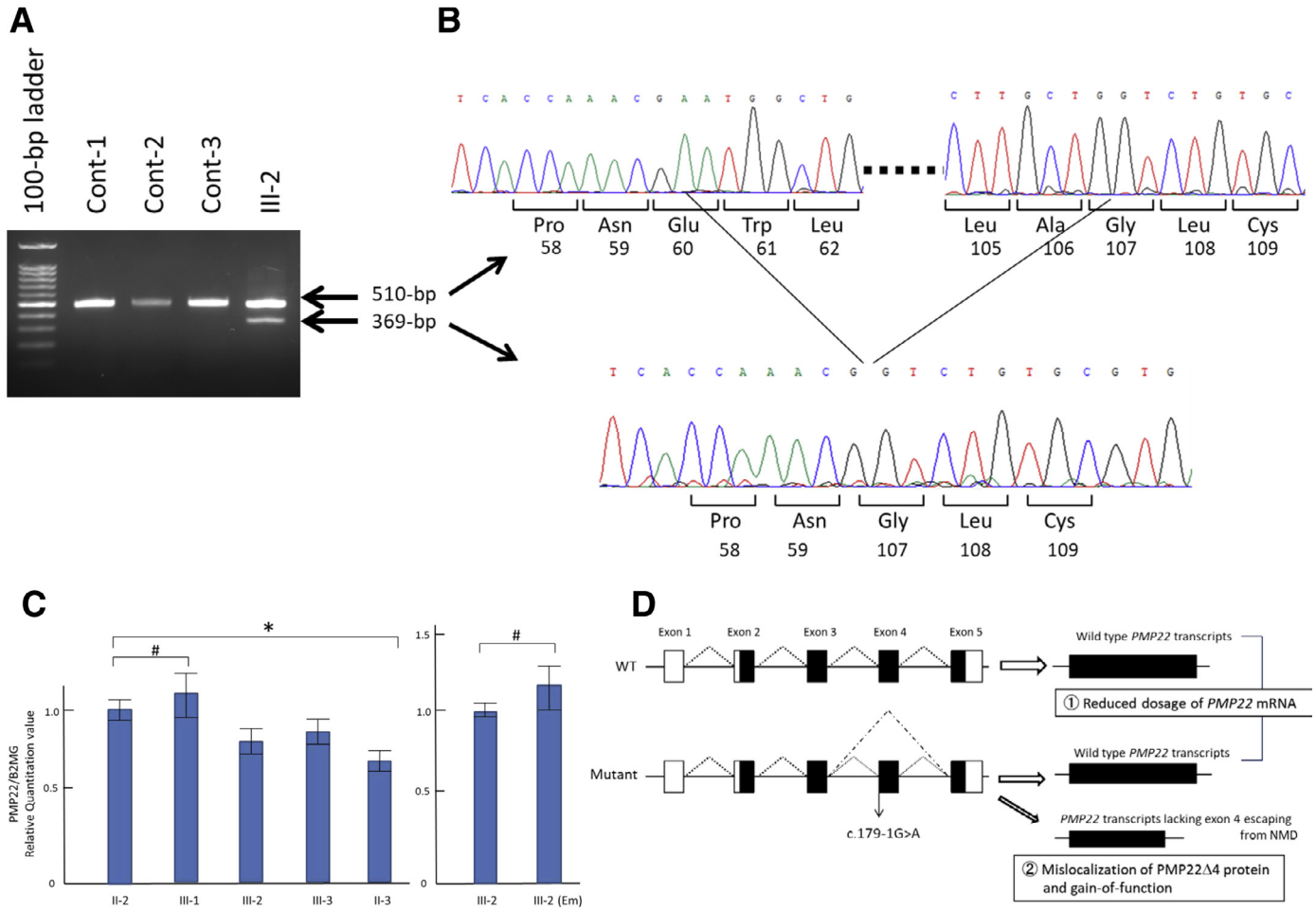


Fig. 2. RT-PCR experiment and sequence analysis of *PMP22* aberrant transcripts. (A) Agarose gel electrophoresis of the RT-PCR products revealed that the cDNA fragment of 510-bp corresponds to the predicted wild-type *PMP22* transcripts, whereas the cDNA small fragment of 369-bp corresponds to the transcript lacking exon 4. Amplification of cDNA via RT-PCR using RNA extracted from; Cont = lymphocytes from control individual, III-2 = lymphocytes from the affected member III-2. (B) Sequence chromatograms from RT-PCR-amplified cDNA of *PMP22* from the patient (III-2). Full-length sequence containing exons 2, 3, 4 and 5 is obtained from the PCR products with 510-bp (upper panel). Sequence lacking exon 4 is demonstrated from the PCR products with 369-bp (lower panel). Amino acid residue position numbering is based on human longest transcript (NM_000304.3). Methionine encoded by the translation initiation site (start codon) is numbered as residue 1. (C) RT-qPCR experiment. No difference of *PMP22* mRNA expression level in lymphocytes (#$p > 0.1$) is observed between the two members without the *PMP22* splice site acceptor variant, II-2 and III-1. Significant difference is shown between the members with and without the variant (*$p < 0.05$). Comparison of expression level of *PMP22* transcripts in cultured T cells from the patient (III-2) demonstrates an increasing through NMD inhibitor, emetin (Em), however, no significant difference is shown by statistical analysis (#$p > 0.1$). (D) Schematic figure representing the structure of the PMP22 gene, splicing pattern and possible pathomechanisms by the intronic variant. Solid square, coding region; Open square, non-coding region. Dotted line shows splicing pattern in wild type *PMP22*. Dotted line shows a possible mechanism of aberrant splicing by the slice site acceptor variant, c.179–1G>*A*. As a result, two possible pathological events would be elicited by the intronic variant. ① Alteration of *PMP22* mRNA dosage, and ② Mislocalization and gain-of-function by PMP22Δ4 protein.

*PMP22* transcripts was different between the members with and without the intronic variant. The expression level in the three members carrying the variant was lower than that in the two members without variant. Expression level of *PMP22* transcripts in cultured T cells was increased via the treatment of specific NMD inhibitor, emetin; however, the difference did not reach statistical significance (Fig. 2-C).

## 3. Discussion

Clinical features observed in the proband includes adult-onset and slowly progressive clinical course, foot deformity, and mild functional disability. The other two members carrying the intronic variant were asymptomatic without any functional disability, but had mild to moderate foot deformities. Progressive deformity of leg or foot is frequently observed in CMT type 1A, and mild to moderate deformity is also seen in 20% of HNPP patients [6]. Broad clinical variations were observed amongst the members carrying the *PMP22* intronic variant. Nerve conduction study demonstrated predominant demyelinating rather than axonal dysfunction, but the conduction velocity was not severely delayed in comparison to typical form of CMT type 1A. The amplitude of compound muscle action potential (CMAP) at median nerve was reduced but the lower limits of normal. Prolongation of the median nerve proximal latency was

observed at the wrist. Distal latency was prolonged at median nerve. Conduction velocity at ulnar nerve was delayed in the proximal part of the elbow joint, indicating liability to pressure at common compression site. Enlargement of nerve trunk was observed by neurosonography around the carpal and cubital tunnels. These data indicate mild and uniform demyelination, accompanying focal morphological changes and conduction slowing. Enlargement of nerve trunk is often observed in HNPP, presumably reflecting soft tissue swelling or oedema [7]. Clinical and pathophysiological features of the family partially overlapped with those reported in CMT type 1A or HNPP (Fig. S3 in Supplemental Material).

The intronic variant would cause two molecular pathomechanisms (see Fig. 2-D). Dosage of full-length *PMP22* mRNA would be decreased by the variant, presumably between 1 and 2 at the level of gene copy number. Transcripts lacking exon 4 sequence were mostly escaped NMD, which would eventually be translated to PMP22 proteins lacking 47 amino acids encoded by exon 4 (PMP22Δ4). The PMP22 protein with in-frame deletion would localize at the endoplasmic reticulum and exert gain-of-function [8].

In summary, pathophysiological findings in the proband and asymptomatic members largely overlapped with those reported in HNPP, but clinical features were distinct, containing broad variation. Further accumulation of genotype-phenotype correlations and biological analyses, including *PMP22* mRNA dosage in peripheral nerve, are a prerequisite for better understanding of pathogenesis and designing effective therapeutic approach for *PMP22*-related disease.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.03.010.

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