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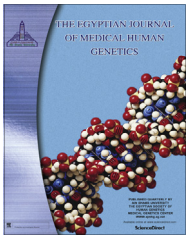

CASE REPORT

Co-occurrence of Xp21 microduplication encompassing the DMD locus in conjunction with 17p12/*PMP22* microduplication in a female with Charcot–Marie–Tooth disease type 1A

Alpa Sidhu [a], Michael Hankerd [b], Kelly Kennelly [d], Melissa Kristofice [b], Salah Ebrahim [b,c,*]

[a] *Division of Genetics & Metabolic Disorders, Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI, USA*
[b] *Detroit Medical Center University Laboratories, Cytogenetics Laboratory, Detroit, MI, USA*
[c] *Department of Pathology, Wayne State University School of Medicine, Detroit, MI, USA*
[d] *Department of Pediatric Neurology, Wayne State University School of Medicine, Detroit, MI, USA*

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Abstract We report on the molecular detection of two microduplications involving chromosomes Xp21.1–Xp21.2 and 17p12 in a 35-year-old female with clinical phenotype of Charcot–Marie–Tooth disease type 1A (CMT1A) documented by chromosomal microarray analysis. The 17p12 microduplication was approximately 1.32 Mb in size and contained eleven genes including the peripheral myelin protein 22 (*PMP22*), while the Xp21.1–Xp21.2 microduplication was estimated to be 626 Kb in size and contained part of the dystrophin (*DMD*) gene. Constitutional interstitial microduplication of 17p12 segment encompassing the *PMP22* gene has been reported in individuals with Charcot–Marie–Tooth disease type 1A. Defects in the *DMD* gene (deletion, duplication, or mutation) are associated with Duchenne and Becker muscular dystrophies (DMD and BMD). Combined microduplications of Xp21/*DMD* with 17p12/*PMP22* are extremely rare with only one published report of a male patient with changes in both the *DMD* and *PMP22* genes.

1. Introduction

Charcot–Marie–Tooth disease type 1A (CMT1A) and Duchenne muscular dystrophy (DMD) are neurogenetic conditions with variable and distinct phenotypes. CMT1A is a demyelinating peripheral neuropathy inherited in an autosomal dominant manner. Clinical features include progressive peripheral

* Corresponding author at: Cytogenetics Laboratory, Department of Pathology, Wayne State University School of Medicine, Detroit, MI 48201, USA. Tel.: +1 313 966 0680; fax: +1 313 966 0687.
E-mail address: sebrahim@med.wayne.edu (S. Ebrahim).
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motor and sensory neuropathy, pes cavus, peroneal muscle atrophy, inability to walk on the heels, deep tendon areflexia and distal sensory disturbance with slow nerve conduction velocity [1]. CMT1A is a slow progressive disease with age of onset from 5 to 25 years. About 70–80% of CMT1A cases are due to microduplication of about 1.4 Mb in the short arm of chromosome 17p12 region that includes the peripheral myelin protein gene (*PMP22*; OMIM# 601097) [2–6].

DMD is an X-linked recessive disorder caused by mutations in the *DMD* gene located on Xp21 region with an incidence in males of 1 in 3500. The clinical phenotype includes a spectrum of disorders. On the mild end of the spectrum, symptoms include asymptomatic increase in serum creatine kinase (CK) concentration, myoglobinuria, and isolated quadriceps myopathy. The other end of the spectrum includes DMD-associated dilated cardiomyopathy without evidence of skeletal muscle disease, Becker muscular dystrophy (BMD), and the most severe form, DMD [7]. Women are usually asymptomatic carriers but can develop muscle weakness and cardiac abnormalities [8]. Clinical features of DMD include progressive symmetric muscle weakness more in the proximal muscles than distal, absence of sensory symptoms, speech delay, delayed motor milestones, with wheelchair dependency in most boys before age 13 years. Molecular etiologies for DMD include deletions of one or more exons (60–70%), point mutations (25–35%), and microduplications [9].

Co-occurrence of an Xp21 microduplication encompassing the *DMD* locus in conjunction with a 17p12/*PMP22* microduplication is extremely rare with only one published report of a male patient with changes in both the *DMD* and *PMP22* genes [10]. Here, we describe the second patient with combined microduplication of the Xp21.1–21.2 region including part of *DMD* gene and the 17p12 region including the *PMP22* gene identified by chromosomal microarray in a female with clinical phenotype of CMT1A.

2. Clinical report

We report on a 35-year-old right-handed Caucasian female who came for an initial evaluation to the CMT clinic at our hospital. She was noted to have hypotonia and club feet at birth. She gave a history of delayed motor milestones, multiple knee surgeries, high arched feet, and back pain. At age 16 years she was diagnosed with CMT1A based on clinical signs and nerve conduction velocity (NCV) study. She reported worsening of symptoms over the last year with inability to perform fine motor tasks with her hands, pain in the upper extremities upon holding her son up, and decreased sensation and motor function of ankles and toes. Physical examination showed normal cognition, absent reflexes bilaterally, bilateral atrophy of thenar eminence, hammertoes, pes cavus, with decreased power at the wrist and intrinsic hand muscles, ankle and toes. There was decreased sensation to vibration and pin-prick, with absent joint position sense up to the wrists and ankles bilaterally. NCV evaluation showed evidence of demyelinating neuropathy. Her serum CK level was within normal limits.

The patient reported to have two healthy daughters aged 3 and 6, and a 4 year-old son recently diagnosed with DMD (microduplication of dystrophin gene exons 45–61). Her son presented with hypotonia at birth, delayed motor milestones with walking at 18-months, speech delay, elevated CK level, and calf pseudohypertrophy. Family history was significant for distal limb atrophy and weakness in the patient's brother, father, paternal aunt, and paternal grandfather (Fig. 1). At the present time, none of the symptomatic individuals had a molecular diagnosis for CMT1A. There was no known consanguinity and no known family history of muscle weakness, delayed milestones, cardiomyopathy, or sensory symptoms other than what is mentioned above.

3. Materials and methods

3.1. Chromosomal microarray

Genomic DNA was isolated from the patient peripheral blood using the Puregene kit (Gentra Systems, Minneapolis, MN). Chromosomal microarray using an oligonucleotide + single nucleotide polymorphism based microarray containing 180K-features (SurePrint G3 GGXChip + SNP v1.0 4x180k manufactured by Agilent Technologies, Santa Clara, California) was performed according to the manufacturer's protocol. The microarray slide was scanned on an Agilent G2565 CA microarray scanner system. Data were extracted from the microarray image; the background subtracted, and then normalized using Agilent Cytogenomics software (v2.5.8.1). Data were imported into array CGH Analytics Software (Genoglyphix™; Signature Genomic Laboratories, Spokane, WA). The array design and genomic coordinates are based on NCBI build 37 (hg19).

3.2. Fluorescent in situ hybridization (FISH)

FISH was performed on metaphase and interphase cells derived from the PHA-stimulated peripheral blood from the patient using BAC probes from the duplicated 17p12 region and the duplicated Xp21.1–p21.2 region. For the duplicated 17p12 region BAC probe RP11-849N15 (HG18 chr17:15,037,103-15,270,554) was used and the RP11-599B13 probe (HG18 chr17:7,849,167-8,045,204) from the 17p13.1 region distal to the duplicated region was used as a control. For the duplicated Xp21.1–p21.2 region BAC probe RP11-1083A21 (HG18 chrX:31,354,660-31,533,379) was used and the RP11-141B6 probe (HG18 chrX:57,316,603-57,480,676) from the Xp11.1 region proximal to the duplicated region was used as a control. The hybridization and posthybridization washes were performed according to standard protocol. Blood samples from other relatives were not available for FISH or microarray analysis.

4. Results

Chromosomal microarray detected a 1.32 Mb microduplication/copy number gain involving chromosome 17p12 short arm region (Fig. 2A). The duplicated region contains at least eleven genes including the peripheral myelin protein 22 (*PMP22*). The 17p microduplication was confirmed by FISH analysis using the RP11-849N15 probe (Fig. 2B). In addition, microarray also detected a 626 Kb microduplication/copy gain involving chromosome Xp21.1–Xp21.2 short arm region including part of the dystrophin gene (*DMD*) (Fig. 3A). The Xp21.1–Xp21.2 microduplication was confirmed by FISH

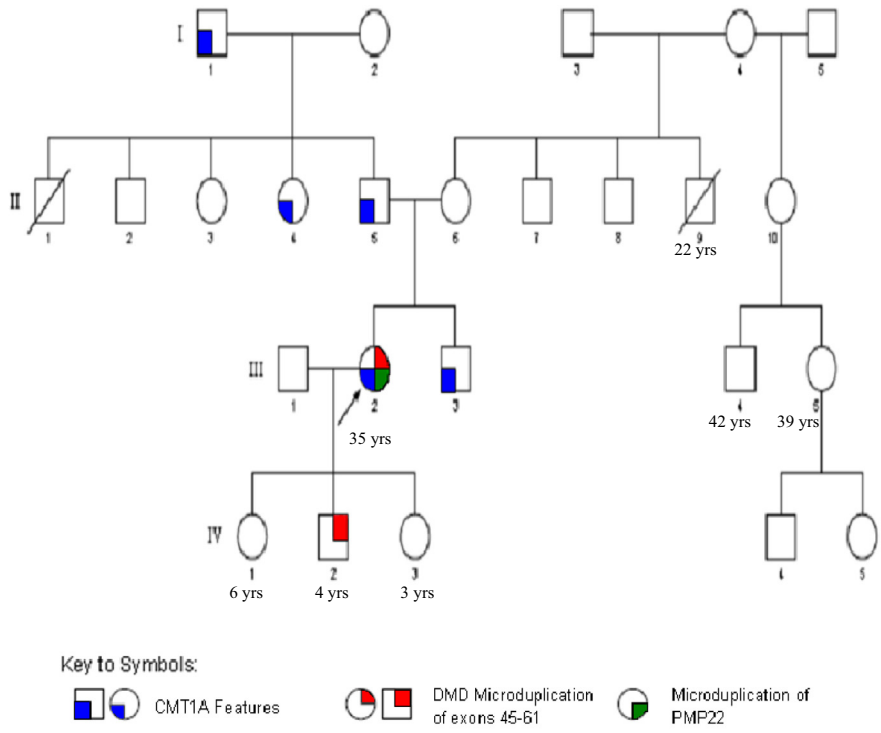

Figure 1 The family pedigree. Family history showed that the CMT1A was most likely inherited from the paternal side of the family. The proband (marked with arrow), her brother, father, paternal aunt and paternal grandfather exhibited phenotypic features of CMT1A (shaded in blue). *DMD* microduplication (shaded in red) was passed from the proband to her son, age 4 years. The green shaded area indicates molecular confirmation for 17p12/*PMP22* microduplication. Empty squares indicate unaffected males, empty circles unaffected females.

analysis using the RP11-1083A21 (Fig. 3B). Additional FISH or CMA testing on at risk family members was recommended to determine whether the microduplications were inherited or de novo, but was not performed due to lack of availability and the age of the children.

5. Discussion

CMT1A and DMD are inherited neuromuscular disorders with distinct clinical features. The combination of both CMT1A and DMD has been described in only one patient in the literature, a male who showed characteristics of both the disorders [10]. The *PMP22* microduplication and frame-shift mutation in the *DMD* gene were both inherited from his mother. His phenotypic presentation included features of both peripheral neuropathy and muscular dystrophy. His mother exhibited a CMT1A phenotype. We present the second case of a female patient who was found to have changes in genes responsible for both conditions. Chromosomal micro-array was performed due to her clinical features, strong family history of CMT1A and the recent diagnosis of her son with DMD. Though molecular confirmation has not been made for any other family members due to lack of familial availability and the age of the children, the CMT1A microduplication appears to be coming from the paternal side of the family based on reported symptoms. It is unknown if the proband's mother is a carrier for the *DMD* microduplication although the risk may be decreased since neither her brother nor her three maternal uncles demonstrate any symptoms of DMD.

Clinically, DMD can be distinguished from CMT1A by complete lack of sensory symptoms, normal nerve conduction velocity, proximal muscle weakness and wasting versus distal in CMT1A, calf pseudohypertrophy, elevated serum creatine kinase, speech delay, classic Gower maneuver (use of distal muscles to rise from a squatting position) and dilated cardio-myopathy. Motor milestones delayed at 6 months with later manifestations of bilateral pes cavus, absent deep tendon reflexes, and sensory loss are indicative of CMT1A. Upon review of literature, Charcot–Marie–Tooth neuropathies in association with various forms of muscular dystrophy have been reported in four cases [10–13]. Butefisch et al. first reported on a 22-year-old female who inherited both facio-scapulohumeral muscular dystrophy (FSHD) and CMT1A from each of her parents leading to shortened life span and early death [11]. The second report is of a 27-year-old male who inherited the X-linked Charcot–Marie–Tooth disease from his mother caused by a mutation in the connexin32 gene and acquired a de novo in-frame deletion in his DMD gene leading to a milder form, BMD [12]. His clinical course was changed with severe proximal and distal muscle wasting and rapid progression of both the diseases. Recent publication by Schreiber et al. [13] describes a 53-year-old male with both CMT1A and FSHD mutations presenting with an atypical phenotype. There is one further report similar to our case on

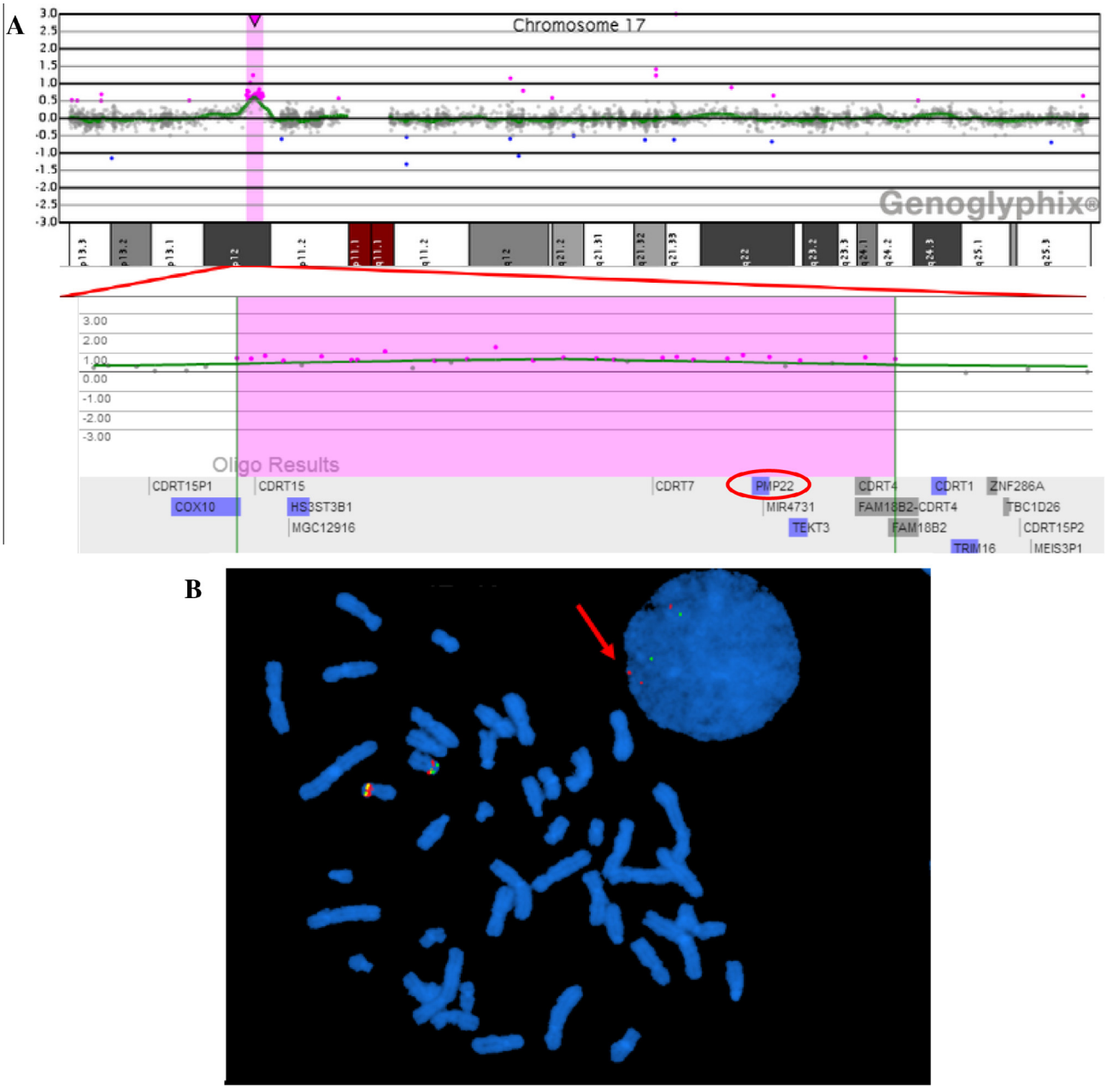

Figure 2 Analysis of 17p12 region encompassing the *PMP22* gene. (A) CMA oligonucleotide data plot for chromosome 17 depicting a 1.32 Mb microduplication (shaded in pink) of the 17p12 region including the *PMP22* gene region (circled in red). (B) FISH image showing microduplication of 17p12 region on the interphase cell. Clone RP11-849N15 from the 17p12 region was labeled in orange and RP11-599B13 clone from the 17p13.1 region was labeled in green as a control. The three orange signals present on the interphase cells indicate microduplication of the 17p12 region.

a family with combined alterations of *PMP22* and *DMD* genes [10]. Vondracek et al. reported a male patient who inherited the CMT1A/*PMP22* microduplication and frameshift mutation in the *DMD* gene from his mother [10]. Within the scope of the published report by Vondracek et al. we can deduce that the mother of the proband showed phenotype similar to our patient with features of CMT1A. To the best of our knowledge, our case is the second report of combined changes in both the *DMD* and *PMP22* genes and the fifth case describing two alterations on different chromosomes involving unrelated neuromuscular diseases in the same patient.

Our patient demonstrates clinical signs and symptoms of CMT1A with a significant family history. Her chromosomal microarray results showed that she passed on the *DMD* microduplication to her 4-year-old son who presented with high serum CK, speech delay, and the classic Gower maneuver on examination. Interestingly, the finding of delayed milestones with hypotonia at birth for her son makes it highly likely that he may carry the CMT1A/*PMP22* microduplication although it would be somewhat early presentation. Based on his risk, her son should be monitored for symptoms of CMT1A until molecular testing for the 17p12 microduplication is performed.

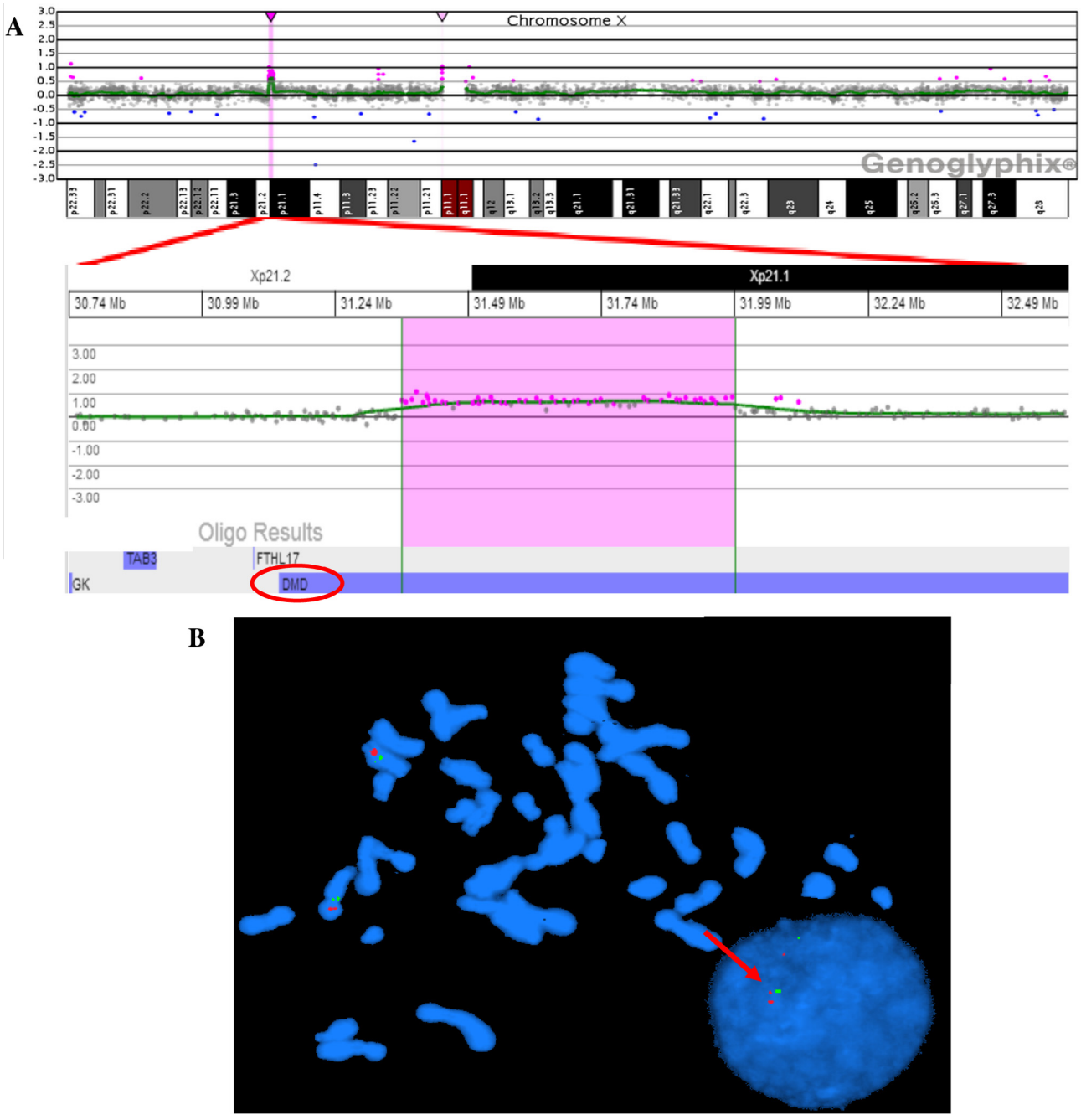

Figure 3 Analysis of the Xp21 region encompassing part of the DMD gene. (A) CMA oligonucleotide data plot for the X chromosome depicting a 626 Kb microduplication (shaded in pink) of the Xp21.1–Xp21.2 region including part of the DMD gene region (circled in red). (B) FISH image showing microduplication of the Xp21 region on the interphase cell. Clone RP11-1083A21 from the Xp21 region was labeled in orange and RP11-141B6 clone from the Xp11.1 region was labeled in green as a control. The three orange signals present on the interphase cells indicate microduplication of the Xp21 region.

His phenotype would be different from our proband's since he would have the disadvantage of a severe manifestation due to both the DMD and CMT1A mutations. Our patient was offered molecular FISH or chromosomal microarray testing for her other family members but additional testing was declined.

This report demonstrates two alterations on different chromosomes involving unrelated neuromuscular diseases in the same patient and adds to the present information for this rare occurrence. Literature evidence shows alteration of disease course with worse prognosis in the presence of Charcot–Marie–Tooth neuropathy and a muscular dystrophy [10,11,13]. Hence even if a mutation in a disease gene has been detected, further genetic testing may be warranted in unusual clinical presentations. Given the unusual phenotype of our patient's son not explained by DMD alone, we highly suspect

him to carry the maternal CMT1A microduplication and hope that the family might re-consider molecular testing. We expect our patient to be protected from an alteration in clinical course of CMT1A despite the presence of her *DMD* microduplication carrier status (likely due to subtle differences in X inactivation patterns). However, diligent follow up evaluations would elucidate the natural course of the combination of the two microduplications in a female.

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