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A male infant with Xq22.2q22.3 duplication containing *PLP1* and *MID2*

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Abstract

Background: The Xq22.2 q23 is a complex genomic region which includes the genes *MID2* and *PLP1* associated with FG syndrome 5 and Pelizaeus–Merzbacher disease, respectively. There is limited information regarding the clinical outcomes observed in patients with deletions within this region.

Methods: We report on a male infant with intrauterine growth retardation (IUGR) who developed head titubation and spasticity during his postnatal hospital course.

Results: Chromosome microarray revealed a 6.7 Mb interstitial duplication of Xq22.2q22.3. Fluorescence in situ hybridization showed that the patient's mother also possessed the identical duplication in the Xq22.3q22.3 region. Among the 34 OMIM genes in this interval, the duplication of the *PLP1* (OMIM# 300401) and *MID2* (OMIM# 300204) appears to be the most significant contributors to the patient's clinical features. Mutations and duplications of *PLP1* are associated with X-linked recessive Pelizaeus–Merzbacher disease (PMD). A single case of a Xq22.3 duplication including the *MID2* has been reported in boy with features of FG syndrome. However, our patient's clinical features are not consistent with the FG syndrome phenotype.

Conclusion: Our patient's clinical features appear to be influenced by the *PLP1* duplication but the clinical effect of other dosage sensitive genes influencing brain development cannot be ruled out.

KEYWORDS
FG syndrome, *MID2*, Opitz-Kaveggia syndrome type 5, Pelizaeus–Merzbacher disease, *PLP1*

1 | INTRODUCTION

MID1 and *MID2* code for RBCC (RING-finger, B-boxes, and Coiled-coil) proteins localized on chromosome Xp22 and Xq22, respectively. Mutations in *MID1* have been associated with X-linked Opitz syndrome patients, characterized by hypertelorism in addition to craniofacial changes,

urogenital, laryngotracheal, and cardiac malformations (Li, Zhou, & Zou, 2016) A duplication in *MID2* have been postulated to be associated with a single case of FG syndrome 5, a condition characterized with macrocephaly, relatively small ears, frontal hair upsweep fetal fingertip pads, and intellectual disability (Jehee et al., 2005). More recently a missense mutation in *MID2* was identified as the cause

for X-linked intellectual disability associated with the dysmorphic facial features which overlap but are distinct from FG syndrome including elongated face, short philtrum, prominent forehead, large ears, and a squint (Geetha et al., 2014). The expression patterns similar to the craniofacial expression of *Mid1* and *Mid2* have been observed during embryonic mouse development, which adds to the complexity in delineation of clinical features which may be unique to *MID1* or *MID2* mutations (Li et al., 2016). We describe a male infant with an Xq22.2q22.3 duplication that contains the *MID2*. We review this patient's phenotypic features and discuss the dosage sensitivities of genes within the regions potentially contributing to this patient's phenotypic features.

2 | CASE PRESENTATION

This patient was born at 38 weeks gestation to a 32-year-old gravida 1 para 0 female who had intermittent prenatal care and gestational diabetes treated with metformin and insulin. There is no history of maternal alcohol, drug usage, or cigarette smoking. Fetal ultrasound revealed intrauterine growth retardation (IUGR). Both parents had macrocephaly. The patient's mother and father had a head circumference of 62.8 cm (hair braided) and 59.8 cm, respectively. The baby was delivered via caesarean section for abnormal fetal heart tracings and had Apgar scores of $9^1 9^5$. Birth weight was 2,370 g (<3%), length was 43.5 cm (<3%), and head circumference was 32.5 cm (5%).

With respect to the family history, the patient's mother required help in high school with math and social studies, but graduated on time. She reports being able to read, write, and perform basic mathematics. She was able to answer all questions at her son's visit and checked appropriate review of system boxes. The mother's brother (maternal uncle to patient) has been living in a residential facility since early childhood. There is limited confirmed information but he is thought to have significant intellectual disability. The mother works as a full time personal care assistant in a nursing home. She does not have any visual or neurological dysfunction.

There is no known maternal family history of birth defects, autism, or seizures.

Early in the NICU course, the patient exhibited some salient physical features including pendular nystagmus, mild micrognathia, small jaw, and head titubation.

Initially he was breast fed and soon was noted to be hypoglycemic with a blood glucose of 30 mg/dl. He was transferred to the neonatal intensive care unit and received intravenous fluids with 10% dextrose (D10). He was successfully weaned off the fluids by day of life 3 and continued to feed on formula with normoglycemia and was switched to Pregestimil formula later.

On day of life 2, he was noticed to have acholic stools. Further diagnostic workup revealed elevated direct bilirubin of 2.4 and aspartate aminotransferase (AST) level of 120. Liver ultrasound revealed a very small gall bladder. Due to the presence of direct hyperbilirubinemia, an infectious workup including urine CMV, toxoplasma antibodies, HIV, Hepatitis B and C, HSV, and CSF HSV, enterovirus was performed with negative test results.

On day of life 10, his oral feeding declined which necessitated placing an NG tube. A repeat ultrasound showed a very contracted gall bladder with a relatively thick wall and a diminutive size of the common bile duct which was concerning for biliary atresia. Head ultrasound and brain MRI were both normal. Chest x-ray was negative for butterfly vertebrae or other spinal defects. EKG showed evidence of right atrial enlargement. Echocardiogram showed a small secundum atrial septal defect with left to right shunt. A pH probe study and upper GI study was normal with no evidence of malrotation. Ceruloplasmin level was 15.8 mg/dl. Alpha-1 antitrypsin level was normal. Alpha-1 antitrypsin Pi phenotype was MM (normal). Hepatobiliary iminodiacetic acid scan (HIDA) at day of life 30 showed poor hepatocellular extraction and no definite excretion into the biliary tree or small bowel at the end of 24 hr (no biliary flow). Autoimmune hepatitis panel was negative. Next generation sequencing jaundice panel showed no pathogenic mutations in *ABCB4*, *ABCB11*, *ATP8B1*, *JAG1*, and *TJP2*. G-tube placement and liver biopsy were performed which showed cholestatic hepatitis with giant cell transformation, compatible with neonatal hepatitis. Cholangiogram showed a normal gall bladder. Renal ultrasound was normal. He was also noted to have metabolic acidosis with a bicarbonate of 16 mmol/L and was started on sodium carbonate supplementation. An amino acid quantitative profile was unremarkable. His initial and repeat newborn screen was normal. The etiologies for the patient's elevated liver function tests and metabolic acidosis were not identified.

SNP microarray analysis was performed using Affymetrix CytoScan Dx microarray. This microarray contains over 2.69 million probes, including 1.9 million unique non-polymorphic probes and 740,000 single nucleotide polymorphism (SNP) probes. The average inter-probe distance is 1,148 base pairs. Thresholds for genome-wide screening set at >500 kb for gains and >200 kb for losses. The SNP microarray also detects the regions of homozygosity (ROH) which potentially increases the risk for autosomal recessive disorders.

This microarray analysis revealed a 6.7 Mb interstitial duplication of Xq22.2q23; arr[hg19] Xq22.2q23 (102,890,822–109,572,085) × 2. (Figure 1a) This duplication interval included 34 OMIM annotated genes including *PLP1* and *MID2* (Table 1). Of note, mutations, deletions, and duplications of the *PLP1* are associated with X-linked recessive
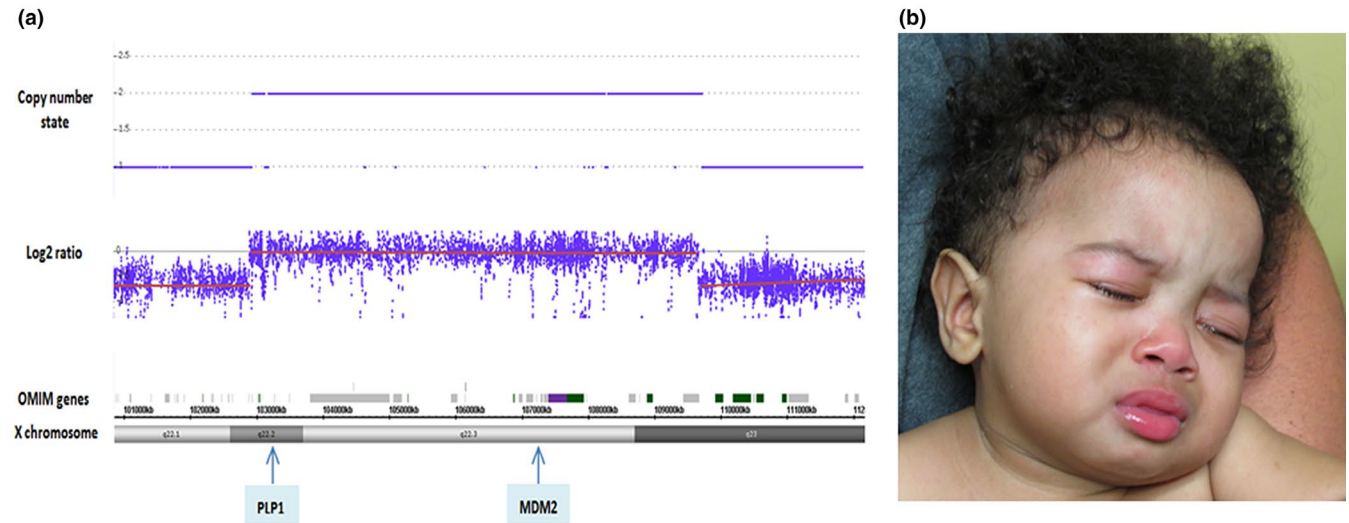
FIGURE 1 (a) Patient at 10 months of age with broad forehead, flat nasal bridge, and anteverted nares (b) SNP array showing a 6.7 Mb interstitial duplication on X chromosome at Xq22.2q23 of this male patient, where *PLP1* and *MID2* and other 32 OMIM annotated genes are in duplicated interval

(XLR) Pelizaeus–Merzbacher disease (OMIM: 312080); and mutations of *MID2* is associated with XLT mental retardation (OMIM: 300928). A single case of a Xq22.3 duplication including the *MID2* has been reported in boy with features of FG syndrome. Duplication in *MID2* may contribute to FG syndrome 5.

Confirmatory FISH analyses were performed on parents' peripheral blood lymphocytes using standard procedures (Rooney, 2001). The BAC clone RP11-1-1J13, which was within the duplication interval of the proband, was selected for the probe. DNA isolated from this BAC clone and labeled directly with fluorochrome conjugated-dUTP by nick translation (Vysis, Downer Grove, IL). The probe was hybridized to metaphase spreads which were prepared from the parents derived lymphocytes. Chromosomes were visualized by counterstaining with DAPI.

Maternal blood FISH analysis revealed the duplication of this region (Rooney, 2001). This is consistent with maternal transmission of this duplication to the male proband.

The patient returned for follow up at 10 months of age. The head titubations and nystagmus were no longer present. He was exclusively G-tube fed and had delayed milestones. Physical examination at 10 months (Figure 1b) was significant when length was 58.5 cm (<3%; 50% for 8 weeks); weight was 4.9 kg (<3% 50% for 6 weeks); and head circumference was 44 cm (8%). There was relative macrocephaly with bitemporal narrowing and a prominent forehead. The anterior fontanelle was closed. The palpebral fissures were horizontal and there was absence of nystagmus. Outer canthal distance measured 6.3 cm (3%–25%), inner canthal distance measured 2.2 cm (3%–25%), and right and left palpebral fissure measured 2.1 cm. The calculated interpupillary distance was 4.1 cm (3%–25%). Right and left pinna measured 4.7 cm

(approximately 50%) and the left outer pinna was slightly irregular. The bridge of the nose was flat and the nasal tip was depressed. There was anteversion of nares. The philtrum was grooved and measured 7 mm. The hairline appeared to be slightly low. Nipples were spaced normally. There was no murmur on auscultation. A gastrostomy tube was present on the abdomen.

There were tri-radiate palmar creases. Dimples were evident on the knuckles. The right second toe overlapped the third toe and the third toe over the fourth toe. The left first toe overlapped the second toe. The penis was normal and both testes were descended. The anus was normally positioned. Neurologic examination was significant for decreased truncal tone and increased tone in the extremities. There were brisk deep tendon reflexes.

At age 3, the patient was able to hold an object placed in hand but unable to grab or transfer. He was able to roll with assistance. If placed in a seated position, he was able to lean to the side. He was using a standing device at home. He was able to coo but did not babble. He reportedly likes music and will cry if his favorite show is turned off. He was receiving speech, physical and occupational therapy, and has nursing care.

At age 3, his length was 81.9 cm (0%, 50% = 1.5 years), weight was 11.2 kg (0%), and head circumference was 47.8 (13%). He had bifrontal narrowing. Palpebral fissures were neutral. There was no nystagmus but strabismus was evident. There was no ptosis or lid eversion. Calculated interpupillary distance was 5.8 cm (>97%) Ears were normally positioned and formed. Knee contractures were present. Hands deviated laterally. Distal phalanges were tapered. Marked hypotonia was present. Deep tendon reflexes were very brisk (Figure 2).

TABLE 1 OMIM genes in Xq22.2q22.3 duplication region

Gene	OMIM number	Function/disease
MORF4L2	300409	Protein which is declined in normal senescent cells (Bertram et al., 1999)
PLP1	300401	Pelizaeus–Merzbacher disease (Diehl, Schaich, Budzinski, & Stoffel, 1986)
TMSB15B	301011	Member of the thymosin-beta family of actin-binding molecules which deregulate motility in prostate cells (Bao et al., 1996)
RAB9B	300285	Regulated intercellular vesicle trafficking (Seki et al., 2000)
H2BFWT	300507	Interacts with DNA histones (Churikov et al., 2004)
SLC25A53	300941	Inner mitochondrial membrane transporter (Palmieri, 2013)
ESX1	300154	Imprinted gene involved in placental morphogenesis, located on X chromosome-fetal growth (Li & Behringer, 1998)
IL1RAPL2	300277	IL1Receptor (Sana, Debets, Timans, Bazan, & Kastelein, 2000)
TEX13A	300312	Testis expressed gene (Wang, McCarrey, Yang, & Page, 2001)
NRK	300791	Has activity against myelin basic protein (Nakano, Yamauchi, Nakagawa, Itoh, & Kitamura, 2000)
SERPINA7	314200	Act at inflammatory sites and release thyroid binding globulin (TBG) (Jirasakuldech et al., 2000)
RNF128	300439	Functions as E3 ubiquitin ligase (Anandasabapathy et al., 2003)
TBC1D8B	301027	Tre2-Bub2-Cdc16 (TBC) domain family protein that involved with cellular recycling processes and is expressed in human podocytes. Mutations associated with nephrotic syndrome 20 (Dorval et al., 2019)
RIPPLY1	300575	Expressed in anterior presomitic mesoderm and somites of zebrafish (Kawamura et al., 2005)
CLDN2	300520	Regulates tissue-specific properties of tight junctions (Sakaguchi et al., 2002)
PRPS1	311850	Arts (cognitive impairment, hypotonia, ataxia, hearing impairment, and optic atrophy), Charcot Marie Tooth, X-linked deafness Purine and pyrimidine synthesis (Roessler et al., 1990)
MORC4	300970	Zinc finger proteins with presently unknown function (Liggins et al., 2007)
PIH1D3		Involved in cytoplasm assembly of axonemal dynein; Mutations associated with X-linked primary ciliary dyskinesia 36 (Paff et al., 2017)
MYCL2	310310	Has DNA sequence homology to MYC family of proto-oncogenes (Morton et al., 1989)
FRMPD3	301005	Protein expressed in expression in adult and fetal brain, adult spinal cord, and ovary. Function unknown (Nagase, Nakayama, Nakajima, Kikuno, & Ohara, 2001)
PRPS1	300681	Gout associated with increased levels of phosphoribosylpyrophosphate synthetase (Roessler et al., 1993). Allelic disorders include X-linked recessive Charcot-Marie-Tooth Disease 5 and X-linked sensorineural hearing loss.
TSC22D3	300506	Modulates T-lymphocyte response (Ayroldi et al., 2001)
MID2	300204	Opitz FG syndrome Type 5 (Buchner et al., 1999)
TEX13B	300313	Testis-expressed gene (Wang et al., 2001)
VSIG1	300620	Junction adhesional molecule (Scanlan et al., 2006)
ATG4A	300663	Cysteine protease involved in autophagy (Mariño et al., 2003)
COL4A6	303631	X-linked deafness (Rost et al., 2014)
COL4A5	303630	Alport hereditary nephritis (Lemmink, Schröder, Monnens, & Smeets, 1997)
AMMECR1	300195	Gene member of contiguous gene complex AMME (Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis) (Meloni et al., 2002). Genes in this region include COL4A5 (303630) and FACL4 (300157) (Andreoletti et al., 2017)
IRS4	300904	Tyrosine phosphorylated in response to insulin and IGF1 (Fantin et al., 1998)
GUCY2F	300041	Expressed largely in photoreceptors (Yang, Fülle, & Garbers, 1996) Candidate for X-linked retinitis pigmentosa
NXT20	300320	RNA export factor and binds to nucleus pore-associated proteins NXF1 and NXF2 (Herold et al., 2000)
KCNE1L	300328	Shares 56% homology with potassium channel KCNE1 and deleted in AMME (Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis) (Meloni et al., 2002)
ACSL4	300157	X-chromosome-imprinted regulator of placental development in mice (Fohn & Behringer, 2001)

FIGURE 2 Patient at 3 years of age

3 | DISCUSSION

We describe a male infant with a maternally inherited Xq22.2q22.3 duplication containing the *PLP1* and *MID2*. This child has clinical features that appear to be influenced by *PLP1* duplication although the contribution of other dosage sensitive genes within the region cannot be ruled out. His mother's neurocognitive phenotype is less clear and both parents have macrocephaly. Thus, both the *MID2* duplication and other familial factors may contribute to our patient's macrocephaly.

Table 1 illustrates the OMIM genes that are present in the Xq22.2 q23 (102,890,822–109,572,085) region. Only duplications of *PLP1* and *MID2* are associated with known clinical disorders which potentially overlap with our patient's clinical features.

TABLE 2 Comparison of Patient clinical features to features described by Jehee et al. and PLP-1 related phenotypes

			PLP-1-related phenotypes	
Clinical feature	**Patient**	**Jehee et al. (2005)**	**Connatal type**	**Classic type**
Gestation	38 weeks	32 weeks	N/A	N/A
Birth length	43.5 cm (<3%)	38 cm (<3%)	N/A	N/A
Birth weight	2,370 g (<3%)	1,800 g (50%)	N/A	N/A
Head circumference	32.5 cm (5%)	NR	N/A	N/A
Craniofacial	Micrognathia	Trigonocephaly		
Muscle Tone	Hypotonia	Hypotonia	Hypotonia	Hypotonia
Pendular nystagmus	Present initially but disappeared in a few months	Unknown (not mentioned in the case report)	Present	Present
Pharyngeal weakness/stridor	Absent	Unknown	Present	Absent
Ataxia	Absent	Not described	Absent	Present
Cognitive impairment	Present	Present	Present	Less severe
GI Symptoms	Direct hyperbilirubinemia (unknown cause), G-tube dependent for nutrition	Severe constipation	Dysphagia more prevalent, constipation	Less prevalent
Significant Maternal history	Intellectual disability, Gestational diabetes, Identical duplication of Xq22.2q22.3	Severe anemia	N/A	N/A
Genetics	Duplication of Xq22.2q22.3	Duplication in Xq22.3	Duplication in Xq22 including *PLP1*	Duplication in Xq22 including *PLP1*
Brain MRI	Normal in infancy	Unknown	Diffuse leukoencephalopathy – noted after the age of 2 years	Diffuse leukoencephalopathy – noted after the age of 2 years
Death	Alive	At age 4 years secondary to multi-organ failure	Infancy to 3rd decade	Can survive up to 3rd−7th decade

Abbreviation: NR, not recorded.

Proteolipid protein (PLP-1)-related CNS disorders include many disorders including PMD and Spastic paraplegia 2 (SPG-2). PMD is an X-linked recessive hypomyelinative leukodystrophy presenting with nystagmus, spastic quadriplegia, ataxia, and developmental delay usually in infancy or early childhood. Most of these manifestations including nystagmus and mild spasticity were noted in our patient. PMD can present in different forms including severe (connatal) PMD which presents in infancy with pendular nystagmus, hypotonia and pharyngeal weakness. In childhood, affected children develop short stature, poor weight gain, spasticity and developmental delays. Patients with classic PMD present with nystagmus in late infancy, are hypotonic and develop titubation and tremors of head and neck with spastic quadriparesis in the first few years of life. A transitional form also exists between the connatal and the classic PMD forms.

First described by Opitz and Kaveggia, FG syndrome (Opitz & Kaveggia, 1974) is an X-linked multiple congenital anomalies (MCA) syndrome. FG syndrome has been mapped to five distinct loci: _FGS1_ (OMIM305450) encoded by _MED12_ on Xq13 (Risheg et al., 2007); _FGS2_ (OMIM 300321) localized to Xq28, caused by _FLNA_ mutations (Unger et al., 2007); _FGS3_ (OMIM 300406) mapped to Xp22.3 (Dessay et al., 2002); and mutations in _CASK_ at Xp11 are responsible for FGS4 (OMIM300422) (Piluso et al., 2009).

Jehee et al. (2005) described a male child with clinical features of FG syndrome including trigonocephaly, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, long philtrum, diastema of upper central incisors, strabismus, and hypospadias. He had hypotonia and developmental delay and died at 4 years of age due to generalized infection and multiple organ failure. A microduplication of approximately 4 Mb was identified at Xq22.3 and included the _MID2_. _MID2_ is highly homologous to _MID1_, a gene is known to be mutated in Opitz G/BBB syndrome, and thus _MID2_ was proposed as a candidate gene for FG syndrome 5.

PLP1 tandem duplications are postulated to be mediated by homologous and nonhomologous recombination mechanism through which there is repair of a double-stranded DNA breakage event by one-sided homologous strand sister chromatid invasion followed by DNA synthesis and subsequent joining with the other end of the break through a nonhomologous mechanism (Woodward et al., 2005). The utilization of capture and single molecule real-time sequencing (cap-SMRT-seq) has revealed three groups of copy number variants (CNVs) including (a) small template insertions with insertions above 20 bp; (b) intra CNV rearrangements; (c) inter-chromosomal and interlocus rearrangements (Zhang et al., 2017). The clinical phenotype associated with _PLP1_ duplications does not appear to be mediated by size of the duplication as patients with large size _PLP1_ duplications had relatively mild clinical features (Regis et al., 2008). Additional brain expressed genes in the _PLP1_ genomic region including _SERPINA7_, _FRMD3_, _AMMECR1_, _ILRAPL2_, _CLDN2_, _TSC22D3_, and _PRPS1_ may contribute to previously reported and our patient's clinical features, although it is difficult to determine which gene(s) apart from duplicated _PLP1_ contribute to our patient's clinical phenotype (Carvalho et al., 2012; Regis et al., 2008). _ACSL4_ may contribute to the patient's IUGR, given its association with placental development (Fohn & Behringer, 2001). As our patient and his mother do not have hearing loss or nephritis, the contributions of duplicated _COL4A6_ and _COL4A5_ are of minimal significance. We summarize our patient's clinical features and compare them to PLP-1 conatal and classic types in Table 2 and FG syndrome. Our patient has clinical features that appear to be more closely aligned with PLP-1 conatal.

Some females who carry Xq22 _PLP1_ duplications have exhibited failure to thrive, developmental delay, dysmorphic features, and abnormal MRI findings which may be attributed to altered X chromosome inactivation in brain cells, dosage-sensitive genes escaping X chromosome inactivation and depending on the size of the Xq22 deletion, genes which when duplicated exhibiting altered expression patterns, and dosage-sensitive genes which escape X chromosome inactivation (Carvalho et al., 2012). It is difficult for us to determine if the _PLP1_ duplication has any subtle effects on the patient's mother as she has not had a neuropsychiatric evaluation and a brain MRI.

The patient described in this communication has a duplication in the Xq22.3 region and displays clinical features influenced by the _PLP1_ duplication. The effect of the _MID2_ duplication and other genes in this interval on the patient's overall phenotype appears less evident.

CONFLICT OF INTEREST
The authors declare no financial or otherwise relevant conflict of interest related to this manuscript.

AUTHORS CONTRIBUTION

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