The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: 1476-7058 (Print) 1476-4954 (Online) Journal homepage: https://www.tandfonline.com/loi/ijmf20

First trimester ultrasound features of X-linked Opitz syndrome and early molecular diagnosis: case report and review of the literature

Laura Sarno, Giuseppe Maria Maruotti, Antonella Izzo, Cristina Mazzaccara, Luigi Carbone, Giuseppina Esposito, Marco Di Cresce, Gabriele Saccone, Angelo Sirico, Rita Genesio, Nunzia Mollo, Pasquale Martinelli, Anna Conti, Fulvio Zullo & Giulia Frisso

To cite this article: Laura Sarno, Giuseppe Maria Maruotti, Antonella Izzo, Cristina Mazzaccara, Luigi Carbone, Giuseppina Esposito, Marco Di Cresce, Gabriele Saccone, Angelo Sirico, Rita Genesio, Nunzia Mollo, Pasquale Martinelli, Anna Conti, Fulvio Zullo & Giulia Frisso (2019): First trimester ultrasound features of X-linked Opitz syndrome and early molecular diagnosis: case report and review of the literature, The Journal of Maternal-Fetal & Neonatal Medicine, DOI: 10.1080/14767058.2019.1677594

To link to this article: https://doi.org/10.1080/14767058.2019.1677594

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
https://doi.org/10.1080/14767058.2019.1677594

Taylor & Francis
Taylor & Francis Group

SHORT REPORT

First trimester ultrasound features of X-linked Opitz syndrome and early molecular diagnosis: case report and review of the literature

Laura Sarno[a], Giuseppe Maria Maruotti[a], Antonella Izzo[b], Cristina Mazzaccara[b,c], Luigi Carbone[a], Giuseppina Esposito[a], Marco Di Cresce[a], Gabriele Saccone[a], Angelo Sirico[a], Rita Genesio[a], Nunzia Mollo[b], Pasquale Martinelli[a], Anna Conti[b], Fulvio Zullo[a] and Giulia Frisso[b,c]

[a]Department of Neurosciences, Reproductive Sciences and Dentistry, University Federico II, Naples, Italy; [b]Department of Molecular Medicine and Medical Biotechnologies, University Federico II, Naples, Italy; [c]CEINGE, Advanced Biotechnologies, Naples, Italy

ABSTRACT

X-linked Opitz G/BBB syndrome (XLOS) is a multiple congenital disorder inherited in an X-linked manner. XLOS may be suspected, in prenatal age, on the basis of sonographic findings in the second and/or third trimester of gestation. Pathogenetic variants in *MID1* gene have been reported in individuals with XLOS. Prenatal genetic testing is offered for pregnancies at risk, in which the mutation in the family has been identified. To date no cases of prenatal diagnosis, based on first-trimester ultrasound data, have been reported. We present a case of a fetus at 12 gestational weeks with ultrasound multiple anomalies, including increased nuchal translucency, heart defects, cleft lip and palate, enlarged fourth ventricle absence of ductus venosus and family hystory of XLOS. The genetic prenatal test detected the c(0).1286-1G > T mutation of *MID1* gene. Data about prenatal ultrasonographic findings consistent with XLOS are limited to second and third trimester. This is the first case reporting ultrasound detectable midline defects suggestive of XLOS as early as the first trimester of gestation. This case also suggests that when multiple anomalies are detected in a fetus with normal chromosomal structure, the possibility of a monogenic disorder must be considered.

ARTICLE HISTORY
Received 3 July 2019
Revised 26 September 2019
Accepted 4 October 2019

KEYWORDS
First trimester; molecular analysis; prenatal diagnosis; ultrasound; XLOS

Introduction

X-linked Opitz G/BBB syndrome (XLOS) was first described by Opitz in 1969. Its prevalence ranges from 1:50,000 to 1:100,000 males [1]. XLOS is a multiple congenital disorder characterized by facial anomalies, heart defects, laryngo-tracheo-esophageal defects and genito-urinary abnormalities. Neurodevelopmental delay and intellectual disability are observed in about 50% of affected males. These manifestations have been associated with mutations of *MID1* gene, mapping to the short arm of the X chromosome (p22.2 band) (OMIM # 300552). Approximately 100 mutations have been found along the entire length of the *MID1* gene, in both sporadic and familial cases of XLOS [2–6]. MID1 protein acts as an E3 ubiquitin ligase associated with the microtubules implicated in the control of phosphatase 2A protein, integrin alpha-4, and serine/threonine-protein kinase 36 levels [7–9].

Prenatal diagnosis by chorionic villus sampling (CVS) and amniocentesis can be offered to the couple when the mother carries a known mutation.

In the last decade, the importance of the first-trimester diagnosis of fetal malformations has been highlighted especially in fetuses with abnormal nuchal translucency (NT). An increased NT is generally associated with increased risk of genetic anomalies. Cytogenetic and cytogenomic analyses identify aneuploidy, chromosomal rearrangements, copy number variants (CNVs) in about 30–40% of pregnancies with increased NT [10,11]. However, if these analyses are normal, the possibility of Mendelian disease, due to single-gene mutation(s) must be considered.

The case we describe is the first report of a diagnosis of XLOS in a fetus with ultrasonographic evidence of heart and facial defects in the first trimester.

Materials and methods

Chorion villi sampling (CVS)

Written informed consent to perform genetic prenatal analysis was obtained by the couple, according to the internal procedure for Prenatal Diagnosis.

CONTACT Antonella Izzo antonella.izzo@unina.it Department of Molecular Medicine and Medical Biotechnologies, University Federico II, Via S. Pansini 5, Naples 80131, Italy

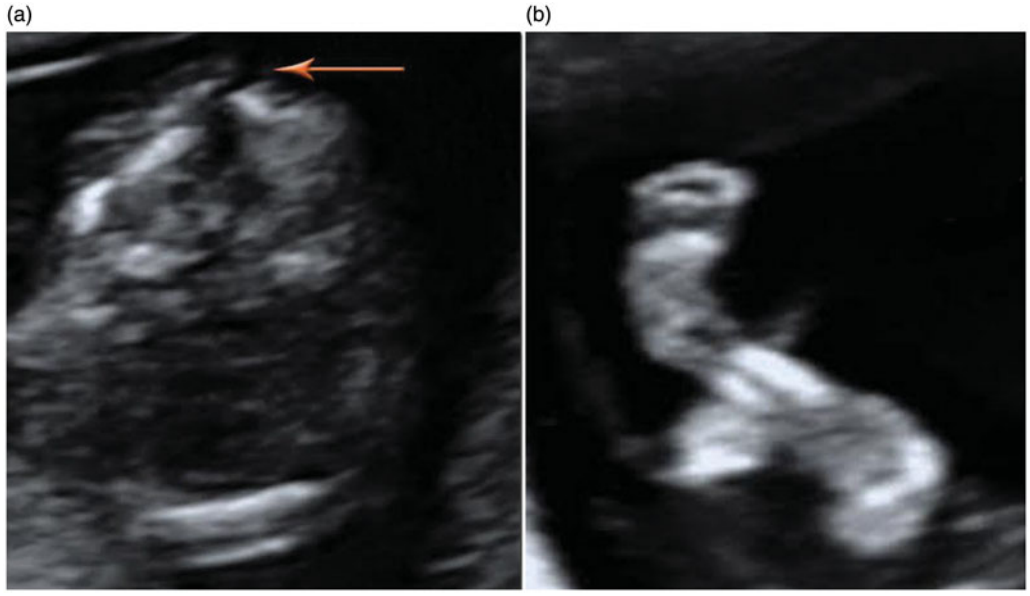

Figure 1. (a) Transverse view showing the cleft in the alveolar ridge and upper lip (arrow). (b) Sagittal view of the lower limb with tibia and fibula and the twisted foot.

Chorionic villi (CV) selection, purification and DNA extraction from CV was performed, as previously described [12].

Karyotype and comparative genomic hybridization array (a-CGH)

Karyotype analysis was performed on GTG-banded metaphases from CVS (short and long-term cultures) at a resolution of 400 bands according to standard cytogenetic protocols.

The high-resolution a-CGH analysis was performed on DNA extracted from cultured villi using Agilent HD 4×180-K chips (Agilent Technologies Italia SpA, Cernusco sul Naviglio, Italy) in accordance with the manufacturer's guidelines for the whole genome screening. The arrays were scanned by the Agilent SureScan Dx microarray Scanner and analyzed using Cytogenomics (Agilent Technologies Italia SpA, Cernusco sul Naviglio, Italy) and Genoglyphix software (Signature Genomics, Spokane, WA, USA), referring to the GRCh37/Hg19 Genome Assembly.

MID1 mutation screening

Genomic DNA sample from mother was extracted from peripheral blood leukocytes using the "Nucleon" procedure (GE Healthcare, Little Chalfont, UK). To detect maternal cell contamination, the CVS DNA and the maternal DNA were analyzed together for a larger spectrum of short tandem repeats, as previously reported [12,13].

Exon 8 of the *MID1* gene (ENST00000317552.8) was amplified by PCR using novel primers, including neighboring flanking regions, to detect the familial *MID1* mutation.

Case presentation

A 31-years-old pregnant woman, gravida 3, nulliparous, at 12 weeks of gestation, was referred to the Prenatal Diagnosis Unit of Federico II University Hospital in Naples. The transabdominal ultrasound scan showed a single viable intrauterine pregnancy. The crown-rump length (CRL) was 64.6 mm and the fetal heart rate was within the normal range (153 beats per minute). The NT was 2.8 mm, which resulted above 95th centile for gestational age. Head shape, cerebral falx, ventricles, and choroid plexi were normal. However, in both cross-section and sagittal views, the posterior fossa appeared abnormal, with an enlarged fourth ventricle. Ductus venosus was absent. A cleft lip and palate were found (Figure 1(a)). The abdominal wall was intact, the bladder and kidneys were visible but there was a single umbilical artery. A unilateral club foot was observed (Figure 1(b)).

The woman reported that during her second pregnancy, she delivered a male baby affected by XLOS, carrying the mutation in exon 8 of *MID1* gene c(0).1286-1G > T. She was found to be the carrier of the same mutation.

Due to the ultrasound findings and maternal anamnesis, an assumption of XLOS was formulated and an invasive procedure for prenatal molecular testing

Table 1. Previous cases of prenatal suspicion of XLOS described in literature and their features.

		Ultrasound findings							
References	Gestational age	Cranial	Facial	Cardiac	Uro-genital	Vascular	Other	Outcome/delivery	Additional postnatal findings
Hogdall et al. [14]	19 weeks	Enlarged cisterna magna Abnormal cerebellum	Hypertelorism		Hypospadias			TOP	Low set and posteriorly rotated ears, Imperforate anus, Criptorchidism
Tajima et al., [15]	27 weeks		Cleft lip	Tricuspid valve regurgitation			Polyhydramnios	Emergency CS at 35 weeks of DCDA twins: an affected male fetus (2076 g) and an unaffected female fetus (2234 g)	Cleft palate, Hypertelorism, Hypospadias, Hoarsness, Dysphagia
Cheng et al., [16]	20 weeks			Hypoplastic left heart, DORV, Hypoplastic pulmonary artery				TOP	Absent corpus callosum
Spinelli et al., [17]	19 weeks		Hypertelorism	Complex CHD (partial atrioventricular canal defect, PLSVC)	Bilateral pielectasia, Hypospadias	Absent DV		Preterm SVD at 30 weeks of a male fetus (1254 g)	Crossed fused renal ectopia, Imperforate anus
This study	12 weeks	Enlarged fourth ventricle	Cleft lip and palate			Absent DV, Single umbilical artery	Increased NT, Unilateral clubfoot	TOP	Not possible to perform

TOP: termination of pregnancy; CS: cesarean section; DCDA: dichorionic diamniotic; DORV: double outlet right ventricle; CHD: congenital heart disease; PLSVC: persistent left superior vena cava; SVD: spontaneous vaginal delivery; NT: nuchal translucency; DV: ductus venosus.

was suggested. After adequate counseling, the couple opted for prenatal diagnosis by CVS. Karyotype, Comparative Genomic Hybridization array (a-CGH) and gene-specific DNA analysis were performed from CVS. The fetus exhibited a normal male karyotype (46, XY) and a normal a-CGH result.

The DNA analysis showed the mutation c(0).1286-1G > T in *MID1* gene, thus confirming, within 1 week, the sonographic XLOS suspicion by molecular diagnosis.

After counseling, the couple opted for terminating the pregnancy.

Discussion

Very few cases describing ultrasound features of fetuses affected by XLOS were previously reported (Table 1) [14–17]. All data refer to fetuses in the second or third trimester of gestation; they include brain (enlarged cisterna magna, abnormal cerebellum), facial (cleft lip, hypertelorism) and cardiac anomalies and hypospadias.

Some of these defects were detected in the presented case, who as early as at 12 weeks of gestation presented with an increased NT associated with a cleft lip and palate, an enlarged fourth ventricle, single umbilical artery, a unilateral club foot and agenesis of ductus venosus. Some of these findings could be suggestive of Patau or Edward's Syndromes, which were excluded by the karyotype analysis.

Cleft lip and palate is the most common facial abnormality characterizing XLOS with an incidence of around 50% in males with identified *MID1* mutations [3,18]. Diagnosis of cleft lip and palate in the first trimester is feasible, and a detection rate for genetic anomalies of about 24% has been reported [19].

Brain abnormalities, including increased fourth ventricle, have an incidence ranging from 6.3 to 51% in males with *MID1* mutations [3,18]. Also abnormalities of posterior fossa as well agenesis of ductus venosus have been previously reported in maternally inherited XLOS [14,17]. NT increase might be ascribed to this last defect.

Associations between single umbilical artery, club foot, and XLOS have not yet been described, even though skeletal abnormalities are reported in 5.9% of affected males [18].

To the best of our knowledge, this is the first case reporting ultrasound features of XLOS in the first trimester. The clinical history and the abnormal thickness of NT have induced the accurate study of the anatomy of the fetus, with particular attention to districts generally interested in XLOS. The malformations detected during pre-CVS ultrasonography were in themselves nonspecific, which makes it difficult to characterize the condition based on the clinical signs alone. In this case, the personal history strongly suggested the prenatal diagnosis of XLOS, even though karyotype and a-CGH were required to exclude the presence of chromosomal abnormalities.

This case report demonstrates that in a fetus carrying a *MID1* mutation, ultrasound abnormalities related to XLOS may be possible also in the first trimester if anatomical regions poorly studied during a standard early ultrasonographic examination are investigated. Indeed an expert operator may identify a significant proportion of fetal anomalies during the first-trimester scan. For example, detection rate might reach up to 100% for cleft lip and palate and around 40% for limb defects.

In conclusion, in agreement with recent literature [20], this report highlights that, in cases of fetal multiple malformations in which chromosome analysis fails to detect a structural anomaly, exome sequencing can add clinically relevant information. This suggests that an integrated ultrasound-molecular approach is necessary to allow proper genetic counseling and decision making in the pregnancy.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Antonella Izzo http://orcid.org/0000-0002-2672-4803
Gabriele Saccone http://orcid.org/0000-0003-0078-2113

References

[1] Meroni G. X-linked Opitz G/BBB syndrome. In: Adam MP, Ardinger HH, Pagon RA, editors. GeneReviews. Seattle (WA): University of Washington, Seattle; 1993.
[2] Cox TC, Allen LR, Cox LL, et al. New mutations in MID1 provide support for loss of function as the cause of X-linked Opitz syndrome. Hum Mol Genet. 2000;9(17):2553–2562.
[3] De Falco F, Cainarca S, Andolfi G, et al. X-linked Opitz syndrome: novel mutations in the MID1 gene and redefinition of the clinical spectrum. Am J Med Genet. 2003;120A(2):222–228.
[4] Mnayer L, Khuri S, Merheby HA, et al. A structure-function study of MID1 mutations associated with a mild Opitz phenotype. Mol Genet Metab. 2006;87(3):198–203.
[5] Pinson L, Augé J, Audollent S, et al. Embryonic expression of the human MID1 gene and its

mutations in Opitz syndrome. J Med Genet. 2004; 41(5):381–386.
[6] So J, Suckow V, Kijas Z, et al. Mild phenotypes in a series of patients with Opitz GBBB syndrome with MID1 mutations. Am J Med Genet. 2005;132A(1):1–7.
[7] Schweiger S, Foerster J, Lehmann T, et al. The Opitz syndrome gene product, MID1, associates with microtubules. Proc Natl Acad Sci USA. 1999;96(6): 2794–2799.
[8] Short KM, Hopwood B, Yi Z, et al. MID1 and MID2 homo- and heterodimerise to tether the rapamycin-sensitive PP2A regulatory subunit, alpha 4, to microtubules: implications for the clinical variability of X-linked Opitz GBBB syndrome and other developmental disorders. BMC Cell Biol. 2002;3(1):1.
[9] Trockenbacher A, Suckow V, Foerster J, et al. MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. Nat Genet. 2001;29(3):287–294.
[10] Normand EA, Braxton A, Nassef S, et al. Clinical exome sequencing for fetuses with ultrasound abnormalities and a suspected Mendelian disorder. Genome Med. 2018;10(1):74.
[11] Cicatiello R, Pignataro P, Izzo A, et al. Chromosomal microarray analysis versus karyotyping in fetuses with increased nuchal translucency. Med Sci. 2019;7(3):1–8.
[12] Maruotti GM, Frisso G, Calcagno G, et al. Prenatal diagnosis of inherited diseases: 20 years' experience of an Italian Regional Reference Centre. Clin Chem Lab Med. 2013;51(12):2211–2217.
[13] Carsana A, Frisso G, Tremolaterra MR, et al. A larger spectrum of intragenic short tandem repeats improves linkage analysis and localization of intragenic recombination detection in the dystrophin gene: an analysis of 93 families from southern Italy. J Mol Diagn JMD. 2007;9(1):64–69.
[14] Hogdall C, Siegel-Bartelt J, Toi A, et al. Prenatal diagnosis of Opitz (BBB) syndrome in the second trimester by ultrasound detection of hypospadias and hypertelorism. Prenat Diagn. 1989;9(11):783–793.
[15] Tajima H, Itoh H, Mochizuki A, et al. Case of polyhydramnios complicated by Opitz G/BBB syndrome. J Obstet Gynaecol Res. 2010;36(4):876–881.
[16] Cheng YK, Huang J, Law KM, et al. Prenatal diagnosis of maternally inherited X-linked Opitz G/BBB syndrome by chromosomal microarray in a fetus with complex congenital heart disease. Clin Chim Acta. 2014;436:140–142.
[17] Spinelli M, Sica C, Dallapiccola B, et al. The challenge of prenatal diagnostic work-up of maternally inherited X-linked Opitz G/BBB: case report and literature review. Case Rep Obstet Gynecol. 2015;2015:1–5.
[18] Li B, Zhou T, Zou Y. Mid1/Mid2 expression in craniofacial development and a literature review of X-linked opitz syndrome. Mol Genet Genomic Med. 2016;4(1):95–105.
[19] Chaoui R, Orosz G, Heling KS, et al. Maxillary gap at 11–13 weeks' gestation: marker of cleft lip and palate. Ultrasound Obstet Gynecol. 2015;46(6):665–669.
[20] Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet. 2019; 393(10173):758–767.