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CASE REPORT

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MED12-related Hardikar syndrome: Two additional cases and novel phenotypic features

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Abstract

Hardikar syndrome (HS) is a *MED12*-related ultra-rare multiple congenital malformation syndrome known to affect the gastrointestinal, cardiac, and genitourinary systems among other features including cleft lip/palate and pigmentary retinopathy. Only 10 patients affected with HS have been previously described in literature, of which seven were molecularly confirmed. We report a 20-year-old and a 13-month-old patient with HS diagnosed by exome sequencing bringing the total number of clinically diagnosed cases to 12 and *MED12* associated to 9. We describe previously unreported molecular and clinical findings associated with HS and review all reported cases to permit prompt diagnosis, appropriate management, and genetic counseling of HS patients.

KEYWORDS
aneurysm, cholangiocarcinoma, Hardikar syndrome, *MED12*, X inactivation

1 | INTRODUCTION

Hardikar syndrome (HS; OMIM #301068) is an exceptionally rare pattern of malformation syndrome which was first described by Hardikar et al. in 1992 in two patients (Hardikar et al., 1992). Cardinal features of HS include cleft lip and/or cleft palate, biliary anomalies, liver disease, intestinal malrotation, pigmentary retinopathy, coarctation of the aorta, hydronephrosis, and ectopic ureters. Following the initial report by Hardikar et al. describing two unrelated girls with obstructive hepatic cholestasis and cholangitis, as well as cleft lip/palate, hydronephrosis/hydroureter, and retinal pigmentation, Cools and Jaeken in 1997 reported similar phenotype in a newborn girl (Cools & Jaeken, 1997; Hardikar et al., 1992). Later, Maluf et al. in 2002 and Poley and Proud in 2008 described two girls with a clinical diagnosis of HS who shared the phenotype of cleft lip/palate, biliary system abnormalities, growth retardation, and pigmentary retinopathy (Maluf et al., 2002; Poley & Proud, 2008). In 2021, Li et al. identified nonsense or frameshift variants in *MED12* by exome sequencing in seven females with HS, including two previously reported patients confirming the *MED12* gene–disease relationship (Cools & Jaeken, 1997; Li et al., 2021; Poley & Proud, 2008). Five of these seven girls underwent further X-inactivation studies and they all showed a skewed pattern. HS is now understood to be a female specific multiple congenital anomaly syndrome associated with nonsense or frameshift pathogenic variants in *MED12*. MED12 (mediator complex subunit 12) (OMIM* 300188) is a component of Mediator which is a multiprotein complex that can function in transcriptional activation or repression. In contrast to other previously described *MED12*-related disorders (Optiz Kaveggia/FG syndrome, OMIM#305450; Lujan syndrome, OMIM#309520; X-linked Ohdo syndrome, OMIM#300895; and nonspecific intellectual disability), cognitive abnormalities are not typical for HS.

Here, we describe two additional patients with HS with novel pathogenic variants in *MED12*. This brings the number of individuals

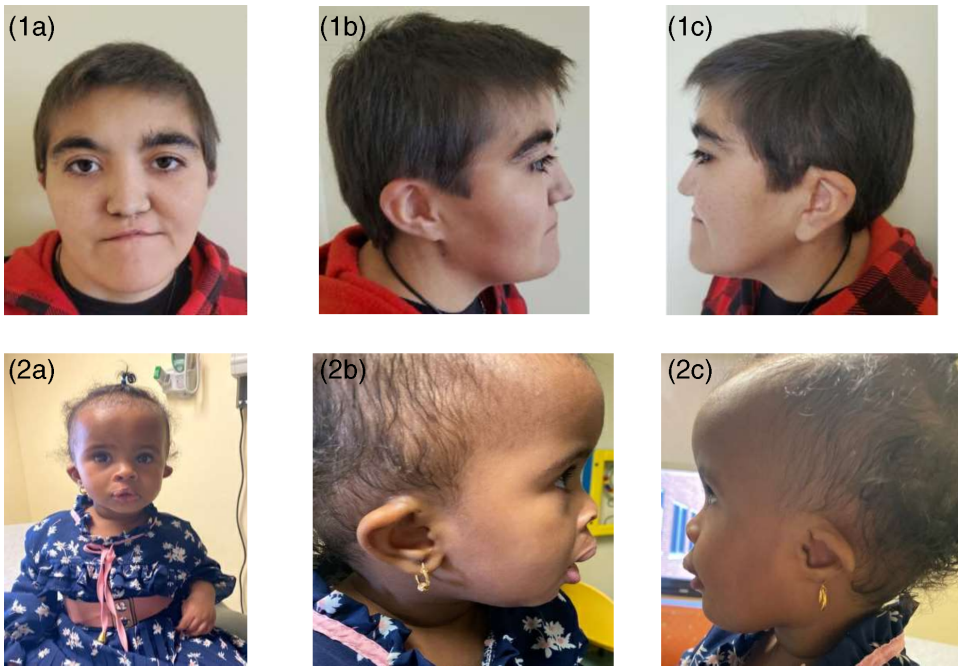

FIGURE 1 Craniofacial features of P1 (a) Short forehead, low anterior hairline, long eyelashes, thick and arched eyebrows, low hanging columella, short philtrum, thin upper lip, broad mouth, and prominent chin. (1b) and (1c) Low-set ears, overfolded superior helix, bilateral preauricular pits, and short neck. Craniofacial features of P2 showing (2a) Broad forehead, deep set eyes, telecanthus, and cleft lip s/p repair. (2b) Low set posteriorly rotated ears, ear pit near superior helix, overfolded superior helix, hypoplastic crux helix and ear lobe. (2c) Low set, posteriorly rotated ears, overfolded superior helix, ear pit near superior helix, underdeveloped antihelix, ear lobe and crux helix

in the literature with *MED12*-related HS to nine. In addition, we describe previously unreported clinical features expanding the phenotypic spectrum of this rare genetic disorder.

2 | CASE REPORT

2.1 | Patient 1

Patient 1 (P1) is a transgender individual (sex assigned at birth was female) who presented to genetics clinic at the age of 18 years for evaluation of complicated medical history, which includes cleft lip and palate, coarctation of aorta, intestinal malrotation, ureteral stenosis, chronic kidney disease, uterine anomalies, growth hormone deficiency, short stature, and chorioretinal lesions. The patient was noted to have multiple dysmorphic facial features (Figure 1). Growth parameters at the time of initial evaluation included height 151.1 cm (3rd centile; Z score $= -1.88$), weight 44.5 kg (2nd centile; Z score $= -1.99$) and head circumference 53.5 cm (15th centile; Z score $= -1.02$). Mid-parental height is at 163.8 cm (50th percentile).

In terms of past medical history, the patient was born at 34 weeks' gestation. Pregnancy history is otherwise unknown to adoptive family. The patient was admitted in the NICU for about 3 months and was diagnosed with ureteral stenosis requiring ureterostomy at 4 days of life. Cystoscopy revealed partial bladder agenesis. Patent Ductus Arteriosus (PDA) ligation was performed at Day 6 of life. Ladd's procedure and gastrostomy tube placement were performed at the age of 2 months for intestinal malrotation. The patient also underwent cleft lip and cleft palate repair in infancy.

Kidney transplant was performed at age 4 years for end stage renal disease secondary to obstructive uropathy. P1 underwent pharyngeal flap closure for velopharyngeal insufficiency at 6 years. Growth parameters at age 8 years 7 months were height 116 cm (<3rd centile; Z score $= -2.61$), and weight 24.9 kg (27th percentile; Z score $= -0.61$). Growth hormone deficiency was diagnosed and growth hormone was started around this time. Brain magnetic resonance imaging (MRI) revealed a benign appearing cystic structure in the sella displacing the pituitary posterosuperiorly, which remained stable over the years.

Spine X rays were obtained at age 11 years for concern for spine deformity. These revealed anomalies of the spine including C2/C3 retrolisthesis, incomplete fusion of S1 posterior elements, Sprengel deformity, and kyphosis. Neuropsychology evaluation performed at age 12 years showed perceptual reasoning, working memory, and processing speed to be within normal ranges. Full-scale intellectual quotient was 89. Our patient's verbal comprehension fell in the slightly below average range. There was no history of behavioral or emotional difficulties. P1 completed high school.

Atypical coarctation of aorta was diagnosed at age 12 years and needed stenting. At 14 years, an abdominal ultrasound undertaken for work up of mild bilirubin elevation revealed mildly large bile duct for age and small, unusual appearance of gall bladder with potential internal septations and wall thickening. The bilirubin levels down trended and normalized spontaneously. Liver enzymes were normal. During an evaluation for chronic lower abdominal pain, asymmetric rudimentary bicornuate noncommunicating uterus with hematosalpinx and hematometra were identified. The patient underwent abdominal hysterectomy and bilateral salpingectomy at 16 years. An ophthalmology evaluation was performed at age 18 years due to vision concerns. This led to the identification of large areas of photoreceptor loss in the inferior macula and superior arcade with trace intraretinal fluid bilaterally, chorioretinal lesions and esotropia.

Prior genetic testing included chromosome analysis, comparative genomic hybridization and RASopathies/*GDF6* next generation sequencing panel, which were negative. Proband-only exome sequencing performed at GeneDx revealed a mosaic (31.58%) pathogenic variant *MED12*: c.4018 C>T p.Q1340X (NM_005120.2). Follow-up X-inactivation studies at Greenwood Genetics Center on blood were highly skewed with ratio of 94:6. Based on the clinical and molecular findings, diagnosis of HS was given.

Brain magnetic resonance angiography (MRA) recommended following diagnosis of HS identified a stable 3 mm left vertebral artery aneurysm and 2 mm right distal posterior cerebral artery aneurysm. Digital subtraction angiography showed a 3.82×3.49 mm left [V4] segment vertebral artery aneurysm. Referral to neurosurgery was made and endovascular aneurysm treatment was planned by neurosurgery.

At age 19 years, the patient presented with right upper quadrant pain, fatigue, poor appetite, and weight loss. A liver ultrasound identified multiple hypoechoic lesions throughout the liver, the largest measuring up to 5.3 cm which was confirmed on MRI. Liver function panel had been normal. Cancer antigen 19-9 (CA 19-9) was elevated at 12,774 (ref: 0–37 U/mL) and normal alpha-fetoprotein and carcinoembryonic antigen levels. Targeted liver biopsy of the larger lesion in the right hepatic lobe revealed an adenocarcinoma, most likely consistent with an intrahepatic cholangiocarcinoma. Origin of the cancer was presumed to be from previously unidentified choledochal cyst. The lesion was deemed unresectable, and patient was considered not to be a candidate for a liver transplant. Palliative chemotherapy was declined by the patient and family. Endovascular brain aneurysm treatment was deferred due to diagnosis of unresectable cholangiocarcinoma. The patient passed away at the age of 20 years.

2.2 | Patient 2

Patient (P2) is a 13-month-old female who was born at 39w3d gestation via C-section to a 29-year-old G1P1 mother. Diagnoses of cleft lip with palate, bilateral urinary tract dilation, and right upper quadrant cyst were made prenatally. The patient's birth weight was 3.2 kg (47th centile; Z score $= -0.1$), length was 51 cm (84th centile; Z score $= +0.99$), and head circumference was 35.5 cm (91st centile; Z score $= +1.37$). Her Apgar scores were 2 and 8 at 1 and 5 min, respectively. She spent 21 days in the hospital. Clinical course was initially complicated by respiratory failure, which required 12 h of continuous positive airway pressure therapy. She was noted to have dysmorphic facial features (Figure 1). No family members had similar features or any known genetic conditions.

Postnatal renal ultrasound showed bilateral severe hydronephrosis with marked bilateral hydroureter, mild dysplasia with parenchymal thinning, and small right renal cyst. Voiding cystourethrogram was significant for bilateral vesicoureteral reflux (VUR; Grade 4–5 on the left and 2 on the right) with incomplete contrast opacification of the upper collecting system. She had a normal renal panel. MRI of the abdomen was significant for cystic structure at the porta hepatis suggestive for an enlarged and irregular gallbladder, as well as additional cystic foci adjacent to the porta hepatis and common bile duct which likely represent choledochal cyst.

Electrocardiogram showed frequent atrial ectopy, whereas echocardiogram was normal. The patient did not pass newborn hearing screening in the left ear. Sedated auditory brainstem response testing at that time demonstrated normal hearing sensitivity in the right ear and severe mixed hearing loss in the left ear. She was fit with a left hearing aid and had pressure equalization tubes placed. Her ophthalmology evaluation in the neonatal intensive care unit was suggestive of subretinal/choroidal depigmented area in clusters. At 12 months of age, her growth parameters were weight: 8 kg (17th centile; Z score $= -0.94$), length: 74.2 cm (51st centile; Z score $= +0.03$) and head circumference: 45.5 cm (66th centile; Z score $= +0.42$). There are no developmental concerns.

Genetic testing for CHARGE, orofaciodigital, and Townes-Brocks syndromes was initially recommended using a custom panel with sequencing and deletion/duplication analysis (*C2CD3*, *C5ORF42*, *CHD7*, *DACT1*, *DDX59*, *IFT57*, *INTU*, *KIAA0753*, *OFD1*, *SALL1*, *SEMA3E*, *TCTN3*, and *TMEM107*). Three variants of uncertain significance were identified: two in *DDX59*: c.536A>G; p.Glu179Gly and c.1200T>A; p.His400Gln (NM_001031725.4) and one in *OFD1*: c.2078G>A; p.Gly693Glu (NM_003611.2). Subsequent parental studies demonstrated that the variants in *DDX59* and the variant in *OFD1* were inherited from asymptomatic mother. Chromosomal microarray was negative. Trio exome sequencing performed at GeneDx identified a de novo heterozygous pathogenic variant in *MED12*: c.2224C>T; p.Gln742X (NM_005120.2). Follow-up X-inactivation analysis on blood at Greenwood Genetic Center, were highly skewed with a ratio of 100:0.

3 | DISCUSSION

Hemizygous missense variants in *MED12* in males are associated with different X-linked intellectual disability phenotypes: Lujan Fryns syndrome (a neurodevelopmental disorder with marfanoid habitus), X-linked Ohdo syndrome (a neurodevelopmental disorder with blepharophimosis) and Optiz Kaveggia/FG syndrome (neurodevelopmental disorder with dysmorphism). These are now considered to be a spectrum of *MED12*-related disorders due to the variability in presentation (Graham Jr. & Schwartz, 2013). Females with missense *MED12* variants were earlier considered to be asymptomatic, though increasing number of cases of affected females with nonsyndromic intellectual disability have now been reported (Bouazzi et al., 2015; Prontera et al., 2016; Charzewska et al., 2018; Wang et al., 2020;; Popp et al., 2017; Polla et al., 2021). In 2013, Lesca et al. described multiple females with a phenotypic spectrum from asymptomatic to profound intellectual disability in a family with a single nucleotide duplication in exon 41 resulting in frameshift in *MED12* (c.5898dupC; p.Ser1967GlnfsX84) but, none of them were described to have the typical phenotype seen in HS (Lesca et al., 2013). Li et al.

TABLE 1 Phenotypic and genotypic features present in all Hardikar patients reported to date

Cases	Hardikar et al. (1992)		Cools and Jaeken (1997) (also P5 from Li et al.)	Maluf et al. (2002)	Poley and Proud (2008) (also P4 from Li et al.)	Li et al. (2021)					Current patients		Percentage of individuals affected
	P1	P2				P1	P2	P3	P6	P7	P1	P2	NA
Age at diagnosis	11 years	2 years	1 year	12 months	7 years	1 week	14 months	1 year	8 months	19 months	20 years	13 months	NA
Genotype (variant in *MED12*)	NA	NA	c.5111G>A; p.W1704*	NA	c.4903_4906delinsCCAGCA; p.V1635Pfs*61	c.322C>T; p.R108* (mosaic 15% in blood)	c.2207_2210del; p.T736Ifs*43	c.2663dup; p.L889Pfs*11	c.5622C>A; p.Y1874*	c.6169C>T; p.Q2057*	c.4018C>T; p.Q1340X (mosaic 31.58% in blood)	c.2224C>T; p.Q742X	NA
X-inactivation	NA	NA	99:1	NA	97:3	89:11	99:1	NA	97:3	ND	94:6	100:0	NA
Development delay	NA	NA	NA	No	No	NA	No	No	No	No	No	NA	0/12 (0%)
Growth failure	+	−	+[a]	+	+	−	−	+	+	−	+[a]	−	7/12 (58%)
Cleft lip/palate	+	+	+	+	+	+	+	+	+	+	+	+	12/12 (100%)
Dysmorphic facial features	−	−	−	−	+	−	+	+	+	+	+	+	7/12 (58%)
Pigmentary retinopathy	+	+	+	+	+	−	−	+	+	−	+	+	9/12(75%)
Cardiac abnormalities	+	+	+	−	+	+	+	+	+	+	+	+	11/12 (92%)
Biliary system anomalies	+	+	+	+	+	+	+	+	+	−	+	+	11/12 (92%)
Intestinal anomalies	+	+	+	+	+	−	+	−	+	+	+	−	9/12 (75%)
Genitourinary anomalies	+	+	+	+	+	+	+	+	+	−	+	+	11/12 (92%)

Note: Cardiac anomalies including aortic coarctation or dilation, pulmonic stenosis, patent ductus arteriosus (PDA), carotid artery aneurysm, and partial anomalous pulmonary venous return. Biliary anomalies reported: cholestasis, congenital absence of gall bladder, choledochal cyst, biliary atresia, cholangiocarcinoma. Intestinal anomalies reported: malrotation, Meckel's diverticulum, imperforate anus, constipation. GU anomalies reported: hydronephrosis, megaureter, vaginal atresia, bladder exstrophy, ectopic ureters.

[a]Growth failure due to growth hormone deficiency.

recently described the association of loss-of-function variants in *MED12* with HS in females (Li et al., 2021). Polla et al., also recently described seven females with de novo nonsense variants in the last four exons of *MED12* who presented with intellectual disability, muscular hypotonia and brain anomalies including partial hypoplasia of the corpus callosum, short stature, skeletal abnormalities and dysmorphic facial features (Polla et al., 2021). Interestingly, none of them had the classic phenotype of Hardikar syndrome. It is believed that nonsense or truncating variants when present in the N-terminal end of *MED12* results in Hardikar syndrome while, in the C-terminal end results in a different syndromic form with intellectual disability, dysmorphic features, short stature, and skeletal anomalies (Plassche & Brouwer, 2021).

HS is a well-recognized syndrome characterized by the presence of multiple congenital anomalies including cleft lip and palate, pigmentary retinopathy, liver and biliary tract disease, intestinal malrotation, foregut malformations, intestinal malrotations, and genitourinary (GU) anomalies. Similar to the previously described cases on HS, both of our patients had dysmorphic facial features, cleft lip and palate, chorioretinal, genitourinary, and biliary tract anomalies (Table 1). Patient (P1) also had coarctation of aorta, which has been described in association with HS before. Interestingly, follow up of aortic coarctation in the patient described by Poley and Proud led to the detection of carotid artery aneurysm. Similarly, the patient initially described by Cools and Jaeken had an unprecipitated intracranial hemorrhage at 21 years of age which was fatal, the cause of which remains unknown. Hence, Li et al. proposed that these individuals are at increased risk of aneurysms in head and neck. Here, we describe a third patient (P1) who was found to have vertebral artery aneurysm and cerebral aneurysm reiterating the necessity for evaluation of vascular anomalies once the diagnosis of HS is confirmed.

There were some previously undescribed features we noted in P1. P1 is the first patient to our knowledge with uterine involvement in HS presenting as asymmetric uterine horns causing hematosalpinx and hematometra. Similarly, vertebral anomalies and cystic structure in sella have not been reported before. Even though growth failure has been well documented in individuals with HS, our patient P1 is only the second in the medical literature known to be diagnosed with growth hormone deficiency requiring growth hormone supplementation.

Biliary ductal abnormalities and ductal plate malformations have been reported in most patients diagnosed with HS including intra- and extra-hepatic ductal dilatations, choledochal cyst, biliary atresia, and hepatic vascular anomalies. There have been two reported cases of a liver transplant performed for biliary cirrhosis (Hardikar et al., 1992; Maluf et al., 2002). Our patient P1 was found to have a cholangiocarcinoma at 19 years of age presumed to be related to a previously undiagnosed choledochal cyst (CC). CC is a known risk factor for biliary malignancy. The most common cancer associated with CC is an adenocarcinoma. There is a 20–30-fold risk for cholangiocarcinoma (CCA) compared to the general population with a mean age of diagnosis at 32 years and the youngest reported case in a 10-year-old (Søreide & Søreide, 2007). In a recent review, the risk of malignancy was reported to increase with every decade with lowest of 0.4% in 0–18 years of age to 38.2% at 60 years and older (Sastry et al., 2015). Surgical intervention in the form of resection appears to mitigate the risk of cancer. Mortality rates are high for CCA presumably due to late diagnosis in most cases and high risk of recurrence. Liver transplant could be considered in highly selective group of patients with early stage perihilar type of CCA. In most cases, palliative chemotherapy with or without biliary stenting is performed to prolong survival.

Evaluation for liver disease has been proposed as a standard of care for patients with HS (Li et al., 2021). This can range from a liver panel to a screening ultrasound. Patients with symptomatic liver disease are usually evaluated early on in their course. However, in patients with asymptomatic liver disease in the presence of biliary ductal or gall bladder anomalies seen on screening ultrasound, further imaging should be obtained e.g., computed tomography (CT) or magnetic resonance cholangiopancreatography (MRCP) to better characterize the biliary tree.

We recommend (1) annual screening with liver tumor markers in presence of persistent biliary ductal abnormalities on imaging or unresected choledochal cysts. In addition, we recommend (2) clinical evaluation of spine at each visit and spine X rays as needed. (3) Growth monitoring at each visit through childhood to allow for detection and treatment of growth hormone deficiency. We suggest these three additions to the previous standard of care evaluations in HS proposed by Li et al (Li et al., 2021) (Table S1, Supporting Information). To the best of our knowledge, this case series is the second report of HS associated with nonsense or frameshift variants in *MED12*. Moreover, this is the first report that describes the increased risk for CCA associated with CC in HS reiterating the importance of annual screening for the same. Furthermore, P1 is one of the few adults with HS described in literature, providing more prognostic information for clinicians and families.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Nishitha R. Pillai: Conceptualization; writing – original draft; review and editing. **Dana Miller**: Writing – original draft; review and editing. **Grace Bronken**: Writing – original draft; review and editing. **Amrita Kahlon Salunke**: Writing – original draft; review and editing. **Anjali Aggarwal**: Conceptualization; writing – original draft; review and editing. All authors have reviewed and approved the final version of the article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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