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Dandy-Walker Malformation in a Girl with *DDX3X*-Related Intellectual Disability

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Established Facts

- Patients with DDX3X syndrome show variable clinical features with different degrees of intellectual disability and/or developmental delay, hypotonia, epilepsy, movement disorders, autism spectrum disorder, and aggressiveness.
- Brain imaging of these cases showed corpus callosum hypoplasia, ventricular enlargement, and polymicrogyria. Moreover, few cases showed small anterior commissure, small pons, and small inferior vermis.

Novel Insights

- We describe new clinical and brain imaging findings such as congenital diaphragmatic hernia and Dandy-Walker malformation which were not reported before in patients with *DDX3X* variants.

Keywords
Dandy-Walker malformation · DDX3X · Autism spectrum disorder · Intellectual disability

Abstract

Introduction: We report on a 4-year-old female patient who presented with severe intellectual disability, autistic features, hyperlaxity of joints, and progressive scoliosis. Whole-exome sequencing identified a de novo missense variant (c.976C>T; p.Arg326Cys) in *DDX3X*. ***Case Presentation:*** The girl was born with congenital diaphragmatic hernia a finding which had not previously been associated with variants in *DDX3X*. Her brain MRI showed hypogenesis of corpus callosum, ventriculomegaly, frontal and perisylvian polymicrogyria, and hypoplastic pons in addition to Dandy-Walker malformation. ***Conclusion:*** Our results confirmed the phenotype and genotype correlation of missense variants and the polymicrogyria. Moreover, it further expands the knowledge of the phenotypic and molecular features of *DDX3X*-related intellectual disability.

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Introduction

Patients with *DDX3X* variants (OMIM #300958) show variable clinical features with different degrees of intellectual disability (ID) and/or developmental delay, microcephaly, hypotonia, epilepsy, movement disorders, autism spectrum disorder, and aggressiveness. Brain imaging of these cases showed corpus callosum hypoplasia, ventricular enlargement, and polymicrogyria (PMG). Moreover, few cases showed small anterior commissure, small pons, and small inferior vermis [Nakata et al., 2009; Calviello et al., 2019]. All reported pathogenic *DDX3X* variants identified in affected females were de novo, while in males variants were maternally inherited [Snijders Blok et al., 2015; Tang et al., 2021].

In mouse model, *DDX3X* was required for embryogenesis, hindbrain development, corticogenesis, and synaptogenesis [Lennox et al., 2020]. Altered somatosensation [Scala et al., 2019] and motor dysfunction were emerging as a critical symptom and one of the earliest signs in neurodevelopmental disorders [Patmore et al., 2020] and were captured in the phenotype of several mouse models that raised the clinical concern and a prominent trait in *DDX3X* syndrome. Disorganized radial glial cells basal processes, aberrant proliferation, and defective neuronal migration were hypothesized to cause PMG [Jamuar and Walsh, 2015]. Likewise, impaired neuron generation and survival underlie microcephaly.

This report describes a new female patient with *DDX3X*-related ID. In addition to the characteristic clinical and brain imaging findings, our patient presented with diaphragmatic hernia and Dandy-Walker malformation which were not reported before, thus expanding the phenotypic spectrum of the disorder.

Clinical Report

A female patient aged 4 years and 6 months was referred to the Outpatient Clinic of Clinical Genetics Department, National Research Centre, because of developmental delay. She was born to a healthy consanguineous couple from Egypt, a 35-year-old father and a 29-year-old mother at her birth (Fig. 1a). She was born at term by caesarean section without complications after an uneventful pregnancy. However, reduced fetal movements were noticed by the mother. Her birth weight was 1.750 kg (−4.6 SD), but the height and head circumference at birth were not documented.

On examination, her weight was 14.5 kg (−1.6 SD), height was 103 cm (−1.5 SD), and head circumference 50.5 cm (mean). The girl had triangular face, broad prominent forehead, prominent supraorbital ridges, arched brushy eye brows, deep-set eyes, prominent nasal bridge, with prominent columella, long philtrum, straight corners of mouth with thin lips (Fig. 1b), low-set and posteriorly rotated ears (Fig. 1c) with thick helix and prominent antihelix, neck webbing, and kyphoscoliosis at back. In addition, hyperextensibility of all joints, bilateral simian creases and abnormal dermatoglyphic, clinodactyly of 5th finger, and overriding between 2nd and 3rd toes of the feet were noted. She had delayed milestones of motor and mental development; supported the head at the age of 1 year, supported at 2 years, crawled at 4 years but unable to walk alone till now. The girl had no speech and dependency upon others for all activities of daily living. She had poor visual attentiveness. Also, there was poor response to name-calling. Drooling was also present. No history of seizures until now. Hypertonia, brisk reflexes, and positive Babinski were documented. She had a normal 46,XX karyotype on chromosome banding analysis.

Ophthalmologic evaluation revealed abnormal upper corneal opacity of left eye and bilateral oval disc. Echocardiography showed atrial septal defect. Hearing test, auditory brainstem response, and abdominopelvic ultrasound showed normal results. X-ray of the spines showed kyphoscoliosis (Fig. 1d). The patient experienced an operation for diaphragmatic hernia at the age of 4 months (Fig. 1e). Brain MRI showed cortical atrophic changes, hypogenesis of corpus callosum, ventriculomegaly, frontal and perisylvian PMG and hypoplastic pons in addition to the new finding of Dandy-Walker malformation (Fig. 1f–i).

Methods

Genomic DNA was extracted from blood lymphocytes of the patient and her parents after having a signed informed consent aligned with the guidelines of the Research Ethical Committee of the NRC (Approval number: 19259). DNA was extracted using Qiagen Blood DNA Kit (Qiagen, Germany). Whole-exome sequencing was performed for the patient using SureSelect Human All Exome 50 Mb Kit (Agilent, Santa Clara, CA, USA) and Illumina HiSeq2000 (Illumina, San Diego, CA, USA). The sequence reads (~100× coverage) were aligned to hg19 and variants using Bowtie2 aligner. Variants were called using SAMtools and annotation was done using Illumina BaseSpace Variant Interpreter Server. Allele frequencies of identified variants were checked against public genetic databases like gnomAD (https://gnomad.broadinstitute.org/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), Exome Variant Server (http://evs.gs.washington.edu/EVS/). Sanger sequencing was then used for confirmation and co-segregation of the causative variant in the patient and available family members.

Results

Exome sequencing revealed a heterozygous missense variant of the *DDX3X* gene (NM_001356.4: c.976C>T: p.Arg326Cys). This variant results from the substitution of arginine at codon 326 by cysteine. The c.976C>T (p.Arg326Cys) variant affects a highly

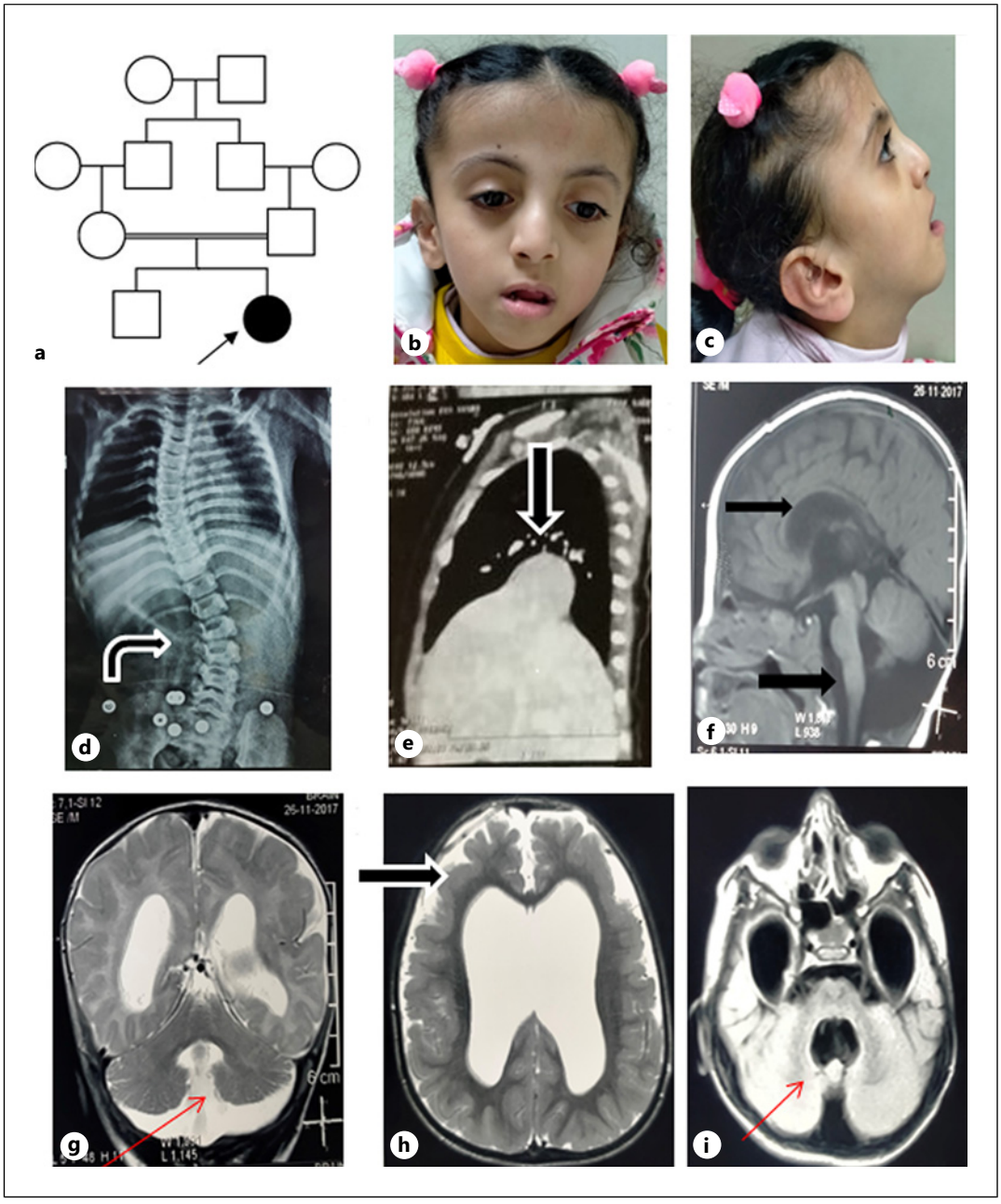

Fig. 1. a Pedigree of the family. **b**, **c** Facial photos of our patient at the age of 4 years showing triangular face, broad and prominent forehead, arched eyebrows, deep-seated eyes (**b**), and-low-set posteriorly rotated ears (**c**). **d** X-ray of the spine showing the kypho-scoliotic changes. **e** The dome shaped diaphragm showing diaphragmatic hernia. **f-i** Brain MRI of our patient showing hypogenesis of corpus callosum, hypoplastic pons (**f**), cerebellar hypoplasia (**g**), frontal, perisylvian polymicrogyria, and ventriculomegaly (**h**), cystic dilatation of the 4th ventricle (Dandy-Walker malformation) (**i**).

conserved residue and is not found in gnomAD or our in-house database of more than 1,500 exomes of Egyptian origin. Furthermore, different bioinformatic tools supported its pathogenicity (online suppl. Table 1; see https://doi.org/10.1159/000531715). Segregation analysis using Sanger sequencing confirmed that the variant is "de novo" as it was not found in the mother's sample (Fig. 2). According to ACMG recommendations of variant classifications, this variant should be classified as "Likely Pathogenic."

Discussion

DDX3X-related ID is a rare condition, and it is difficult to recognize clinically because of the overlapping features with other ID syndromes. The diagnosis is usually established by the identification of pathogenic variants in *DDX3X*. *DDX3X*-related disorders are characterized by a relevant phenotypic variability (Table 1). As regard to the severity of neurodevelopmental involvement and the associated congenital anomalies, our patient had most of the findings

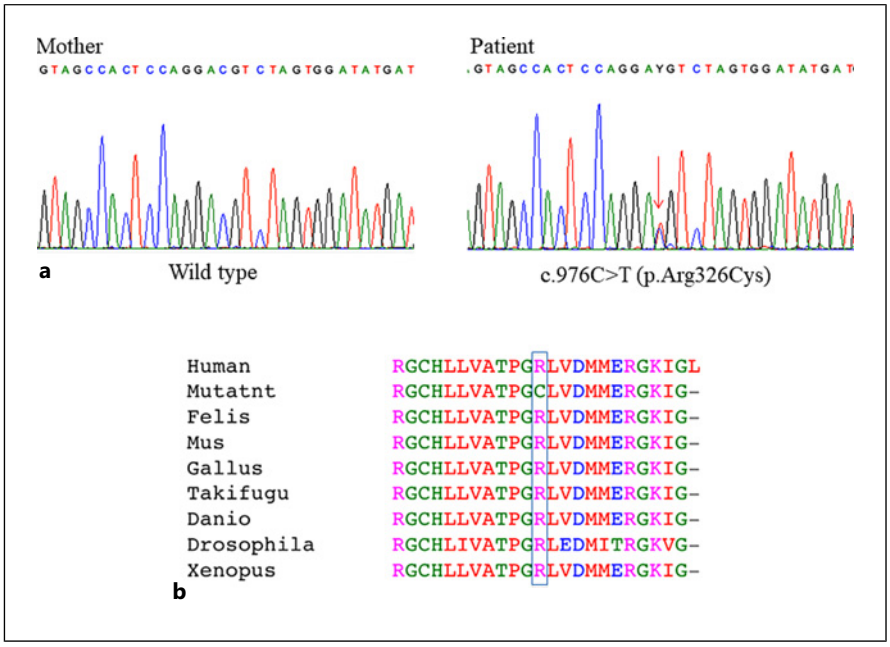

Fig. 2. a Portion of the sequencing chromatogram showing the missense variant c.976C>T (p.Arg326Cys) identified in the *DDX3X* gene in our patient. The arrow indicates the site of variant. **b** The conservation of the p.Arg326 residue across different species.

reported previously in the literature including triangular face, webbing of neck, kyphoscoliosis, and hyperextensibility of all joints, in addition to severe ID/DD. Developmental delay and/or ID are present in the vast majority of patients in previous reports, while 53% of affected individuals showed significant behavioral abnormalities and autistic behavior [Leonard and Wen, 2002: Hu et al., 2015). Diaphragmatic hernia was not reported before in the literature, although severe gastrointestinal symptoms were described before in a Japanese girl with the de novo missense variant (c.1574A>G, p.Tyr525Cys) [Okano et al., 2020].

All patients in previous reports with missense variants displayed a common brain malformative pattern characterized by bilateral frontal and perisylvian PMG and/or dysgyria, callosal hypo-dysgenesis and may be pontine and inferior vermis hypoplasia. This elaborates a striking association between missense variants and PMG [Lennox et al., 2020]. In line with previous reports, our proband showed nearly similar brain malformation pattern. In addition, she had Dandy-Walker malformation which has not been previously reported in *DDX3X*-related disorders. However, Nicola et al. [2019] reported a boy with the missense variant (c.1486G>A, p.Val496Met) and MRI showed a unilateral cerebellar hypoplasia.

Of note, 16% of reported patients with missense variants in the case series of Snijders Blok et al. [2015] developed seizures in whom three cases had PMG. Despite that our proband had PMG, she had not developed seizures until now (Table 1).

In 2015 and 2018, two large case series of 38 and 31 affected females harboring de novo or presumably de novo *DDX3X* variants had been reported by Snijders Blok et al. [2015] and Wang et al., [2018], respectively. Out of the 38 females studied in the series, 35 cases had distinct de novo variants in *DDX3X*. Of them, 15 cases had missense variants and all variants were located in the helicase ATP-binding domain or helicase C-terminal domain. The c.1126C>T (p.Arg376Cys) was recurrent in three unrelated females [Snijders Blok et al., 2015]. Although these two studies suggested that *DDX3X* variants may function via a haploinsufficient mechanism through Wnt signaling [Snijders Blok et al., 2015; Kellaris et al., 2018], more recent observations reported a new mechanism in which some pathogenic missense variants induce the formation of cytoplasmic RNA-protein granules that, in a dominant-negative manner, disrupt translation in neuronal progenitors and neurons [Lennox et al., 2020]. To our knowledge, majority of the reported *DDX3X* variants map within the two helicase domains [Lennox et al., 2020].

The DDX3X protein exhibits a significant level of RNA-independent ATPase activity which is thought to be stimulated by both DNA and RNA. It plays a crucial role in regulating transcription, pre-mRNA splicing and export, translation, and cellular signaling. Therefore, DDX3X deficiency is thought to be associated with impaired neurogenesis and influencing the early embryonic cortical development [Phung et al., 2019]. To date, more than 75 different variants have been reported in the *DDX3X* gene. Majority of these

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Table 1. Comparing the clinical and radiological findings in patients with *DDX3X* variants in the literature

	Snijders Blok et al. (2015)	Dikow et al. (2017)		Kellaris et al. (2018)		Nicola et al. (2019)			Scala et al. (2019)	Okano et al. (2020)	This study
		patient 1	patient 2	patient 1	patient 2	patient 1	patient 2	patient 3	patient 1	patient 1	patient 1
Variant	15 missense/ 9 frameshift/ 6 nonsense/ 4 splice site/ 1 in-frame	missense c.1703C>T (p.Pro568Leu)	missense c.1600C>G (p.Arg534Gly)	missense c.236G>A (p.Arg79Lys)	missense c.236G>A (p.Arg79Lys)	missense c.1127G>A (p.Arg376His)	missense c.1486G>A (p.Val496Met)	missense c.1702C>T (p.Pro568Ser)	missense c.1511G>Ap.(Gly504Glu)	missense c.1574A > G (p.Tyr525Cys)	missense c.976C>T (p.Arg326Cys)
Gender	35F/3M	F	F	M	M	M	M	M	F	F	F
ID/DD	38/ 38 (100%)	+	+	+	+	+	+	+	+	+	+
Microcephaly	12/38 (38%)	+	+	–	–	–	–	+	–	–	–
Growth retardation	12/38 (32%)	NA	NA	NA	NA	NA	NA	NA	+	–	–
Short stature	NA	+	–	NA	NA	NA	NA	NA	–	–	–
Triangular face	+	–	–	–	–	–	–	–	–	+	+
Epilepsy	6/38 (16%)	+	+	NA	NA	NA	NA	NA	NA	NA	–
Behavioral problem/ autistic features	20/38 (53%)	–	–	+	–	–	+	+	+	NA	+
Hypotonia	29/38 (76%)	+	+	–	–	+	–	+	–	NA	–
Spasticity	17/38 (45%)	–	–	+	+	+	+	+	+	NA	+
Intracranial abnormalities (brain MRI)											
Polymicrogyria	4/37 (11%)	–	+	NA	NA	NA	NA	NA	+	–	+
Corpus callosum agenesis\hypogenesis	13/37 (35%)	+	+	+	+	–	–	+	+	–	+
Ventricular enlargement	13/37 (35%)	+	+	+	+	–	–	–	+	+	+
Pontine hypoplasia	NA	NA	NA	NA	NA	NA	NA	NA	+	–	+
Cerebellar malformations (Dandy-Walker variant)	NA	NA	NA	–	–	–	Posterior fossa cyst	–	–	–	Dandy-Walker malformation
Others											
Hyperlaxity of joints	14/38 (37%)	+	+	NA	NA	NA	NA	NA	NA	NA	+
Scoliosis	4/38 (11%)	NA	NA	NA	NA	NA	NA	NA	+	NA	+
Diaphragmatic hernia	NA	NA	NA	NA	NA	NA	NA	NA	–	NA	+

ID, intellectual disability; DD, developmental delay; F, female; M, male; +, present; –, absent; NA, not available.

variants are clustered in the two helicase domains (ATP binding and C-terminal) which are crucial for the protein function [Moresco et al., 2021; Sun et al., 2022].

In this study, we identified a missense variant (c.976C>T, p.Arg326Cys) in *DDX3X* gene. This variant resulted from the substitution of arginine by cysteine at position 326 which is located in the helicase ATP-binding domain. The p.Arg326 is highly conserved across different species. Interestingly, another missense variant affecting the same position (p.Arg326His) had been described before in 3 unrelated patients [Lennox et al., 2020]. Therefore, the p.Arg326 seems to be a hot-spot region for mutations in the gene. Functional studies of the p.Arg326His have shown that it is associated with impaired helicase activity. Deficient *DDX3X* helicase activity results in the enzyme inability to release the bound RNA after ATP hydrolysis and the altered translation of *DDX3X*-dependent targets [Phung et al., 2019].

PMG is strongly linked to missense variants and significant clinical findings. Ten patients with PMG had variants at recurrent amino acid residues (p.Arg326, p.Iso415, and p.Thr532). Variants affecting the p.Arg326 residue were previously associated with PMG, confirming this strong correlation [Snijders Blok et al., 2015). Nevertheless, the most common recurrent variant (p.Arg376Cys) showed an overall mild clinical phenotype. As such, our results support the notion of the recent study by Lennox et al. [2020] on the association of recurrent *DDX3X* missense variants and severe brain malformations including PMG and complete or partial agenesis of the corpus callosum and existence of genotype-phenotype correlations. In conclusion, we believe that our report expands the genotypic and phenotypic disease spectrum and refines the core clinical features associated with *DDX3X* missense variants.

Statement of Ethics

The study was approved by the Medical Research Ethics Committee of the NRC (Approval number: 20105) and followed standard Helsinki Declarations. A written informed consent was obtained from the parents for publication of the details of the medical history of the case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ghada Abdel-Salam and Karima Rafat performed the clinical and neuroradiological evaluation of the patient. Mohamed Abdel-Hamid performed the molecular studies including whole-exome sequencing and segregation analysis by Sanger sequencing. The first draft of the manuscript was written by Karima Rafat. Ghada Abdel-Salam and Mohamed Abdel-Hamid had critically reviewed the manuscript and approved the final version.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon request.

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