DDX3X mutations in two girls with a phenotype overlapping Toriello–Carey syndrome

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Recently, de novo heterozygous variants in *DDX3X* have been reported in about 1.5% of 2659 females with previously unexplained intellectual disability (ID). We report on the identification of *DDX3X* variants in two unrelated girls with clinical features of Toriello–Carey Syndrome (T-CS). In patient 1, the recurrent variant c.1703C>T; p.(P568L) was identified when reconsidering X-linked de novo heterozygous variants in exome sequencing data. In patient 2, the *DDX3X* variant c.1600C>G; p.(R534G) was also detected by exome sequencing. Based on these data, de novo heterozygous *DDX3X* variants should be considered not only in females with unexplained ID, but also in individuals with a clinical diagnosis of T-CS.

KEYWORDS
DDX3X, exome sequencing, intellectual disability, Toriello–Carey syndrome

1 | INTRODUCTION

Mutations in X-chromosomal genes account for approximately 5–10% of the causes of intellectual disability (ID) in males, with fragile X syndrome being the most frequent X-linked ID (XLID) syndrome (Lubs, Stevenson, & Schwartz, 2012). Females with mutations in XLID genes tend to have much lower penetrance and disease severity than hemizygous males (Dobyns et al., 2004),

Web resources: Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

but there is limited data on the proportion of X-linked genes that account for ID in females. Mutations in more than 100 genes on the X chromosome have been identified in males with ID, most of them accounting for a small percentage of patients only (Piton, Redin, & Mandel, 2013). Therefore next generation sequencing (NGS) technologies including large gene panels or exome sequencing (ES) came up as helpful diagnostic tools in males with suspected XLID (Hu et al., 2016; Tzschach et al., 2015). Recently, an increasing number of X-linked genes, previously associated with phenotypes in males, were identified to carry de novo mutations in females with an overlapping but often distinct phenotype. Examples are *PHF6* (Zweier et al., 2013) or *USP9X* (Reijnders et al., 2016). The so far most commonly affected gene is *DDX3X* [MIM: 300160] in which de novo mutations were identified in 1–3% of females with unexplained ID (Snijders Blok et al., 2015). This study was based on the examination of ES data on three different cohorts of individuals with developmental disorders counting up for a total of more than 6.000 individuals.

ES data of a small group of female individuals with unexplained syndromic ID from our institution were filtered for heterozygous autosomal de novo variants as well as for compound heterozygous or homozygous variants. Reconsideration of X-linked de novo heterozygous variants showed a *DDX3X* variant in patient 1, previously diagnosed clinically with Toriello–Carey syndrome (T-CS [MIM: 217980]). Subsequently, a second female patient (patient 2) with syndromic ID and a de novo *DDX3X* variant was noted to share clinical features with T-CS.

2 | MATERIALS AND METHODS

Clinical data, photographs, and results of laboratory testing were obtained with informed consent, including the consent to use the photographs in this report, which has been documented in protocols approved by institutional review boards at the participating institutions.

High resolution molecular karyotyping was performed on a clinical basis for evaluation of ID, multiple congenital anomalies and dysmorphic features using an Affymetrix® 500 K SNP array in patient 1 and Agilent 180 K oligonucleotide array (resolution 70–100 kb) in patient 2, to exclude genomic imbalances.

ES was performed at the German Cancer Research Center (DKFZ), Heidelberg, on DNA of patient 1 and both parents as previously described; analysis of the sequence data was performed using the previously described Heidelberg exome data analysis bioinformatics pipeline (Granzow et al., 2015). ES and variant filtering pipeline are summarized in Table S1.

Patient 2 and both unaffected parents were tested by trio–ES on an Illumina HiSeq system after enrichment with the SureSelect Target Enrichment v5 technology (Agilent Technologies, Santa Clara, CA). After analysis and filtering steps (Table S1), de novo, compound heterozygous, and homozygous variants in 777 known ID-associated genes (status March 2016) (Kochinke et al., 2016) were evaluated.

3 | RESULTS

3.1 | Clinical report

Clinical data of patients 1 and 2 are summarized in Table 1; facial appearance is depicted in Figure 1. Patient 1 was born to healthy non-consanguineous German parents after an uneventful pregnancy with normal birth measurements and multiple congenital anomalies: anal atresia, congenital heart defect (CHD; left coronary artery fistula, ventricular septal defect), laryngomalacia, and malformation of two vertebral bodies. She experienced neonatal respiratory distress. Hypotonia and psychomotor delay were noted from the beginning. She developed infantile spasms at 6 months of age as well as hyperopia. Neonatal hearing screening was inconspicuous; however, at the age of 6 months, bilateral sensorineural hearing loss was detected by brainstem evoked response audiometry (BERA). Cranial MRI was performed at 19 months of age and showed corpus callosum hypoplasia (CCH), ventricular dilatation, and delayed myelination. At the age of 4 years, she showed microcephaly (−3 SD), short stature (−3 SD), dysmorphic facial features (see Fig. 1) and a high and narrow palate. At 10 years of age, she was a severely intellectually disabled girl without speech, unable to sit unsupported with marked muscular hypotonia, scoliosis, and contractures of the knees. A clinical diagnosis of Toriello–Carey syndrome (T-CS) was considered.

Patient 2 is the second child of German non-consanguineous parents. Nuchal hygroma was noted at 10 weeks and intrauterine growth retardation at 24 weeks of pregnancy. She was born after 35 weeks of pregnancy with low normal birth measurements and had surgery for congenital medial diaphragmatic hernia and CHD (atrial septal defect and patent ductus arteriosus). The neonatal period was complicated by necrotizing enterocolitis. Respiratory insufficiency was initially treated with CPAP; at the age of 3 years a tracheostomy became necessary due to hypoventilation. Bilateral sensorineural hearing loss was detected at the age of 3 months by BERA. She showed psychomotor delay, hypotonia, and was tube fed since birth, via gastrostoma tube since 1 year of age, due to severe feeding problems. Xerophthalmia was caused by bilateral lagophthalmos. Since the age of 3 years, she had recurrent luxation of the right knee and developed severe lumbal scoliosis. Seizures developed at the age of 1 year. cMRI at the age of 3 months showed CCH, ventricular dilatation, delayed myelination, and a simplified gyral pattern. At the age of 4 years and 9 months, she presented with severe ID. She had no speech and hardly any head control, microcephaly (−3.4 SD), short stature (−2 SD) and dysmorphic facial features (see Fig. 1). She had small hands with ulnar deviation, adducted thumbs, and a ventral position of the anus.

3.2 | Molecular testing

In patient 1, routine chromosome analysis, Affymetrix 500 K SNP array and targeted sequence analysis of *UBE3B* showed normal results. ES was performed (see Table S1). Evaluation of ES data for X-linked de novo heterozygous *DDX3X* variants showed the variant c.1703C>T; p.

TABLE 1 Clinical features of patients 1 and 2, published females with de novo *DDX3X* variants and published individuals with Toriello–Carey syndrome (T-CS)

	Patient 1 Age 10 y	Patient 2 Age 4 y	DDX3X, Snijders Blok et al (2015)	T-CS Toriello and Carey (1988)	T-CS Toriello et al (2003)	T-CS Toriello et al (2016)
ID or DD	Severe	Severe	38/38	2/2	45/45	3/x
Microcephaly	+ Postnatal −3 SD	+ Postnatal −3.4 SD	12/38	2/2	+ 12/25 (prenatal: 7/x)	2/6
Short stature	+ Postnatal −5 SD	+ Postnatal −2 SD	n.a.	2/2	+ 25/28 (prenatal: 7/x)	4/7 postnatal
Hypotonia	+	+	29/38	4/4	25/x	4/7
Movement disorder (including spasticity)	−	−	17/38	n.a.	n.a.	n.a.
Epilepsy	+	+	6/38	1/x	3/x	2/x
Behavior problems	−	−	20/38	n.a.	n.a.	n.a.
CCH or CCA	+ CCH	+ CCH	13/37 CCH	3/4 CCA	+ CCA 19/x CCH10/x Partial CCA 8/x	5/7
Cortical malformation	−	+	4/37	n.a.	−	n.a.
Ventricular enlargement	+	+	13/37	1/x	4/x	n.a.
Hypermobility	+	+	14/38	1/x	5/x	n.a.
Visual problems	+	+	13/38	n.a.	±	2/x
Hearing loss	+	+	3/38	1/x	11/x	5/7
CL(P) or CP	−	−	3/38	CP 4/4	CP: 25/34	5/7
Precocious puberty	−	n.a.	5/38	n.a.	n.a.	n.a.
Respiratory distress	+	+	n.a.	2/2	8/x	4/7
Laryngeal/Tracheal anomalies	+	−	n.a.	2/2	16/x	3/7
Anal abnormalities	+	+	n.a.	n.a.	5/x	n.a.
Cardiac defects	+	+	−	¾	33/45	4/6
Skeletal anomalies	− bd; + sc	− bd; + sc	n.a. sc: 4/38	1/2; bd: 4/4	19/45; bd: 12/x	4/7
Face						
Short palp. fissures	+	+	n.a.	4/4	44/45	4/7
Hypertelorism/ telecanthus	−	+	5/x	4/4	+/4	5/7
Short/small nose	+	−	n.a.	4/4	40/45	4/7
Thin lips and or downturned	+	+	n.a.	2/2	"generally"	n.a.
Full cheeks	+	+	n.a.	2/2	33/45	1/7
Micrognathia	+	+	n.a.	4/4	40/40	3/7

Not included are individuals with chromosome aberrations causing a T-CS (like) phenotype published after 2003. Legends: +, present; −, absent; bd, brachydactyly; CCA, corpus callosum agenesis; CCH, corpus callosum hypoplasia; CL(P), cleft lip (palate); CP, cleft palate; DD, developmental delay; ID, intellectual disability; n.a., not available; sc: scoliosis; /x, total number of investigated patients not available.

(P568L) in *DDX3X* (NM_001356.4), confirmed with Sanger sequencing. In patient 2, routine chromosome analysis and array CGH showed normal results. ES with analysis of 777 known ID-associated genes showed a de novo heterozygous *DDX3X* variant c.1600C>G, p.(R534G), which was confirmed with Sanger sequencing. Other variants in both patients were unlikely to contribute to the phenotype due to the results of in silico prediction on the variants' functional effects and are summarized in Table S2. Trio data indicated correct paternity in both patients.

4 | DISCUSSION

In patients 1 and 2, two girls with severe ID and a phenotype resembling TC-S, ES revealed de novo *DDX3X* variants. The herewith presented patient 1 carries the same mutation as one of the previously published individuals (individual 37) (Snijders Blok et al., 2015). The *DDX3X* variant p.(R534G) detected in patient 2 has not been reported so far. In silico analyses predicted a damaging effect, and the same amino acid was affected by another missense mutation p.(R534H) in individual five

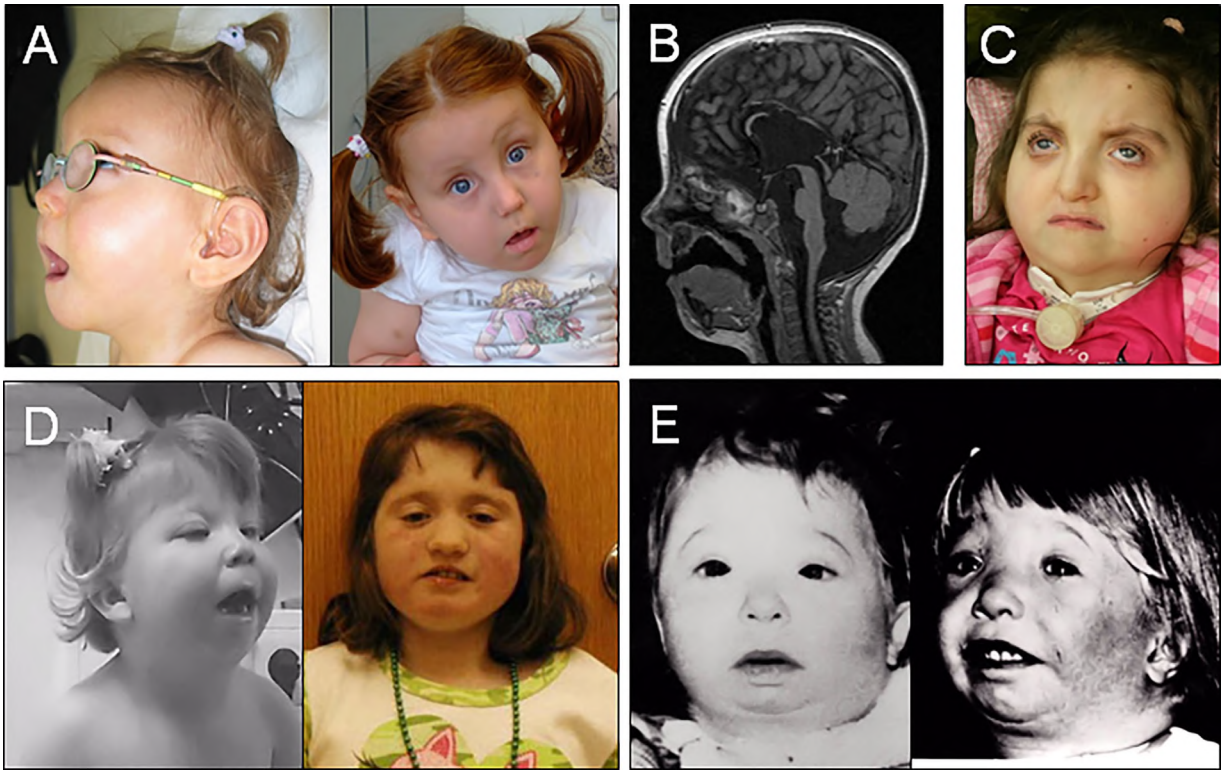

FIGURE 1 (A) Patient 1 at 20 months and 4 years of age. Note flat face with full cheeks, short palpebral fissures (18 mm), a short nose with underdeveloped alae nasi, thin upper lip vermillion, downturned corners of the mouth, and micrognathia. (B) MRI of patient 1 at 19 months of age. Note hypoplasia of corpus callosum. (C) Patient 2 at 4 years of age, showing flat face with full cheeks, widely spaced eyes, short and downslanting palpebral fissures, short philtrum, thin upper lip vermillion, downturned corners of the mouth, and micrognathia. (D) Published patients with T-CS (Toriello et al., 2003: Figs. 1 and 4) showing flat face, short palpebral fissures, and full cheeks. (E) The originally described patients with T-CS (Toriello & Carey, 1988: Figs. 3 and 4) [Color figure can be viewed at wileyonlinelibrary.com]

reported by Snijders Blok et al. Furthermore, the phenotypic accordance strongly points to pathogenicity of this *DDX3X* variant. *DDX3X* encodes a conserved DEAD-box RNA helicase involved in numerous cellular processes, in the RNAi pathway and acting as a regulator of the Wnt/ß-catenin pathway (Snijders Blok et al., 2015). The authors reported de novo *DDX3X* variants in 38 female individuals with mild to severe ID and a variable syndromic presentation including microcephaly, hypotonia, movement disorders, epilepsy, behavioral problems, CCH, and cleft lip (CL) or palate (CP). The clinical features of both patients are consistent with the published *DDX3X* associated phenotype including severe ID, postnatal microcephaly and short stature, hypotonia, epilepsy, CCH, ventricular enlargement, visual problems, hearing loss, hypermobility, and skeletal anomalies. Patient 2 also has cortical malformations, reported in 4 of the 37 published patients with *DDX3X* variants. Both patients 1 and 2 were born with CHD and anal anomalies, which have not been associated to *DDX3X* variants so far, thus widening the clinical spectrum.

Initially, a diagnosis of Toriello–Carey syndrome was considered in patient 1. T-CS is a multiple congenital anomaly disorder with ID, first described in 1988 (Toriello & Carey, 1988). The phenotype initially described consisted of postnatal growth delay and microcephaly, ID, CCA, Robin sequence, laryngeal abnormalities (upper airway issues more than CP), cardiac defects, typical facial features (widely spaced eyes, round or full cheeks, micrognathia), and other anomalies. T-CS was reviewed in 2003 by describing 45 individuals (Toriello et al., 2003), extending the phenotype to a variable clinical picture and including both patients with CCH and with micrognathia or palatal anomalies instead of CP. Very recently, 7 individuals published after 2003 and reported to have T-CS were reviewed (Toriello, Colley, & Bamshad, 2016). Comparing patient 1 to these groups of individuals, there is concordance for most of the features listed in Table 1, making T-CS a tempting clinical diagnosis, even in the absence of CP. The phenotype of patient 2 also shows significant overlap with T-CS.

A commonly mutated gene in T-CS has not yet been identified, and the condition shows many overlapping phenotypes without a clear definition of the core phenotype yet. In 2003, Toriello et al already admitted doubts on the existence of a specific T-CS phenotype both concerning the facial gestalt and the associated congenital malformations. Two different hypotheses are possible with regard to the genetic cause(s) of T-CS. First, there might be one or a small number of underlying genes that have still to be elucidated. Such genes would probably affect a subgroup of the patients with a clinical diagnosis T-CS and the associated core phenotype could be delineated more precisely in a second step. However, ES in nine families with at least one member with T-CS gave no common candidate gene (Toriello et al., 2016).

A second possible explanation might be that T-CS is not a distinct clinical and molecular entity but rather a collection of overlapping phenotypes with unrelated causative defects. Several individuals with suspected T-CS phenotypes were found to carry causative cytogenetic aberrations (McGoey, Varma, & Lacassie; Toriello & Hatchwell, 2008; Toriello et al., 2016), or variants in *UBE3B* (Basel-Vanagaite et al., 2014). Other conditions overlapping with TC-S phenotype were recently reviewed, including cytogenetic (micro-)deletions, XL IGBP1-associated syndrome, and Stevenson–Carey syndrome (Toriello et al., 2016). The here presented patients add *DDX3X*-variants to the differential diagnosis in patients with TC-S phenotype. The clinical picture of more patients with underlying *DDX3X*-variants may overlap with TC-S. Published clinical data of 38 female individuals (Snijders Blok et al., 2015) are suggestive, however not detailed enough for a reliable evaluation.

In conclusion, disease causing *DDX3X* variants were detected in two unrelated females with a phenotype resembling TC-S. This report and the review of literature provide evidence that the T-CS phenotype not only significantly overlaps with phenotypes caused by cytogenetic alterations and single gene alterations such as *UBE3B* variants but also with clinical features associated with *DDX3X* variants. X-linked de novo heterozygous *DDX3X* variants should be considered and the gene, if appropriate, sequenced, not only in females with unexplained ID, but also in individuals with a clinical diagnosis of T-CS.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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