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Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in Ulnar-Mammary syndrome

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Abstract

Ulnar-mammary syndrome (UMS) is characterized by ulnar defects, and nipple or apocrine gland hypoplasia, caused by TBX3 haploinsufficiency. Signs of hypogonadism were repeatedly reported, but the mechanisms remain elusive. We aim to assess the origin of hypogonadism in two families with UMS. UMS was suspected in two unrelated probands referred to an academic center with delayed puberty because of the evident ulnar ray and breast defects in their parents. Clinical, biochemical and genetic investigations proved the existence of congenital normosmic IHH (nIHH) associated with pituitary hypoplasia in the two probands who were heterozygous for novel TBX3 pathogenic variants. The mutations co-segregated with delayed puberty, midline defects (nose, teeth and tongue anomalies) and other variable features of UMS in the two families (absent axillary hairs and nipple hypoplasia, asymmetrical features including unilateral ulnar or renal abnormalities). The combined analysis of these findings and of the previous UMS reports showed delayed puberty and other signs of hypogonadism in 79 and 37% of UMS males, respectively. Proband 1 was followed up to adulthood with persistence of nIHH. In conclusion, UMS should be suspected in patients with delayed puberty and midline defects, including pituitary hypoplasia, in the presence of mild cues for TBX3 mutation, even in the absence of limb malformations. In addition, TBX3 should be included among candidate genes for congenital nIHH.

Introduction

The TBX3 gene encodes a member of the T-box family of transcription factors acting as a repressor of target gene expression. Tbx3 has a role in the specification of posterior limb mesoderm and in the development of the dorsoventral limb axis. The same inductive interaction between epithelial tissue and underlying mesenchyme demonstrated for limb buds had been found in breast, tooth and genital development (1). Recently, it has also been reported that Tbx3 functionality is required for the hormone sensing cell lineage in the mammary epithelium (2).

In humans, loss of Tbx3 function causes ulnar ray defects and hypoplasia/aplasia of the breasts/nipples,
an association that characterizes the ulnar-mammary syndrome (UMS, MIM #181450). Expressivity of these features is usually asymmetrical and variable: as for the upper limbs, from shortening or clinodactyly of the fifth finger to the absence of the third to fifth fingers and ulna with radial shortening or complete absence of the radius/ulna and hand in the most severe cases. All patients exhibit diminished or absent axillary hair and reduced or absent perspiration as further signs of the involvement of apocrine glands; lactation may be absent (3).

Despite the lack of association between TBX3 variants with isolated hypogonadotrophic hypogonadism (IHH), signs of hypogonadism, including bilateral cryptorchidism, micropenis and delayed puberty, have been repeatedly reported among patients with UMS (3, 4, 5, 6, 7, 8, 9). Moreover, the constellation of endocrine manifestations in UMS patients includes short stature, growth hormone deficiency and obesity (3, 5, 6, 10, 11, 12); however, information on hormonal parameters is scarce to date in UMS patients.

In this study, we report on two probands who presented with delayed puberty and were then found to have novel TBX3 pathogenic variants segregating in their families with variable UMS phenotype. Hormonal studies were consistent with congenital normosmic IHH (nIHH), while brain/pituitary malformations were recurrently found in affected patients. Since no variants in candidate genes for HH or hypopituitarism were found, these features can be considered part of the TBX3 loss-of-function-related clinical spectrum. Therefore, endocrinologists should consider the possibility of a TBX3 pathogenic variant in patients presenting with delayed puberty or short stature associated with subtle UMS features and peculiar midline defects.

Subjects and methods

Patients

Both probands were referred for delayed puberty and midline defects. The patients underwent clinical evaluation, baseline and dynamic hormonal testing, as well as genetic analyses after informed consent of the parents. Gonadotrophin-releasing hormone (GnRH) test (100 µg, Relefact, gonadorelin; Sanofi) was performed using standard procedures (intravenous injection and blood sample collection for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) determinations at baseline and +15', +30', +60', +90', +120'). Serum LH and FSH concentrations were measured by specific immunoassays (Elecsys, Roche).

As in UMS each pathogenic variant is known to be unique to the family, all family members were enrolled in the study in order to perform a co-segregation analysis of the predicted pathogenic variants.

Sequencing

The genomic DNA was extracted from peripheral blood lymphocytes using GeneCatcher gDNA Automated Blood Kit, 96 × 10 mL (Invitrogen, Life Technologies). Analysis was performed by targeted next-generation sequencing (NGS) on an Illumina MiSeq sequencer (Illumina) following previously reported methods (13) and using a gene panel which includes known causal genes for IHH and hypopituitarism: CHD7, NR0B1, DUSP6, FSHB, FEZF1, NSMF, LHB, FGE17, PROK2, FGF8, PROKR2, FGRF1, SMAD3, FLRT3, SEMA3E, SNRNP1, SOX10, GNRHR, SOX2, HS6ST1, HESX1, SPRY4, IL17RD, TAC3, ANOS1 (KAL1), TACR3, KISS1, WDR11, KISS1R, LHx3, LHx4, PU01F1, PROP1, GH1/2, GHRH, GHRHR, IGFS1. The total coverage of the target genes was >80%. All uncovered regions were recovered by Nextera DNA Library Preparation Kit (Illumina).

TBX3 was sequenced by either Sanger sequencing or NGS, while the 5’ and 3’ UTRs of the gene were sequenced by Sanger.

Variant analysis

Neither of the TBX3-identified genetic variants had been reported in the SNP database or in the Exome Aggregation Consortium database. The missense variant was predicted to be damaging by eight in silico prediction tools – SIFT (scale-invariant feature transform), PolyPhen2 HVAR, PolyPhen2 HDIV, LRT (likelihood ratio test), MutationTaster, Mutation Assessor, FATHMM (functional analysis through hidden Markov models), PROVEAN and CADD (complete annotation dependent depletion) (14).

UMS database

We analyzed the previous clinical reports of UMS patients who were carriers of a TBX3 pathogenic variant, with special focus on endocrine features and midline or asymmetrical defects. Facial resemblance across unrelated UMS patients (broad/beaked/bifid nasal tip and jaw hypoplasia) (12), bilobated tongue tip and teeth anomalies, were classified as midline defects. The clinical data from all UMS families are reported in the supplemental materials.
Results

Clinical reports

Family A

A 14-year-old boy (III-1, Figs 1A and 2A, B, C, D and E) was referred for endocrine evaluation because of delayed puberty, micropenis (3 cm stretched length), dyslipidemia and obesity (BMI 31.2 kg/m², >95th percentile).

He previously underwent cerebral and spinal magnetic resonance imaging (MRI) for urinary incontinence that besides syringomyelia (Fig. 1C) showed pituitary gland hypoplasia (Fig. 3A), a thin pituitary stalk (Fig. 3A and B) and skull base dysmorphism with clivus horizontalization, consistent with an Arnold Chiari (AC) type 1 malformation (Fig. 3C). He also exhibited peculiar asymmetrical features (Fig. 3C and D). He had normal pituitary functions (insulin-like growth factor 1 (IGF1) = 201 ng/mL, n.v. 141–669; thyroid-stimulating hormone (TSH) = 1.85 mU/L, n.v. 0.27–4.5; free T4 = 15.9 pmol/L, n.v. 11.5–24.5; prolactin (PRLb) = 6.1 ng/mL; n.v. 2.5–17) with prepubertal response of LH and FSH to GnRH test, low inhibin B and undetectable total testosterone (TTe) levels (Table 1). He was normosmic at Brief-Smell Identification test (B-SIT) and normal central olfactory structures were confirmed using MRI. His karyotype was 46,XY. At the age of 15 years, he underwent decompression surgery for AC1 malformation, with diuresis improvement.

He received testosterone enanthate treatment for 9 months, but at 16 years and 11 months, the testis volume was −4.9 SDS (according to Joustra et al. (15)), and puberty was induced with FSH priming (75 IU for three times a week for 4 months) (16), followed by an induction scheme with FSH (150 IU for 3 times a week). Human chorionic gonadotrophin was given starting from a dose of 1000 up to 2000 UI twice a week with increments of 500 UI every 6 months and up-titrating the dose according to TTe levels (4, 17, 18). During the puberty induction, a progressive rise of testosterone and inhibin B was seen (Table 1).
After 15 months, he gained an adult testicular volume (testes 20–25 mL) and penis length (9 cm stretched length and 3.5 cm diameter). At 18 years, he presented with a Tanner stage P5G5A1, and the gonadotropin treatment was withdrawn (Fig. 2E and F). Hypo-testosteronemia recurred (Table 1), and he complained of poor school performance and fatigue that improved during subsequent transdermal testosterone replacement therapy. Absent axillary sweating was repeatedly reported.

His maternal grandfather (I-1, Fig. 1A) was reported to have had pubertal spurt at 17 years and finger hypoplasia. We were able to evaluate his clinical records and photographs of his hands, documenting right fifth finger clinodactyly, left fourth finger camptodactyly and hypoplasia of the left fifth finger distal phalanx (Fig. 2M). His mother (II-2), who reported late menarche (at 14 years), had an asymmetric right ulnar deformity and left breast hypoplasia, with other phenotypic features consistent with UMS (Fig. 2G, H, I, J, K and L), such as almost absent axillary hairs with no sweating from the armpits.

Family B
Proband (IV-2, Fig. 1B) was assessed at the age of 14 years and 1 month because of short stature (height = −1.59 SDS; delta SDS target height (TH) = −2.07). His bone age (BA = 12.8/12 on radius, ulnar and short bones (RUS) and carpal (CARP), Tanner–Whitehouse evaluation) was delayed by 1 year and 5 months compared to his chronological age (CA = 14.1/12). Hormonal testing revealed euthyroid hyperthyrotropinemia (TSH = 14.1 mU/L, n.v. 0.27–4.0, FT4 = 17.9 pmol/L, n.v. 11.5–24.5) with negative antithyroid antibodies and without signs of thyroiditis on
ultrasound. The other pituitary hormones were normal (prolactin (PRL) 8.4 ng/mL, n.v.: 2.5–17; IGF1 182 ng/mL; n.v.: 152–324), but serum determinations showed IHH (TTe=0.31 nmol/L; FSH =1.3 U/L, LH <0.3 U/L). The pituitary MRI showed anterohypophysis hypoplasia with normal pituitary stalk (Fig. 3D and E).

The proband had normal neonatal growth parameters, and his karyotype performed on amniotic liquid for advanced maternal age was 46,XY. He underwent surgery at the age of 5 years for short frenulum of the tongue. At 14 years, he was evaluated for a suspected arrhythmia.

Physical examination showed high forehead, epicanthic folds, broad nasal tip, antverted nostrils, bilobated tongue tip, high palate, slight microretrognathia, asymmetric ears, hypoplasia of nipples, more evident on the left side, and absence of axillary hairs. He was normosmic at B-SIT and referred absent sweating. A clinical re-examination at 15 years and 3 months confirmed short stature (height = -1.85 SDS; delta SDS TH = -2.33) with

### Table 1  Evaluation of gonadal axis of patient III-erom family A, before and after puberty induction.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>At diagnosis</th>
<th>After testosterone enanthate priming</th>
<th>After induction of puberty with FSH + hCG</th>
<th>Two years later</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal Peak</td>
<td>Basal Peak</td>
<td>Basal Peak</td>
<td>Basal Peak</td>
</tr>
<tr>
<td>GnRH test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>0.1 0.4</td>
<td>0.5 4.7</td>
<td>2.1 12.1</td>
<td>1.2 1.9</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>0.7 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>0.05 0.65</td>
<td>20.66 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibin B (pg/mL) adult n.v.: 80–400</td>
<td>70 115</td>
<td>170 166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes (mL)</td>
<td>3–3 3–4</td>
<td>20–25 20–25</td>
<td></td>
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</tr>
</tbody>
</table>

n.v., normal value.
a growth velocity still in the prepubertal range (growth rate=4.5 cm/year, SDS growth rate = −1.87). At physical examination, he was prepubertal (testes 3 mL bilaterally, −2.8 SDS), nIH was again confirmed (FSH=1.32U/L, LH=0.32U/L, TTe=0.41 nmol/L) and puberty was then induced at 16.8 years, following the same protocol of proband of family A. Echocardiogram and ultrasound of the abdomen carried out after the genetic evaluation showed normal findings.

On clinical examination, the proband’s elder sister, 20 years old, and the father, 59 years old (IV-1 and III-2, respectively, Fig. 1B), exhibited similar facial features with broad nasal tip and bilobated tongue tip, as well as absent axillary hairs and sweating, and bilateral symmetrical nipple hypoplasia. The father exhibited ulnar hypoplasia and absence of the left fourth and fifth fingers. His puberty was delayed and had been induced by testosterone priming for 4 months at 16 years of age. He was normosmic at B-SIT. The circulating TSH was 3.48 mU/L with negative anti-thyroid antibodies. The proband’s sister had normal menses but exhibited absence of the right breast and moderate hypoplasia of the left one at ultrasound. She had been diagnosed with idiopathic GH deficiency (GH peak was blunted either after clonidine or insulin tolerance tests: 4.3 or 2.0 ng/mL, respectively) and received rhGH (0.2 mg/Kg/7 days a week) for 6 years from the age of 9.9 years. Her karyotype was 46,XX. Brain MRI displayed a slight downward displacement of the cerebellar tonsils but normal pituitary gland and stalk. She had normal thyroid function with absent anti-thyroid antibodies. She reported delayed menarche (at 13.9 years).

The paternal grandfather and the great grandmother (II-2 and I-2, Fig. 1B), exhibited similar facial features with broad nasal tip and bilobated tongue tip, as well as absent axillary hairs and sweating, and bilateral symmetrical nipple hypoplasia. The father exhibited ulnar hypoplasia and absence of the left fourth and fifth fingers. His puberty was delayed and had been induced by testosterone priming for 4 months at 16 years of age. He was normosmic at B-SIT. The circulating TSH was 3.48 mU/L with negative anti-thyroid antibodies. The proband’s sister had normal menses but exhibited absence of the right breast and moderate hypoplasia of the left one at ultrasound. She had been diagnosed with idiopathic GH deficiency (GH peak was blunted either after clonidine or insulin tolerance tests: 4.3 or 2.0 ng/mL, respectively) and received rhGH (0.2 mg/Kg/7 days a week) for 6 years from the age of 9.9 years. Her karyotype was 46,XX. Brain MRI displayed a slight downward displacement of the cerebellar tonsils but normal pituitary gland and stalk. She had normal thyroid function with absent anti-thyroid antibodies. She reported delayed menarche (at 13.9 years).

Kidney, ovary and uterus abnormalities were ruled out by transabdominal US. The circulating TSH was 4.42 mU/L with normal FT4 (13.7 pmol/L) and negative anti-thyroid antibodies. She reported delayed menarche (at 13.9 years). She had normal thyroid function with absent anti-thyroid antibodies. The proband of family A. Echocardiogram and ultrasound of the abdomen carried out after the genetic evaluation showed normal findings.

On clinical examination, the proband’s elder sister, 20 years old, and the father, 59 years old (IV-1 and III-2, respectively, Fig. 1B), exhibited similar facial features with broad nasal tip and bilobated tongue tip, as well as absent axillary hairs and sweating, and bilateral symmetrical nipple hypoplasia. The father exhibited ulnar hypoplasia and absence of the left fourth and fifth fingers. His puberty was delayed and had been induced by testosterone priming for 4 months at 16 years of age. He was normosmic at B-SIT. The circulating TSH was 3.48 mU/L with negative anti-thyroid antibodies. The proband’s sister had normal menses but exhibited absence of the right breast and moderate hypoplasia of the left one at ultrasound. She had been diagnosed with idiopathic GH deficiency (GH peak was blunted either after clonidine or insulin tolerance tests: 4.3 or 2.0 ng/mL, respectively) and received rhGH (0.2 mg/Kg/7 days a week) for 6 years from the age of 9.9 years. Her karyotype was 46,XX. Brain MRI displayed a slight downward displacement of the cerebellar tonsils but normal pituitary gland and stalk. She had normal thyroid function with absent anti-thyroid antibodies. She reported delayed menarche (at 13.9 years).

Genetic findings

Family A

A heterozygous frameshift variant, c.857-858 del CT, in exon 4 of TBX3 gene (NM_005996, hg19), was found in the proband and his mother (Fig. 1C), resulting in the substitution of the second-last amino acid of the T-box domain and a premature stop codon after 19 amino acids (p.T286RfsX19) (Fig. 1E). Benign variants were identified in TBX3 5’ and 3’ UTR and deposited in the Figshare database Repository 2018 (Supplementary Table 1, see section on supplementary data given at the end of this article).

Pathogenic variants in IHH and pituitary candidate genes were ruled out by target NGS. The TBX3 variant was identified in the grandfather I-1, whereas the remaining family members I-2, II-1, II-3, II-4 and III-2 showed a WT genotype (Fig. 1A). Mutation was inferred for subject II-5, who, having been born premature and having died shortly thereafter, was reported to show a hand malformation.

Family B

NGS of TBX3, IHH and pituitary gene panel, and TSHR was performed on the proband, who was found to carry a heterozygous missense variant c.570C>A, p.S190R in exon 2 of TBX3 gene (NM_005996, hg19 g.115118771) (Fig. 1E), which is predicted to be pathogenic by eight different databases, as deposited in the Figshare database Repository 2018 (Supplementary Table 2). The proband’s sister was found to carry the TBX3 missense variant, which was inherited from the father. Benign variants were identified in TBX3 5’ and 3’ UTR (Supplementary Table 1).

Interestingly, the proband and his sister were heterozygous for the variant c.959G>A, p.S320N in TSHR gene (NM_000369, rs772172530, hg19 g.81609361), which was inherited from their father, probably explaining their mild non-autoimmune hypothyroidism.

TBX3 genotype/phenotype correlations

Clinical findings of UMS patients with TBX3 mutations belonging to the 17 previously reported families and the two families here described are shown in Supplementary Table 3. The frequency of endocrine features as well as congenital malformations (classified into midline defects, asymmetric malformations, breast and limb defects) is shown in Fig. 4. Noteworthy, differently from the midline (Fig. 4B) and other congenital malformations (Fig. 4B, C and D), endocrine defects appear more penetrant in males than in females (Fig. 4A). Furthermore, UMS phenotype is frequently characterized by multiple midline defects involving the face and several other areas (Fig. 4B).

Most of the TBX3 pathogenic variants (n. 16) are predicted to cause a premature truncated protein, being frameshift (n. 11) or nonsense (n. 5) mutations. Three missense mutations only have been reported so far, including ours (Fig. 1E). To perform genotype/phenotype correlations, each of the 19 TBX3 pathogenic variant has...
been mapped within the gene. Truncating mutations are distributed all over the gene, whereas the missense ones cluster in exon 2, within the T-box domain (Fig. 1E). There is no clear segregation of particular phenotypes with mutations destroying or truncating the protein before the T-box domain in comparison with those occurring after the DNA-binding domain (6, 21, 22) (Fig. 1E and Supplementary Table 3). In particular, the majority (59%) of \(TBX3\) pathogenic variants are associated with delayed puberty. In addition, the phenotypic manifestations appear highly variable even among individuals from the same family.

Clinical features of UMS vs IHH cohorts

By analyzing the UMS data collected from literature in comparison with those from the IHH cohorts (17, 23, 24), we found that the male-to-female ratio (M:F) of patients with delayed puberty is higher in UMS than in IHH (M:F = 7:1 vs 3–5:1, respectively) (Fig. 4A).

We then found that the prevalence of congenital IHH manifestations in UMS boys, such as bilateral cryptorchidism and micropenis, reaches 22 and 15%, respectively (Fig. 4A). These percentages are even higher than those found in the Italian cohort of normosmic prepubertal-onset IHH (17.7 and 2.4%, respectively) (23), and in Pitteloud cohort of nIHH, that were 7 and 8%, respectively (24).

In addition, the prevalence of orofacial cleft and/or tooth agenesis in the syndrome is 31% and that of renal abnormalities is 6% (Fig. 4B). These percentages are higher than those found in the Italian cohort of prepubertal onset IHH (9.8 and 0.4%, respectively) (23).
**Discussion**

Our findings indicate that delayed puberty due to normosmic hypogonadotropic hypogonadism with pituitary hypoplasia is a recurrent feature in two unrelated families with variable UMS phenotype carrying novel heterozygous variants of *TBX3* gene. Physical and developmental abnormalities including signs of hypogonadism and delayed puberty had been previously described in UMS patients (Fig. 4A), but they were poorly characterized (3, 4, 5, 6, 8, 9, 10, 11, 12). Therefore, *TBX3* loss of function is frequently associated with hypogonadism of central origin.

Consistently, three authors (5, 8, 9) previously described gonadal defects associated with the syndrome (Fig. 1E and Supplementary Table 3). Puberty of patient 1, as described by Schinzl in 1987 (5), was induced by testosterone and gonadotropins without reaching an adult Tanner stage, but hormonal values and brain MRI were not reported. In two prepubertal UMS Japanese brothers, mild gonadotropin deficiency has been described as the cause of the defective development of external genitalia (8), but the absence of clinical and biochemical follow-up does not allow a differentiation between true IHH and congenital delay of growth and puberty (CDGP). Finally, one out of two twins (born from an in vitro fertilization pregnancy) recently described by Tanteles (9) had a prepubertal response to GnRH test at 15 years, but developed a normal puberty after testosterone priming, thus suggesting a CDGP rather than IHH.

We report on two new *TBX3* variants whose deleterious impact is supported by their co-segregation with variable UMS and nIHH features in two families. In patient III-1 (family A), congenital nIHH diagnosis is based on the combination of a prepubertal response to GnRH test after the age of 14 years, the absent clinical response to testosterone priming, the ability to achieve Tanner stage V after gonadotropins treatment and the decrease of testosterone levels associated with signs and symptoms of hypogonadism after treatment withdrawal (17). Therefore, this patient is the first UMS adult reported to have the classic biochemical and clinical features of congenital nIHH. Similarly, the repetitively low gonadotropin and testosterone levels at 16.8 years of age in the patient IV-2 (family B) are also consistent with the diagnosis of congenital nIHH (25, 26). Indeed, we found a large overlap between some features of UMS and congenital IHH. In particular, we show the presence in UMS of a high percentage of midline defects, including those involving brain and pituitary. It is noteworthy that in all four UMS patients, including the present three cases (family A: III-1 and Family B: IV-1 and IV-2), who underwent brain neuroimaging, some sort of brain and/or pituitary malformation has been detected. Both of our probands had a small pituitary, associated in case III-1 with a thin stalk flattened on the posterior wall of sella turcica. Likewise, the only author who previously performed brain MRI in an UMS patient (11) revealed an anterior pituitary hypoplasia with a thin stalk and an ectopic posterior pituitary gland. We found an AC1 malformation in one patient (III-1) and a slight downward displacement of the cerebellar tonsils in a second unrelated one (IV-1). The association of UMS with these variable midline defects at brain MRI is consistent with the known role of T-box family genes in hypothalamic and pituitary development both in humans and in mice (27, 28), and with the expression of *TBX3* gene in pituitary human tissue (6). One previous study in mice by Trowe et al. (29) showed that *TBX3* knockout causes a failure in the infundibulum development, resulting in Rathke's pouch degeneration and pituitary hypoplasia. These effects appear to be the consequence of a de-repression of the sonic hedgehog pathway in the ventral diencephalon combined with an impaired FGF signal from the infundibulum.

In conclusion, midline defects together with absent axillary hairs and nipple hypoplasia are key features of UMS that should be checked in patients with delayed puberty. Neuroimaging seems particularly useful to uncover pituitary and brain malformations that appear specifically associated with the *TBX3* loss-of-function phenotype. In addition, *TBX3* should be included among the candidate genes for congenital normosomic IHH and its involvement can be suggested in one patient with delayed puberty by the presence of ulnar deformities in other family members.

**Supplementary data**

This is linked to the online version of the paper at https://doi.org/10.1530/EC-18-0486.

**Declaration of interest**

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

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Patient consent
Informed consent for genetic studies, including case reports and accompanying images, have been obtained from all patients or their tutors of both families. The treatment of human subjects complies with the Declaration of Helsinki, and the Research Ethics Committee of IRCCS Istituto Auxologico Italiano approved the study.

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