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Array report

Distinct phenotype of *PHF6* deletions in females

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ABSTRACT

We report on two female patients carrying small overlapping Xq26.2 deletions of 100 kb and 270 kb involving the *PHF6* gene. Mutations in *PHF6* have been reported in individuals with Borjeson–Forssman–Lehmann syndrome, a condition present almost exclusively in males. Two very recent papers revealed *de novo PHF6* defects in seven female patients with intellectual disability and a phenotype resembling Coffin–Siris syndrome (sparse hair, bitemporal narrowing, arched eyebrows, synophrys, high nasal root, bulbous nasal tip, marked clinodactyly with the hypoplastic terminal phalanges of the fifth fingers and cutaneous syndactyly of the toes, Blaschkoid linear skin hyperpigmentation, dental anomalies and occasional major malformations). The clinical presentation of these patients overlaps completely with our first patient, who carries a germline deletion involving *PHF6*. The second patient has a mosaic deletion and presented with a very mild phenotype of *PHF6* loss in females. Our report confirms that *PHF6* loss in females results in a recognizable phenotype overlapping with Coffin–Siris syndrome and distinct from Borjeson–Forssman–Lehmann syndrome. We expand the clinical spectrum and provide the first summary of the recommended medical evaluation.

1. Clinical description

1.1. Patient 1 (DECIPHER 278344, Fig. 1A)

The girl was born as a first child of healthy unrelated parents of German origin. The birth measurements were normal – 39 weeks of gestation, length of 47 cm (−1.86 SD), weight of 2790 g (−1.32 SD), and OFC 35 cm (0.31 SD). The girl was referred to a geneticist at the age of 12 weeks because of muscular hypotonia with feeding difficulties and the presence of a single horseshoe kidney. Biometric measurements were still normal but with borderline length (length: 55 cm, −2 SD; weight: 4.69 kg, BMI: 15.5; OFC: 38.5 cm, −1.21 SD). During the first year of life the girl developed a severe obstructive uropathy and presented with recurrent pyelonephritis including long-lasting fever and renal insufficiency. She suffered multiple febrile seizures. At the age of 15 months, the girl underwent the Anderson–Hynes open pyeloplasty that improved the urine flow and prevented further infections.

Developmental milestones were significantly delayed. The patient could sit unsupported at the age of 16 months. At 24 months her length was 81 cm (−2.27 SD), her weight 10.4 kg (BMI 15.85, normal) and her OFC 45 cm (−2.6 SD). At the age of 27 months the girl still could not walk or speak. The hearing test was normal, although she was noted to have a narrow external auditory canal. The ophthalmological examination was unremarkable. No brain MRI was performed. The head ultrasound at the age of 12 weeks did not reveal any morphological changes. The girl showed a distinct pattern of minor anomalies (Fig. 1A) including sparse hair, bitemporal narrowing, arched eyebrow, synophrys, bilateral epicanthic folds, high nasal root, broad nasal tip, and open mouth appearance with extensive drooling. The teeth were small, widespaced and prone to caries. The patient had bilateral clinodactyly of the fourth and fifth fingers. The terminal phalanges were hypoplastic and the nails of the fifth fingers were small. The middle phalanges of all fingers appeared to be short. The girl had a unilateral skin syndactyly between the third and the fourth toes. There was linear skin hyperpigmentation showing a blaschkoid distribution on both thighs, along the left costal arch and under the

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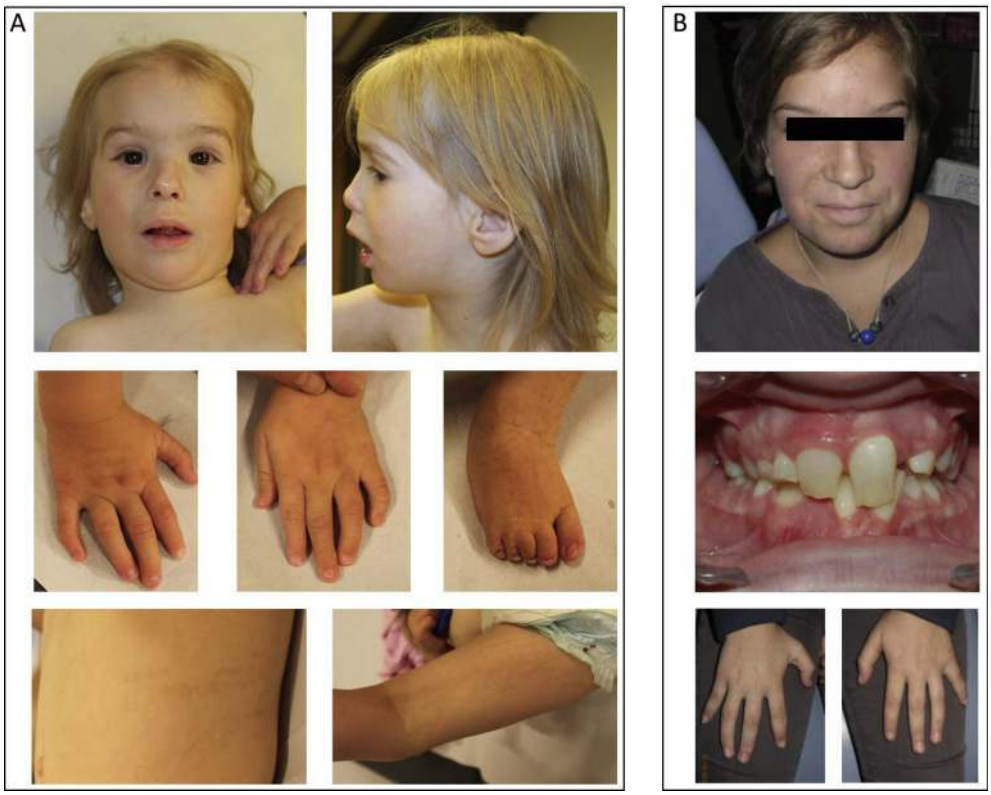

Fig. 1. Phenotypes of patients 1 and 2. (A) Patient 1 at the age of 2 years. Note the characteristic minor anomalies with broad arched eyebrows, synophrys, high nasal root, broad nasal tip, marked clinodactyly of the fourth and fifth fingers, hypoplastic terminal phalanges of the fifth fingers, syndactyly between the right third and fourth toes, and linear hyperpigmentation posteriorly on the right thigh and along the right costal arch. (B) Patient 2 at the age of 12 years. Note mild dysmorphic features with arched eyebrows, broad nasal tip, macrodontia and irregularity of the central incisors, clinodactyly of the fourth and hypoplastic terminal phalanges of the fifth fingers.

right axilla. Additionally, the girl showed hypertrichosis along the spine.

1.2. Patient 2 (DECIPHER 274703, Fig. 1B)

The girl was born after an uneventful pregnancy as a fourth child of healthy non-consanguineous parents. The birth was at term with normal length (51 cm) and weight (3700 g). Mild muscular hypotonia was noted during the neonatal period. No other medical issues had been reported. The independent walking was achieved at the age of 17 months. She showed mild speech delay.

At the age of 12 years and 3 months the patient's height was 155.5 cm (0.34 SD), weight 51.3 kg (BMI 21.22, normal), OFC 57 cm (3 SD). Minor anomalies were noted (Fig. 1B): dolichocephaly, arched eyebrows, broad nasal tip, low set and posteriorly rotated ears. Additionally, the girl presented with marked dental anomalies, macrodontia of the both central incisors, delayed eruption of the anterior teeth and caries. She had a narrow external auditory canal. The metacarpals II and V were short bilaterally and clinodactyly of the 4th fingers was present. The patient showed hyperlaxity of the large joints, and bilateral cubitus valgus. Furthermore, one café au lait spot was noticed on the thorax.

The girl showed mild learning disability. No formal IQ test was performed, but IQ was estimated within borderline to normal range. The dental anomalies required extensive and continuous treatment.

2. Methods

2.1. Molecular karyotyping

Copy number variants for patient 1 were identified by molecular karyotyping using a SurePrint G3 Human CGH microarray 2 × 400K design 021850 (Agilent Technologies). Agilent's enzymatic labeling kit was utilized and all procedures were carried out according to the manufacturer's instructions. Scanning was carried out on an Agilent microarray scanner and raw data were processed by Feature Extraction 9.5. Deleted and amplified regions were determined on Agilent's Genomic Workbench Standard Edition 5.0.14. A minimum of four consecutive probes had to be affected to make a call.

Patient 2 was examined with Agilent Human Genome CGH 60K oligonucleotide array (Agilent, Santa Clara, CA; www.agilent.com) with the ISCA design (www.iscaconsortium.org) following the protocols provided by Agilent. The arrays were analyzed with the Agilent scanner and the Feature Extraction software (v. 9.1.3). Graphical overview was obtained using the CGH analytics software (v.3.5.14).

The aberration detection threshold of the ADM-2 algorithm was set to 5.9 for both patients.

Metaphase and interphase FISH analysis with the specific BAC RP 11-797H09 (GRCh37/hg19 chrX: 133471874–133667334) was used to verify the deletion in patient 2. BAC RP11-141O19 (GRCh37/hg19 chrX: 51343219–51516444) was used as a control probe.

X-inactivation testing: Amplification of the CAG repeat in exon 1 of the androgen receptor (AR) gene was performed by PCR with fluorescence tagged primers. Subsequently, digestion with the

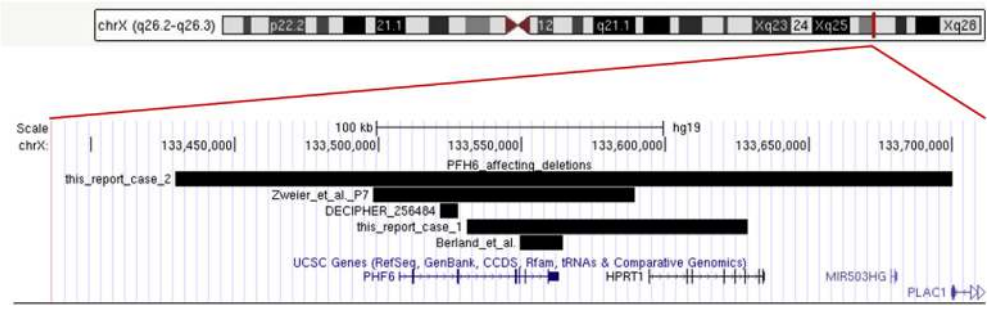

Fig. 2. Chromosomal region Xq26.2-q26.3 from UCSC Genome Browser (build GRCh37/hg19) showing the genes within this region and the deletions revealed in patients 1 and 2 as well as the three deletions of comparable size reported by Zweier et al. (P5 and P7) [Zweier et al., 2013] and Berland et al. [2011].

methylation-sensitive enzyme HpaII was performed, and fragments were analyzed with an automated capillary sequencer (ABI 3100; Applied Biosystems). Genescan and Genotyper Softwares (Applied Biosystems) were used to determine fragment sizes and intensities, and the degree of X-inactivation was calculated as described elsewhere.

3. Results (Fig. 2)

3.1. Patient 1

Molecular karyotyping gave the following result for patient 1:

arr[hg19] Xq26.2q26.3(133527921x2,133530984–133628672x1, 133634029x2)

The 100 kb deletion on the X chromosome affected the last five coding exons of *PHF6* (OMIM *300414) and exons 1–7 (or 8) of *HPRT* (OMIM *308000). This deletion was excluded in the healthy mother. Although the paternal material was not available for analysis, the healthy father is very unlikely to be an asymptomatic carrier of this deletion.

X-inactivation testing on DNA extracted from whole blood showed complete skewing of 100%.

3.2. Patient 2

Molecular karyotyping revealed a 270 kb loss on Xq26.2-q26.3:

arr[hg19] Xq26.2q26.3(133373380x2,133429257–133700128x1~2133751845x2)

Metaphase and interphase FISH analyses of blood lymphocytes showed that the deletion was only present in about 25% of the examined cells:

ish mos del(X)(q26q26)(RP11-797H09-) [51/200].

No other tissue was available for analysis. Parental analysis was not considered because of postzygotic mosaicism.

DECIPHER comprises 14 deletions involving the *PHF6* gene. However, only three of them are of comparable size (two reported patients and DECIPHER 256484 [Zweier et al., 2013]). Sizes of the other reported deletions range from 1.17 to 155.17 Mb.

4. Discussion

We report here on two female patients carrying overlapping deletions involving the genes *PHF6* and *HPRT1*. The *HPRT1* gene is unlikely to influence the phenotype of the patients. The hemizygous mutations in *HPRT1* are the cause of the Lesch–Nyhan syndrome [Nyhan et al., 1993]. Both patients show no features suggestive of Lesch–Nyhan syndrome, such as involuntary movements, dystonia, spasticity, hyperreflexia or deposition of kidney stones. Although patient 1 was still too young to definitely exclude the Lesch–Nyhan syndrome, her serum uric acid concentration was repeatedly normal. Moreover, two reported patients did not show any additional features compared to the other females with the loss of the *PHF6* gene only (Table 1).

Mutations of *PHF6* have been reported in X-chromosomal Borjeson–Forssman–Lehmann (BFLS) [Lower et al., 2002], a condition that is characterized by intellectual disability, obesity, hypogonadism and a recognizable facial phenotype in males. Although most of the female carriers are asymptomatic, there have been reports of mildly affected women in large families.

However, mutations in *PHF6* have recently been associated with another condition, namely Coffin–Siris syndrome. Wieczorek et al. [2013] reported on two female patients with typical features of Coffin–Siris syndrome – coarse facial features; thick and arched eyebrows; synophrys; large mouth with thick lips; hypoplasia/aplasia of the distal phalanges, especially of the fifth fingers; nail hypoplasia; body hirsutism and sparse scalp hair. Moreover, a very recent paper [Zweier et al., 2013] summarized seven female patients (including the two patients described by Wieczorek et al.) with different loss-of-function mutations in *PHF6*, including one patient with a whole gene deletion of *PHF6* (P7) and one patient with a partial deletion including exons 4 and 5 of *PHF6* (P5, DECIPHER 256484). A similar terminal deletion of *PHF6* has been reported previously in a female patient with overlapping clinical presentation [Berland et al., 2011].

The features of our patient 1 significantly overlap with the phenotype of *PHF6* deletions described in the literature (Table 1). Overlapping clinical presentation of the individuals reported to date includes developmental delay with severely affected speech, specific pattern of minor anomalies (sparse hair, bitemporal narrowing, arched eyebrows, synophrys, high nasal root, bulbous nasal tip, marked clinodactyly with the hypoplastic terminal phalanges of the fifth fingers and cutaneous syndactyly of the toes), Blaschkoid linear skin hyperpigmentation, dental anomalies and occasional major malformations (vertebral and kidney anomalies). Horseshoe kidney with severe obstructive uropathy and recurrent pyelonephritis was a life-threatening condition during the first year of life in patient 1. Developmental delay is another point requiring intensive intervention during the first years of life. Also, marked dental anomalies needed regular surveillance in the reported patients. Female patients with loss-of-function mutations in *PHF6* other than deletions (frameshift, intragenic duplication) showed a very similar pattern of minor and

Table 1
Clinical features of the female patients with *PHF6* loss.

	Patient 1	Patient 2	*PHF6* deletion/loss-of-function mutations in 8 females [Berland et al., 2011; Zweier et al., 2013]
Age	2 y	12 y	6–32 y
Height/weight/OFC	81 cm (−2.27 SD)/10.4 kg (BMI 15.85)/45 cm (−2.6 SD)	155.5 cm (0.34 SD)/51.3 kg (BMI 21.22)/57 cm (3 SD)	−0.3 to 2.2 SD/6 normal BMI/2 mildly obese −1 to 2.2 SD
Short stature	Mild	No	No
Obesity	No	No	2 Mild
ID	Moderate	Borderline to normal IQ	2 Mild/4 moderate/2 severe
Speech	Absent, no babbling	Mild delay	1 Almost normal/2 moderate/5 severe impairment
Neurological features	None	None	Seizures (2), behavioral problems (4)
Hair	Sparse	Normal	4 Sparse in infancy, 1 persistent frontotemporal alopecia
Synophrys	Yes	No	Yes (4)/no (2)/not reported (2)
Bitemporal narrowing	Yes	No	Yes (8)
High nasal root	Yes	No	Yes (5)/no (2)/not reported (1)
Bulbous nasal tip		Yes	Yes (7)/not reported (1)
Linear skin pigmentation	Yes	No (1 café au lait spot)	Yes (8)
Dental anomalies	Small, wide-spaced teeth, prone to caries	Macrodontia of the central incisors, delayed eruption, prone to caries	Yes (6)/no (1)/not reported (1)
Finger anomalies	Clinodactyly and brachytelephalangy 5th	Clinodactyly 4th, brachytelephalangy 5th	Yes (8)
Short toes	No	No	Yes (6)
Skin syndactyly of the toes	Unilateral III–IV	No	Unilateral II–III (1), unilateral III–IV (1), bilateral II–III and IV–V (2)
Ear anomalies	Narrow auditory canal	Narrow auditory canal	Hearing loss (3)
Eye features	No	No	Strabismus (3)/retinal dystrophy (1)/nystagmus (1)/hyperopia (1)
Other medical issues	Horseshoe kidney, severe obstructive uropathy	Cubitus valgus	Vertebral anomalies (4)/deep hoarse voice (3)/constipation (2)/advanced bone age (2)/neurogenic bladder (1)/ectopic kidney (1)/recurrent infection (1)

Adapted from Zweier et al. [2013].

major anomalies that strongly suggests the specific effect of *PHF6* loss in females.

The facial gestalt in patient 1 was at first suggestive of Coffin–Siris syndrome. The presence of bilateral hypoplasia of the terminal phalanges and the small nails of the fifth fingers was also considered as a possible Coffin–Siris feature. However, the normal biometric parameters with the absence of the wide mouth and everted lips led us to perform array CGH as a first line genetic test. BFLS has neither been considered in the patients described here, nor in the previously reported patients [Wieczorek et al., 2013]. Berland et al. [2011] published the first report of a female patient carrying the intragenic *PHF6* deletion. She was diagnosed with BFLS in retrospect, after the identification of the deletion. However, the detailed analysis of the reported clinical features of this patient showed more similarities with the other patients with PHF6 loss, than with the BFLS (facial gestalt with the high nasal root and bulbous nasal tip, dental anomalies, brachyclinodactyly, asymmetric toe syndactyly II–III and IV–V as well as linear skin pigmentation), that was also noted by Zweier et al. [2013].

Cutaneous syndactyly between the toes II–III is a consistent feature of BFLS. Five out of ten female patients with *PHF6* loss also presented with toe syndactyly (Table 1). However, only one patient showed syndactyly II–III and this was a unilateral finding [Zweier et al., 2013]. The remaining patients had either III–IV syndactyly, or a combination of syndactyly II–III and IV–V. The syndactyly was either unilateral or asymmetric, if bilateral [Berland et al., 2011; Zweier et al., 2013]. Taken together, the type of toe syndactyly is different in BFLS in males and *PHF6* loss in females and this may be useful as an additional diagnostic feature.

Patient 2 is, to our knowledge, the only patient with a confirmed postzygotic mosaic loss of *PHF6*. All other female patients discussed above and below are considered to carry a *de novo* germline deletion or mutation. The clinical presentation of patient 2 is the mildest in the known cohort, with no intellectual disability or major malformations. Her facial features were considered to resemble neither Coffin–Siris syndrome nor BFLS. Although she showed mild and rather unspecific dysmorphism, she also presented with arched eyebrows, broad nasal tip, finger clinodactyly and hypoplastic terminal phalanges of the fifth fingers. The latter feature is, in retrospect, suggestive of Coffin–Siris syndrome. She showed distinct dental abnormalities, confirming that this is a constant feature and one of the major health issues of *PHF6* loss in females.

Surprisingly, patient 2 did not show the pigmentation pattern that would be expected in a patient with a mosaic situation. A low grade mosaic or even normal situation in the skin tissue could be an explanation for this phenomenon.

Skin hyperpigmentation in a Blaschkoid distribution is a strong indication of the mosaic status of the affected organism. All but one of the ten reported patients with a heterozygous *PHF6* mutation presented linear hyperpigmentation following the lines of Blaschko. All patients showed 96–100% skewed X-inactivation in blood lymphocytes. However, testing of available skin-derived primary fibroblast cell cultures from four patients revealed a random pattern of X-inactivation [Zweier et al., 2013]. The blaschkoid skin features suggest the presence of a functional mosaicism in all patients with *PHF6* loss, even if second tissue could not be tested in some of them. Zweier et al. also proposed that functional mosaicism in females with *PHF6* loss to be a contributing factor in the development of the phenotype [Zweier et al., 2013]. Unfortunately, no data are available about the status of X-inactivation in different tissues from healthy female carriers from families with BFLS. Since skin abnormalities have so far not been reported in these individuals, it can be assumed that the unaffected carriers of the *PHF6* mutations have complete skewing of X-inactivation in the vast majority of the cells. The continuous inactivation of mutated *PHF6* in most of the cells of BFLS carriers may explain their healthy status in contrast to the female patients with *PHF6* loss and pigment changes suggestive of functional mosaicism within a certain percentage of cells lacking *PHF6* function.

Taken together, we report on two female patients with a deletion involving the *PHF6* gene, one of whom is the first female patient who revealed a postzygotic mosaic loss of *PHF6*. The observed clinical features strongly overlap with the recently defined clinical presentation of *PHF6* loss in females, confirming that the loss of *PHF6* in females represent a clinical entity with recognizable phenotype similar to Coffin–Siris syndrome and distinct from BFLS. The very mild clinical picture in patient 2, especially her borderline to normal IQ, together with the variable clinical presentation of the other females with *PHF6* mutations, who show different degree of intellectual disability [Berland et al., 2011; Zweier et al., 2013], supports the hypothesis that (functional) mosaicism and consequently the different proportion of cells lacking the *PHF6* function is an important contributing factor to the variability of the phenotype.

Based on the spectrum of observed medical issues we recommend the following medical evaluations in early childhood: renal ultrasound for the detection of structural anomalies with early and aggressive treatment of the urinary tract infections, cardiac examination, developmental and language evaluation followed by intensive physical and speech therapies, neurological evaluation and EEG, hearing test and ophthalmologic evaluation and dental assessment with early treatment.

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