RESEARCH ARTICLE

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A Microdeletion Encompassing *PHF21A* in an Individual With Global Developmental Delay and Craniofacial Anomalies

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In Potocki–Shaffer syndrome (PSS), the full phenotypic spectrum is manifested when deletions are at least 2.1 Mb in size at 11p11.2. The PSS-associated genes *EXT2* and *ALX4*, together with *PHF21A*, all map to this region flanked by markers D11S1393 and D11S1319. Being proximal to *EXT2* and *ALX4*, a 1.1 Mb region containing 12 annotated genes had been identified by deletion mapping to explain PSS phenotypes except multiple exostoses and parietal foramina. Here, we report a male patient with partial PSS phenotypes including global developmental delay, craniofacial anomalies, minor limb anomalies, and micropenis. Using microarray, qPCR, RT-qPCR, and Western blot analyses, we refined the candidate gene region, which harbors five genes, by excluding two genes, *SLC35C1* and *CRY2*, which resulted in a corroborating role of *PHF21A* in developmental delay and craniofacial anomalies. This microdeletion contains the least number of genes at 11p11.2 reported to date. Additionally, we also discuss the phenotypes observed in our patient with respect to those of published cases of microdeletions across the Potocki–Shaffer interval. © 2015 Wiley Periodicals, Inc.

Key words: Potocki–Shaffer syndrome; haploinsufficiency; microdeletion; PHF21A; BHC80; histone reader; unmethylated histone; H3K4me0; histone demethylase; H3K4me2; H3K4me1; ZMYM2; ZMYM3; histone eraser; KDM1A; LSD1; ZNF217; BRAF35; GTF2I; CoREST; HDAC1; HDAC2; MAPK8IP1; EXT2; ALX4; repressor; developmental delay; craniofacial anomalies; tapering finger; micropenis; 11p11.2; positional cloning

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INTRODUCTION

Genome-wide microarray technology has led to the discovery of numerous microdeletion syndromes [Carvill and Mefford, 2013]. Microdeletions normally encompass 10–30 genes [Lindsay, 2001] and are often the underlying cause of contiguous gene deletion syndromes due to the deletion of multiple genes. Sometimes, however, single-gene mutations are the cause of microdeletion syndromes such as DiGeorge syndrome (MIM 188400), Smith–Magenis syndrome (MIM 182290), and Kleefstra syndrome (MIM 610253). In WAGR syndrome (MIM 194072), characterized by Wilms tumor, aniridia, genitourinary anomalies, and intellectual disability, interestingly, mutations of two genes, *WT1* and *PAX6*, are responsible for the phenotype. The phenotypic features of Potocki–Shaffer syndrome (PSS, MIM 601224) vary depending upon the precise location and extent of the deletion in the proximal short arm of 11p11.2. The full

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spectrum of PSS is manifested when deletions are at least 2.1 Mb in size encompassing 15 annotated genes. Among these genes, haploinsufficiency of three genes is the underlying mechanism for the main phenotypic features of multiple exostoses, parietal foramina, and intellectual disability coupled with craniofacial anomalies. Linkage studies to establish genotype–phenotype relationships have shown that the gene *EXT2* is responsible for multiple exostoses [Stickens et al., 1996], whereas *ALX4* is the cause of parietal foramina [Hall et al., 2001; Mavrogiannis et al., 2001]. Recently, breakpoint mapping in two balanced translocations coupled with deletion mapping, has shown that *PHF21A* is associated with intellectual disability and craniofacial anomalies in PSS. This gene aka *BHC80* was truncated in two unrelated patients with balanced translocations- t(11;19)(p11.2; p13.2)dn and t(1;11)(p21.1;p11.2)dn and fully deleted in five patients with PSS [Kim et al., 2012].

Patients with small microdeletions encompassing only few genes are rare but informative to identify the causative genes involved in a specific microdeletion syndrome [Dode et al., 2003; Vissers et al., 2004]. Here, we report a 4-year-old boy with a microdeletion in the 11p11.2 region involving only five genes including *PHF21A* (Figs. 1 and 6). The main clinical features in this boy are global developmental delay and craniofacial anomalies. We performed qPCR to further refine the breakpoints and quantified the mRNA expression levels of deleted or truncated genes within the microdeletion using RT-qPCR. This microdeletion at 11p11.2 involves the least number of genes reported to date and provides further support that haploinsufficiency of *PHF21A* is the underlying cause of intellectual disability and craniofacial anomalies in PSS.

CLINICAL REPORT

The proband (DGDP262) was born following a natural conception to healthy unrelated white European parents. The pregnancy was monitored intermittently because of maternal concerns regarding reduced fetal movement. Labor was induced at 38 weeks because of maternal hypertension and reduced fetal movements. The patient was born by vaginal forceps delivery and required minimal resuscitation. He had a birth weight of 3.32 kg (25th percentile), a length of 53 cm (75–91th percentile), and an occipitofrontal head circumference of 34.5 cm (25th percentile). He was admitted to the neonatal unit on day 2 because of poor feeding and low blood sugar levels. He was also noted to have a small buried penis with a stretched penile length of 1.7 cm. He was initially managed with intravenous fluid, antibiotics, and hydrocortione, because of suspected hypopituitarism. However, his laboratory assessments were normal, his feeding difficulties resolved, and he was discharged home on day 8 with topical testosterone gel. In early childhood, he had recurrent self-limiting upper airway infections and upper airway obstruction that resulted in snoring at night. He had normal ophthalmology and hearing assessments.

There were concerns about his development from about 6 months of age. He began to roll at 8–9 months, crawled at 18 months, and walked at 2 years. He spoke a few single words, but by 22 months, screamed to gain attention and communicated using gestures. He liked being around children, but had difficulty with social interaction. On developmental assessment, he was found to have moderate global developmental delay with gross and fine motor skills, speech development at the 13–14 month level, and personal–social skills at the 15–17 month level. On examination at 22 months, the patient had a weight of 17.28 kg (99.6th percentile), a height of 89.3 cm (98th percentile), and a head circumference of 50.6 cm (75th percentile). Clinically, he had no skull defects. He had a broad forehead with a slightly prominent metopic ridge, full cheeks, and mild malar hypoplasia. He had mild epicanthic folds. He had a broad flat nasal bridge with a full nasal tip, a small mouth with a thin upper lip, and mild micrognathia. He had large posteriorly rotated ears (Fig. 2A), brachycephaly (Fig. 2B), and scaphocephaly (Fig. 2C). His fingers were tapering and he had bilateral clinodactyly (Fig. 2E and F). He had a mild syndactyly between the second and third toes, more noticeable on the left than the right foot (Fig. 2G and H). His testis were palpable and he had a stretched penile length of 4 cm. No bony exostoses were detected. He displayed similar craniofacial anomalies at 3 years and 10 months of age as evidenced by photographs taken 2 months after his medical examination (Fig. 2B–D, Table I).

When the patient was 3 years and 2 months, he was evaluated by the schedule of growing skills (SGS) II. His speech and language was at 18 months, interactive and social at 18 months, and his self-care was at around 18 months. The patient's hearing and language skills were scored at 12 months, and his locomotive skills were at 18 months, indicating global developmental delay. At 3 years and 10 months of age, he was unsteady on his feet and only able to walk short distances. He communicated with single words and was learning to join words together. He was active with a short

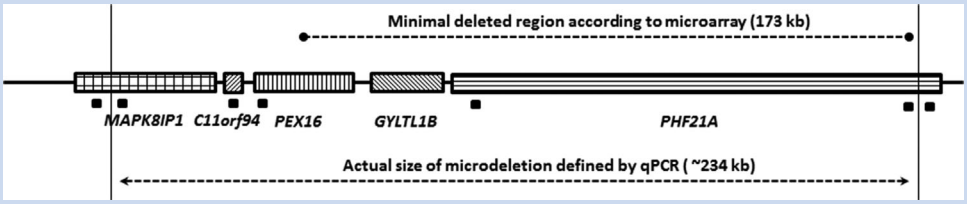

FIG. 1. Schematic diagram showing the organization of five genes at 11p11.2. The minimal size of the microdeletion detected by microarray is displayed as well as the actual size of the deletion defined by qPCR. Black bloxes show approximate locations of loci where primers were designed for qPCR.

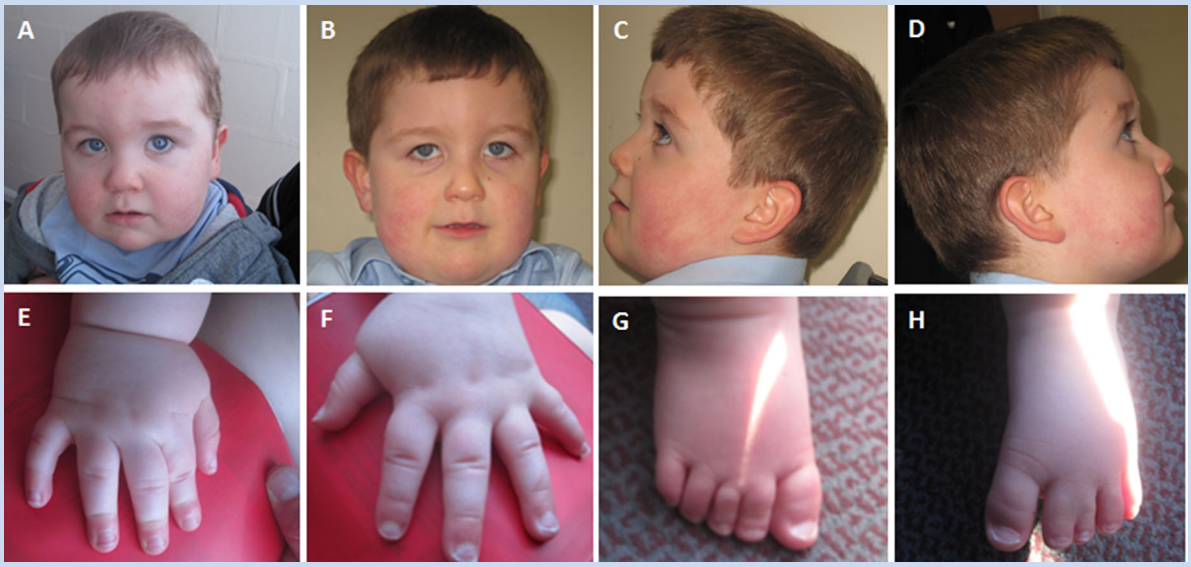

FIG. 2. Facial and limb pictures of patient DGDP262. Facial pictures at (A) 22 months showing his wide forehead, prominent metopic ridge, epicanthic folds, full slightly sagging cheeks with a small mouth and chin; (B–D) 48 months craniofacial features include a flat facial profile due to depressed nasal bridge and malar hypoplasia, and low set posteriorly rotated ears. B shows brachycephaly, whereas C displays scaphocephaly; (E and F) DGDP262 full hands with tapering fingers at 22 months; (G and H). Webbing between second and third toes more prominent on the left one foot at 22 months (H).

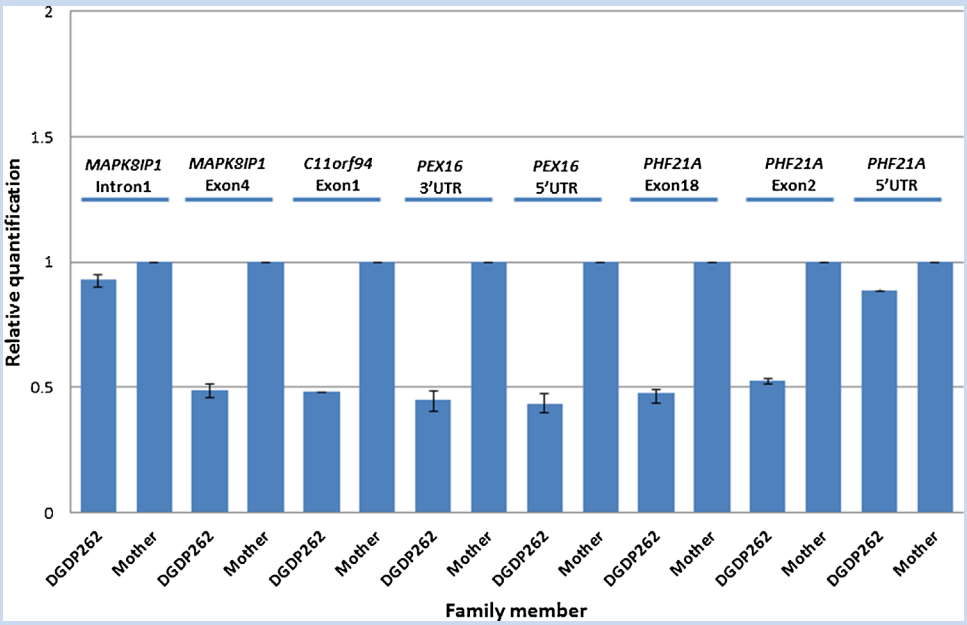

FIG. 3. qPCR assays showing four genes involved in the microdeletion. Gene *C11orf94* and *PEX16* are completely deleted on one chromosome 11, whereas *PHF21A* and *MAPK8IP1* are truncated. A value close to 0.5 indicates deletion on one chromosome 11, whereas a value of close to 1.0 indicates no deletion.

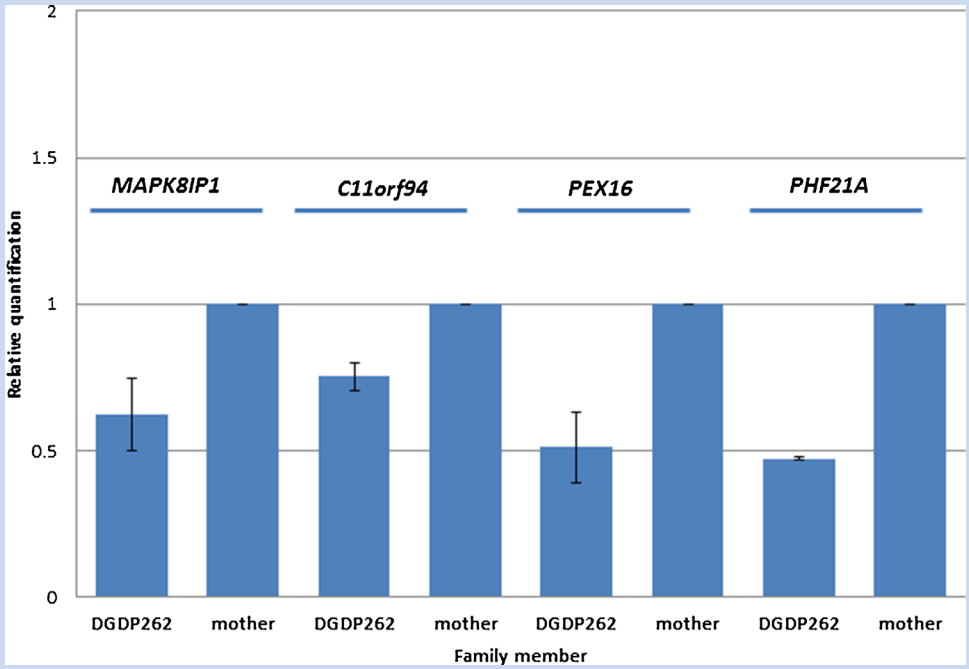

FIG. 4. Transcript levels of four genes implicated in the microdeletion. The transcript levels of all four genes are reduced in the DGDP262 relative to his mother.

concentration span. He had difficulty interacting with other children and preferred adult company. He had no awareness of toileting. He attended mainstream nursery where he had one-on-one support and a special needs school placement was arranged.

The patient had no biochemical evidence of hypopituitarism on laboratory investigation. 7-Dehydrocholesterol, standard karyotyping, and genetic testing for Prader Willi syndrome and Fragile X syndrome were normal. A renal ultrasound, a bone age wrist X-ray, and a brain MRI were normal (he had not had a skull X-ray). Microarrays and follow-up studies were undertaken on the proband and his mother. Unfortunately, the father was unavailable for testing. A summary of his phenotype and commonly observed phenotypes of patients with microdeletions in the PSS interval are provided in Table I and Table II, respectively.

MATERIALS AND METHODS

Cell Culture

Blood samples obtained from patient DGDP262 and his mother were used to create lymphoblastoid cell lines as reported [Nishimoto et al., 2014].

Microarray

Genomic DNA derived from DGDP262 was examined for copy number variations (CNVs) using a BlueGnome 8×60 K ISCA design oligonucleotide microarray.

Real-Time qPCR and RT-qPCR

We performed qPCR to verify and refine the coordinates at both ends of the putative deletion breakpoints. Primers were designed using sequences from *PEX16* 5′ and its 3′ untranslated region (UTR) as well as in *PHF21A* exon 18 and the 5′-UTR. We also designed primers against exonic sequences of *C11orf94* and *MAPK8IP1* for RT-qPCR (Supplementary Table SI). Total RNA was extracted from cell lines established from the patient and his mother using RNeasy Plus Mini kit (Qiagen Valencia, CA) following the manufacturer's instructions. cDNA was synthesized using the RevertAid First cDNA Synthesis Kit (Thermo Scientific Waltham, MA) according to the manufacturer's protocol. All real-time qPCR reactions consisted of 2 μl of sample, 10 ml of Fast Essential DNA Green Master (Roche Indianapolis, IN), and 2.5 μM primers in a total reaction volume of 20 μl.

Western Blot

Protein was isolated from lymphoblastoid cell lines and PHF21A expression was further investigated by Western blot using an anti-PHF21A antibody synthesized in rabbit [Iwase et al., 2004]. The antibody targeted a 93-residue polypeptide located at the N-terminal end of PHF21A. A dilution of 1:500 was used for the primary antibody, while the secondary anti-rabbit antibody (Thermo Scientific, Waltham, MA) was diluted 1:1000. Detection was carried out using the Amersham^{TM} ECL^{TM} Western Blotting Analysis System (GE Healthcare, Pittsburg, PA).

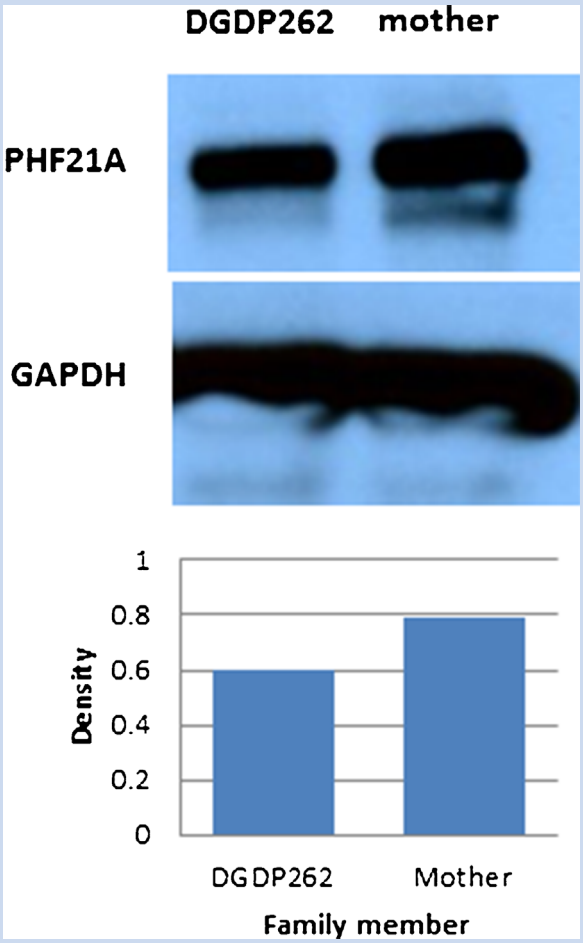

FIG. 5. (A) Western blot showing PHF21A protein levels in patient and mother. The level of GAPDH protein is also displayed as loading control. (B) Densitometry analysis showing PHF21A protein levels in patient and healthy his mother is also displayed.

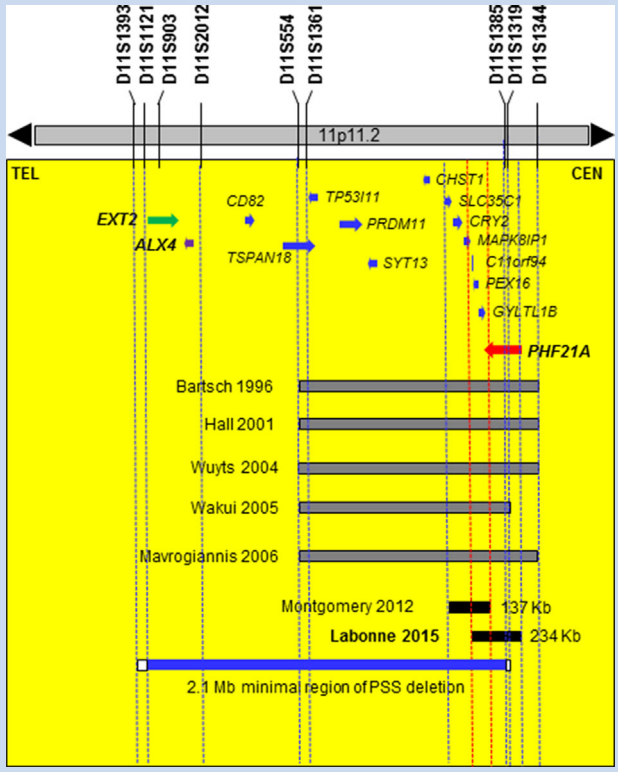

FIG. 6. Schematic diagram showing PSS region at 11p11.2. Fifteen genes residing in the 2.1 Mb minimal deletion region associated with full phenotypes of PSS are displayed with markers. Solid boxes in gray represent the refined ID/CFA candidate gene area deduced from each paper. Two black boxes depict the two smallest microdeletions found so far. Montgomery's deletion contains seven genes including *PHF21A*, whereas our microdeletion contains refined five genes by eliminating *SLC35C1* and *CRY2*. The 62 kb overlapping region predicted to contain the ID/CFA gene is shown by two red dotted lines.

RESULTS

Microarray Analysis

A heterozygous deletion of at least 173 kb at 11p11.2 was detected in DGDP262 arr[hg19] 11p11.2(45,936,954-46,110,572) x1 (Fig. 1). The minimal deleted region involves only three genes (truncated *PEX16*, *GYLTL1B*, and truncated *PHF21A*).

Real-Time qPCR

Assays by qPCR showed that the *PEX16* gene is completely deleted. Exons 2 and 18 of *PHF21A* were found deleted, but the beginning of its 5′-UTR was intact (Fig. 3), indicating that the proximal deletion breakpoint lies within *PHF21A* 5′-UTR. At the distal end of the microdeletion, gene *C11orf94* was found to be completely deleted. The telomeric breakpoint was found to lie between first and fourth exon of *MAPK8IP1*, suggesting this gene was truncated. The refined microdeletion encompasses five genes (Figs. 1, 3, and 6).

Transcript Levels of Genes Involved in Microdeletion

Transcript levels of *C11orf94*, *PEX16*, and *MAPK8IP1* were found to be reduced in DGDP262 relative to maternal transcript levels (Fig. 4). As expected, *PHF21A* in DGDP262 was found to be reduced approximately by half compared to asymptomatic mother. Transcript level of *GYLTL1B* was inconclusive because of the level of variation observed between each assay (data not shown).

Western Blot

Assaying the level of PHF21A protein by Western blot revealed that PHF21A expression level was reduced in DGDP262 compared to his mother (Fig. 5).

TABLE I. Phenotype Displayed by Patient DGDP262

Clinical features of PSS	DGDP262
Developmental delay	+
Craniofacial anomalies	+
Multiple exostosis	−
Parietal foramina	−
Micropenis	+
Seizures	−
Impaired socialization skills	+
Impaired communication skills	+
Clinodactyly	+
Syndactyly	+
Tapered fingers	+

DISCUSSION

PSS is a rare contiguous gene deletion disorder characterized by distinctive clinical phenotypes caused by haploinsufficiency of at least three genes; *EXT2* [Stickens et al., 1996], *ALX4* [Mavrogiannis et al., 2001], and *PHF21A* [Kim et al., 2012]. The main clinical features of the patient (DGDP262) reported in this study are global developmental delay, craniofacial anomalies, micropenis, clinodactyly, syndactyly, and tapered fingers (Table I). We hypothesize that DGDP262 is likely to show intellectual disability as he gets older. Microarray analysis revealed a minimal microdeletion at 11p11.2 involving at least three genes (*PEX16*, *GYLTL1B*, and *PHF21A*) in the patient. Through qPCR, we were able to show that the distal deletion breakpoint extends to two nearby genes (*C11orf94*, and *MAPK8IP1*) underscoring the importance of qPCR in refining the location of microdeletion breakpoints. In the absence of a paternal DNA sample, we cannot determine whether this deletion is a *de novo* or an inherited finding. However, in view of the genomic location of this deletion containing *PHF21A* and the recent evidence in the literature [Kim et al., 2012], it is likely that the healthy father does not have this deletion.

Recently, Montgomery et al. [2012] identified a 137-kb microdeletion in a young male containing seven genes namely, six OMIM genes—*SLC35C1*, *CRY2*, *MAPK8IP1*, *PEX16*, *GYLTL1B*, *PHF21A*—and one uncharacterized gene *C11orf94*. As our case involves only five genes—*MAPK8IP1*, *C11orf94*, *PEX16*, *GYLTL1B*, and *PHF21A*—at 11p11.2, it is a microdeletion containing the least number of genes with the phenotypes of global developmental delay and craniofacial anomalies. Our patient did not have multiple exostoses or parietal foramina on clinical assessment. This is consistent with the previous studies that *EXT2* and *ALX4*, which are not deleted in this patient, are responsible for the latter two phenotypes commonly observed in PSS individuals [Stickens et al., 1996; Wu et al., 2000].

The transcript levels of four genes (*MAPK8IP1*, *C11orf94*, *PEX16*, and *PHF21A*) within the microdeletion were reduced compared to his healthy mother, which is expected because of the loss of one allele of each gene. Of these four genes, *PHF21A* associated with intellectual disability as well as craniofacial anom-

TABLE II. Clinical Phenotypes of Patients With Microdeletions Encompassing *PHF21A*

Patient	Reference	Size	ME	PF	ID	DD	CFA	LA	MP
1	Bartsch et al. [1996], pt 1	>1 Mb	+	+	+	+	+	+	+
2	Bartsch et al. [1996], pt 6	>1 Mb	+	+	−	−	−	+	NA
3	Bartsch et al. [1996], pt 7	>1 Mb	+	+	−	−	−	−	NA
4	Bartsch et al. [1996], pt 8	>1 Mb	+	+	−	−	−	−	NA
5	Bartsch et al. [1996], pt 2	>1 Mb	+	−	+	+	+	+	+
6	Hall et al. [2001], pt 1	>1 Mb	+	+	−	−	−	−	U
7	Hall et al. [2001], pt 2	>1 Mb	+	+	−	−	−	−	NA
8	Yamamoto et al. [2001]	>1 Mb	+	+	+	+	+	+	+
9	Wuyts et al. [2004], pt 1	>1 Mb	+	+	+	+	+	−	+
10	Wuyts et al. [2004], pt 2	>1 Mb	+	−	+	+	+	+	NA
11	Wakui et al. [2005], pt 1	>1 Mb	+	+	+	+	+	−	NA
12	Wakui et al. [2005], pt 1	>1 Mb	+	+	+	+	+	−	NA
13	Wakui et al. [2005], pt 4	>1 Mb	+	+	+	+	+	−	+
14	Wakui et al. [2005], pt 7	>1 Mb	−	+	+	+	+	−	+
15	Wakui et al. [2005], pt 8	>1 Mb	−	+	+	+	+	+	NA
16	Wakui et al. [2005], pt 10	>1 Mb	+	+	+	+	+	+	+
17	Wakui et al. [2005], pt 12	>1 Mb	−	+	+	+	+	+	−
18	Wakui et al. [2005], pt 13	>1 Mb	−	−	+	+	+	+	−
19	Chuang et al. [2005], pt 1	>1 Mb	−	+	+	+	+	−	+
20	Chuang et al. [2005], pt 2	>1 Mb	+	+	+	+	+	−	−
21	Chuang et al. [2005], pt 3	>1 Mb	−	+	+	+	+	−	−
22	Bremond-Gignac et al. [2005]	>1 Mb	+	+	+	+	+	−	NA
23	Montgomery et al. [2012]	>137 Mb	−	−	+	+	+	−	−
24	Our patient, DGDP262	~234 kb	−	−	NA	+	+	+	+

ME, multiple exostoses; PF, parietal foramina; ID, intellectual disability; DD, developmental delay; CFA, craniofacial anomalies; LA, limb anomalies; MP, micropenis; pt, patient; N/A, not applicable (patient is a female); U, undetermined. Mb represents megabase, whereas Kb denotes kilobase.

alies has been shown to be haploinsufficient in patients with balanced translocations and deletions [Kim et al., 2012]. The reduced *PHF21A* expression levels in DGDP262 continue to support and confirm such a pathological role for *PHF21A* in PSS. PHF21A is a component of KDM1A histone demethylase complex including histone deacetylases HDAC1, HDAC2, DNA binding factors, ZMYM2, ZMYM3, ZNF217, BRAF35, GTF2I, CoREST, and a histone demethylase KDM1A [Hakimi et al., 2003]. Recently, a *KDM1A* mutation has been reported in a boy with developmental delay and craniofacial anomalies [Tunovic et al., 2014]. There is a critical interaction between PHF21A and KDM1A as components of a KDM1A repressor complex [Lan et al., 2007]. Identified as a first demethylase, KDM1A (lysine (K)-specific demethylase 1A /aka LSD1) is an "eraser", which removes a methyl group from mono- and di-methylated histones H3K4me1 and H3K4me2 and creates H3K4me0 [Shi et al., 2004]. PHF21A is a "reader" protein, which binds to this unmethylated histone H3K4me0 [Lan et al., 2007]. The functional relationship between these PHF21A and KDM1A in this repressor complex is poorly understood.

Among the genes deleted in our patient, *MAPK8IP1* (mitogen-activated protein kinase 8 interacting protein 1, MIM 604641) is highly expressed in brain and neurons. It is localized to neurite tips in differentiating cells. A neuron-restrictive silencer element (NRSE) was identified in the promoter region of this gene and reported to bind to REST (RE1-silencing transcription factor, MIM 600571), a zinc finger transcription repressor [Abderrahmani et al., 2001]. *MAPK8IP1* is thought to be a susceptibility gene for a type 2 diabetes, because one missense mutation segregating with this phenotype was found in a family [Waeber et al., 2000].

Our microdeletion encompassing only five genes further refines this PSS region and highlights the Potocki–Shaffer interval associated with global developmental delay, craniofacial anomalies, micropenis, minor digital anomalies including tapering fingers, clinodactyly, and syndactyly (Fig. 1, Table I). The digital findings and the micropenis are yet to be ascribed to specific gene(s) at 11p11.2 region. Interestingly, tapering fingers is a phenotypic feature also observed in Coffin–Lowry syndrome [Nishimoto et al., 2014], which shares overlapping phenotypes of developmental delay and craniofacial anomalies with PSS. Micropenis has been observed in several other male patients with 11p11.2 microdeletions [Wakui et al., 2005]. This phenotype in males with PSS was proposed to be caused by haploinsufficiency of *ALX4* due to both a reduction of the pubic bone and a shortened phallus in *Alx4* mutant mice [Wu et al., 2000]. However, as a balanced translocation truncating *PHF21A* was reported in a male patient with micropenis [Kim et al., 2012], we cannot exclude the possibility that this anomaly of the phallus might be caused by *PHF21A* or a gene in close proximity. In the balanced translocation patient DGAP012 [Kim et al., 2012], the causative gene for micropenis might be dysregulated by a position effect, whereas in our microdeletion case a gene near *PHF21A* may be responsible for this phenotype if *PHF21A* is not causative.

The manifestation of clinodactyly and micropenis [Wakui et al., 2005] appears to be complex as male patients with larger deletions including the same 11p11.2 interval as reported in the present study do not always display such phenotypes [Montgomery et al., 2013; Wakui et al., 2005]. It is possible that these phenotypes could be the result of a synergistic effect of several genes involved in the microdeletion or specific combination of deleted genes [Girirajan et al., 2010; Veltman and Brunner, 2010]. Smaller-sized microdeletions containing even fewer genes or point mutations of individual genes within the 11p11.2 region would be invaluable in dissecting phenotype–genotype relationships, thereby providing more insights into the individual contribution of each gene in the manifestation of the PSS phenotype.

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