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ORIGINAL ARTICLE

Combined pituitary hormone deficiency due to gross deletions in the *POU1F1 (PIT-1)* and *PROP1* genes

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Pituitary development depends on a complex cascade of interacting transcription factors and signaling molecules. Lesions in this cascade lead to isolated or combined pituitary hormone deficiency (CPHD). The aim of this study was to identify copy number variants (CNVs) in genes known to cause CPHD and to determine their structure. We analyzed 70 CPHD patients from 64 families. Deletions were found in three Turkish families and one family from northern Iraq. In one family we identified a 4.96 kb deletion that comprises the first two exons of *POU1F1*. In three families a homozygous 15.9 kb deletion including complete *PROP1* was discovered. Breakpoints map within highly homologous *AluY* sequences. Haplotype analysis revealed a shared haplotype of 350 kb among *PROP1* deletion carriers. For the first time we were able to assign the boundaries of a previously reported *PROP1* deletion. This gross deletion shows strong evidence to originate from a common ancestor in patients with Kurdish descent. No CNVs within *LHX3*, *LHX4*, *HESX1*, *GH1* and *GHRHR* were found. Our data prove multiplex ligation-dependent probe amplification to be a valuable tool for the detection of CNVs as cause of pituitary insufficiencies and should be considered as an analytical method particularly in Kurdish patients.
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INTRODUCTION

The human pituitary gland is a central regulator of growth, fertility, stress response and metabolism. Development of the pituitary gland depends on a precise algorithm of interacting transcription factors and signaling molecules. Mutations in any of the genes involved in pituitary development lead to pituitary insufficiency resulting in reduced or entirely missing secretion of pituitary hormones. Deficiency of GH in combination with a deficiency in one or more additional anterior pituitary-derived hormones is called combined pituitary hormone deficiency (CPHD). Epidemiology of CPHD is not entirely clear, in part due to marked regional differences in prevalence;[1] however, genetic causes are not uncommon. It evolves from a diversity of transcription factor mutations in genes such as *PROP1*, *POU1F1 (PIT-1)*, *HESX1*, *LHX3* and *LHX4*.[2–4] Whereas *PROP1* and *LHX3* mutations cause autosomal recessively inherited CPHD, for *HESX1*, *POU1F1*, and most recently also for *LHX4* mutations both dominant and recessively inherited hypopituitarism has been reported.[5–7]

The most frequent genetic cause of CPHD is defects of *PROP1*, which is located on the long arm of chromosome 5 (5q35.3) and consists of three exons spanning a region of ~4 kb. Translation product of *PROP1* gene is a paired-like homeodomain protein, which is involved in the development of pituitary gonadotropes, somatotropes, lactotropes and thyrotropes. Prop1-deficient Ames dwarf mice (*df/df*) are characterized by severe dwarfism and infertility.[8] In humans, defects in PROP1 result in a deficiency of GH, TSH, FSH/LH and prolactin (PRL). ACTH deficiency can evolve in some patients.[9] Onset and severity of hormone deficiencies as well as changes in pituitary morphology are variable with temporary enlargement of the anterior lobe reported especially in pediatric patients.[10–14] To date, various *PROP1* point mutations, small deletions and insertions have been reported with a remarkably uneven regional/ethnic distribution of specific variants.[2,13,15] Complete homozygous deletion of *PROP1* was described six times, but exact deletion breakpoints have yet to be identified.[16–21]

POU1F1 (also known as PIT-1), which belongs to the POU homeodomain protein family, regulates transcription via complex protein–DNA and protein–protein interactions mediated by its POU-specific and POU-homeo domains. *POU1F1* is located on the short arm of chromosome 3 (3p11), contains six exons and spans a region of ~17 kb. POU1F1 is required for formation of pituitary somatotropes, thyrotropes and lactotropes. Snell (*dw/dw*) and Jackson

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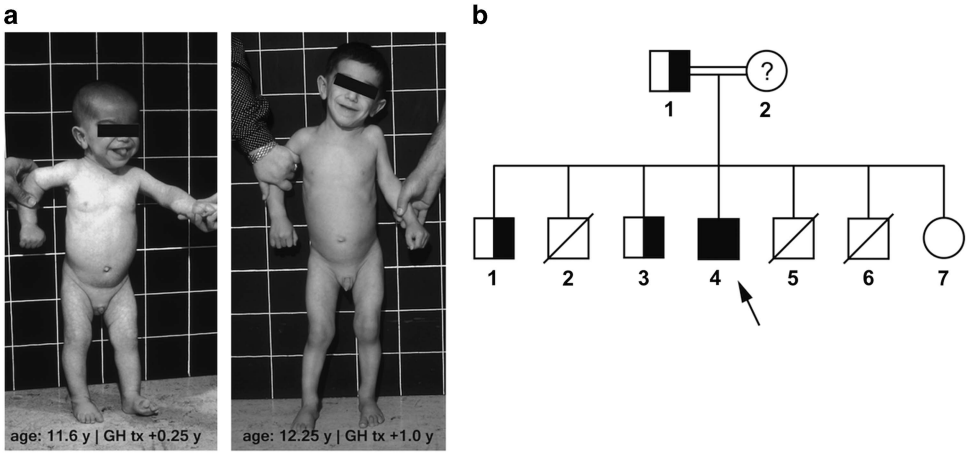

Figure 1 Phenotype and pedigree of case 1. (**a**) Index patient (case 1) at the age of 11 years and 7 months, 3 months after starting rhGH therapy and at the age of 12 years and 3 months, 1 year after starting therapy (left and right photograph, respectively; with consent of the family). (**b**) Pedigree of the family of case 1. Arrow indicates the index patient. Half-black symbols mark unaffected heterozygous carriers of the *POU1F1* deletion. DNA of the healthy mother was not available for analysis. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

(dw^j/dw^j) dwarf mice, harboring Pou1f1 mutations, present with greatly reduced size and hypothyroidism.[22] Comparable clinical characteristics are found in humans carrying *POU1F1* mutations. They often present with severe growth impairment.[23–25] GH and PRL deficiencies are generally complete, but the onset of clinical hypothyroidism is highly variable.[25] Human *POU1F1* mutation carriers display hypoplastic or normal adenohypophysis with normal pituitary stalk and posterior pituitary.[24–26] Several studies have investigated the frequency of *POU1F1* mutations in CPHD patients,[5] but there are only three reports about *POU1F1* gross deletions.[17,23,27]

Aim of the study was to determine the occurrence of homozygous or heterozygous copy number variants (CNVs) in genes known to cause hypopituitarism in a cohort of patients with CPHD.

MATERIALS AND METHODS

A total of 70 patients from 64 families (38 males and 32 females) diagnosed with CPHD were analyzed for the occurrence of CNVs in genes established to cause hypopituitarism (*PROP1*, *POU1F1*, *LHX3, LHX4, GH1* and *GHRHR*). We selected patients at the more severe end of the phenotypic spectrum, who are affected by GH deficiency and at least two additional hormone deficiencies including TSH (94%), gonadotropins (69%), ACTH (63%) and PRL (46%). DNA of seven patients was included in the study, for which PCR amplification of an entire gene or specific exons had failed in the past. Further 63 patients with CPHD were included in the study, in whom previously neither point mutations nor small deletions or insertions were found by dHPLC screening and direct sequencing in the genes for *PROP1*, *POU1F1*, *HESX1* and *LHX3*. Studies were conducted with informed consent of the probands or their parents.

Mutation analysis

Genomic DNA was isolated from peripheral blood cells. Multiplex ligation-dependent probe amplification (MLPA) assays were performed using MLPA kit P216-A2 containing probes for exons of *GH1* (except exon 2), *PROP1, POU1F1* (except exon 5), *GHRHR* (except exon 8), *HESX1, LHX3* (except exon 1) and *LHX4* following the protocol provided by MRC Holland (Amsterdam, the Netherlands). *GH1* and *GHRHR* were included in the analyses because they are part of MRC Holland's growth hormone deficiency multiplex panel and, moreover, specific autosomal-dominant GH1 mutations have been described to cause an evolving CPHD-like phenotype with multiple pituitary hormone deficiencies.[28] MLPA products were run on ABI Prism 310 sequencer and analyzed using PeakScanner version 1.0 and Coffalyser software (Applied Biosystems, Foster City, CA, USA). Direct sequencing of deletion breakpoints was performed according to a slightly modified manufacturer's protocol (ABI 310, Applied Biosystems). Sequence data were compared to the genomic reference assembly (March 2006 NCBI36/hg18; accessed through the UCSC Genome Browser (URL: http://genome.ucsc.edu/)) employing Vector NTI Advance software (Life Technologies, Darmstadt, Germany).

Mapping of *POU1F1* deletion

Primer pairs for PCR markers D1 through D6 upstream of exon 1 and downstream of exon 2 of *POU1F1* (NM_000306) were designed based on the human genomic assembly (March 2006 NCBI36/hg18). Long-range PCR was performed using Roche's Expand Long Template PCR System according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Primer sequences are provided in the (Supplementary Table S1).

Mapping of *PROP1* gross deletion

To narrow down deletion breakpoints we used STS markers and primer pairs described previously[16] with some minor modifications (primer sequences provided in the Supplementary Table S1). In the *PROP1* upstream region this strategy was limited by a 51 kb duplicated region containing the *FAM153C* (family with sequence similarity 153) pseudogene that impedes selection of specific PCR primers (for location of PCR markers compare Figure 4a). Therefore, inverse PCR starting from the R4 downstream region was performed. Briefly, genomic DNA was digested with the restriction enzyme *Nsp*I and restriction fragments were circulated under conditions favoring self-ligation. The resulting circular monomers were used as template for PCR amplification using primers complementary to the R4 sequence but pointing outwards (*inverse,* R4inv). The resulting PCR fragment was re-amplified with nested primers followed by direct sequencing, thereby disclosing the nearest distally located *Nsp*I site that is not affected by the deletion. Knowing the region between the last absent and the first present upstream *Nsp*I recognition sequence facilitated the assignment of the upstream breakpoint to a range of 3.8 kb. Three additional reverse primers (P15R, 15.4 kb distal of R4 in the wild-type sequence; P17R, 17.4 kb distal of R4; P18R, 18.2 kb distal of R4) were designed and applied in long-range PCRs using R4F as forward primer. Data obtained by direct sequencing of the shortest resulting PCR product cloned into pGEM-T vector (Promega, Mannheim, Germany) were aligned with the genomic reference sequence (build NCBI36/hg18) to define the deletion boundaries. Details of inverse and nested PCR primer pairs as well as sequences of long-range PCR primers are provided as (Supplementary Table S1).

Table 1 Hormonal phenotypes of the patients including siblings of case 4

				Family 4			
	Case 1	*Case 2*	*Case 3*	*(IV/1)*	*(IV/5)*	*(IV/6)*	*(IV/12)*[a]
Age (yrs)	11.5	40	3.5	14	34	31	17
Sex (m/f)	m	m	m	f	m	m	m
Gene mutated	*POU1F1*	*PROP1*	*PROP1*	*PROP1*	*PROP1*	*PROP1*	*PROP1*
GH, basal ($\mu g\ l^{-1}$)	NA	<0.05	NA	<0.05	<0.05	0.40	0.40
GH, stim. ($\mu g\ l^{-1}$)	<0.20	0.21	<1.00	NA	NA	NA	NA
TSH ($mIU\ l^{-1}$)	<0.50[b]	0.38 0.94[b]	1.15 (0.3–2.5)	0.01 (0.3–2.5)	<0.005 (0.3–2.5)	0.39 (0.3–2.5)	0.01 (0.3–2.5)
T4							
Total T4 ($nmol\ l^{-1}$)	<10 (60–150)	NA	NA	NA	NA	NA	NA
Free T4 ($pmol\ l^{-1}$)	NA	2.19 (12–25)	5.57 (10–23)	NA	NA	NA	NA
LH ($IU\ l^{-1}$)	0.30 (0.5–5.3)	<0.10[b] (>3.0)	<0.07 (<0.5)	0.89 (0.5–9.0)	0.70 (1.5–9.3)	0.10 (1.5–9.3)	0.10 (0.5–5.3)
FSH ($IU\ l^{-1}$)	1.30 (0.4–6.6)	0.20[b] (>4.9)	<0.07 (<2.2)	0.10 (1.4–9.2)	0.20 (0.3–8.5)	4.45 (0.3–8.5)	0.10 (1.4–9.2)
ACTH ($pmol\ l^{-1}$)	6.82 (1.6–14.2)	3.06 (1.6–13.9)	NA	2.21 (1.6–14.2)	8.43 (1.6–14.2)	6.21 (1.6–14.2)	4.71 (1.6–14.2)
Cortisol ($nmol\ l^{-1}$)	500.0[b] (>500)	537.6[b] (>500)	766[b] (>500)	NA	NA	NA	NA
PRL ($mIU\ l^{-1}$)	2.1 (85.6–322.2)	42.4 (84.0–315.0)	74.2 (25.44–634.5)	92.4 (63.6–305.3)	106.6 (85.6–322.2)	122.9 (85.6–322.2)	25.4 (85.6–322.2)
MRI, anterior pituitary	Hypoplastic	Hypoplastic	Hypoplastic	Near normal	NA	Enlarged	Near normal

Abbreviations: ACTH, adrenocorticotrope hormone; FSH, follicle-stimulating hormone; GH, growth hormone, LH, luteinizing hormone; MRI, magnetic resonance imaging; NA, not available; PRL, prolactin; TSH, thyroid-stimulating hormone.
[a]Index patient.
[b]Stimulated. Laboratory measurements were performed using different assays. Reference values (in parenthesis) are just given for overall guidance.

Haplotype analysis

Haplotypes were constructed using 7 microsatellite (STR; Supplementary Table S2) and 22 SNP markers flanking the *PROP1* deletion and spanning a region of ~9.6 Mb (identified using UCSC genome browser (http://genome.ucsc.edu/, GRCh37/hg19)). Fragment length analysis of STR markers was performed on an automated sequencer (ABI 310, Applied Biosystems) using FAM-labeled primers, data were analyzed using GeneScan version 3.1 and GeneMapper software version 4.1 (Applied Biosystems). SNPs were genotyped by allele-specific quantitative PCR (Applied Biosystems, Carlsbad, CA, USA) and analyzed by Phase software as described previously.[29]

RESULTS

MLPA analysis revealed six patients, belonging to three families, carrying a homozygous deletion of the complete *PROP1* gene. One further patient was identified with a homozygous deletion of exon 1 and 2 of the *POU1F1* gene. CNV analysis of the remaining genes did not yield any aberration in our patients, neither in a homozygous nor in a hemizygous or compound hemizygous state. Detailed clinical case presentations of deletion carriers are available in the Supplementary Information.

Clinical evaluation

Case 1. A boy from a consanguineous Turkish pedigree, the only affected child of seven siblings, presented at first evaluation at 11.5 years of age with an extreme growth retardation of −11.43 SDS (Figure 1a). Laboratory analysis of pituitary hormones suggested complete or nearly complete loss of somatotrope, lactotrope and thyrotrope, but normal corticotrope and gonadotrope function (Table 1). Magnetic resonance imaging (MRI) revealed a hypoplastic anterior pituitary gland. Parents of the patient are first cousins and clinically healthy (Figure 1b). Three brothers died during infancy with no information available about their cause of death. MLPA analysis of the index patient yielded no signal for exons 1 and 2 of *POU1F1*. The father and two brothers, all unaffected, were found to be hemizygous in these exons. DNA of the mother was not available.

Case 2. Clinical evaluation of a 40-year-old patient from northern Iraq showed an apparently hypogonadal man with short stature (139.4 cm, −5.5 SDS). Laboratory results confirmed hypogonadotropic hypogonadism with low stimulated LH and FSH, and testosterone below the detection limit. Further dynamic testing of pituitary function demonstrated somatotrope, thyrotrope and lactotrope insufficiency. The pituitary–adrenocortical axis was not affected (Table 1). X-ray of the left hand revealed a skeletal age of 14.4 years (Figure 2a) in line with juvenile levels of bone-specific alkaline phosphatase ($79.6\ \mu g\ l^{-1}$). MRI showed severe hypoplasia of the anterior pituitary lobe; the posterior pituitary and the pituitary stalk appeared normal (Figure 2b). Failure to amplify genomic DNA of the index patient in the *PROP1* locus was paralleled by MLPA analysis disclosing biallelic loss of all three *PROP1* exons.

Case 3. A boy from the southeast part of Turkey born to consanguineous, normal statured parents showed retarded growth (height 81 cm, −4.17 SDS) at first presentation at 3.4 years of age. Laboratory analyses demonstrated deficiency of GH (Table 1). TSH and fT4 testing revealed secondary hypothyroidism, whereas PRL serum levels were in the lower physiological range. Initial ACTH stimulation test was normal but became pathological when repeated at 13 years of age (peak cortisol of $373.3\ nmol\ l^{-1}$, normal $>500\ nmol\ l^{-1}$). MRI revealed hypoplasia of the anterior pituitary lobe but normal pituitary stalk and neurohypophysis (Figure 2c). No additional family members are affected by CPHD. However a sister of the patient died at 5 months of age and a brother died shortly after birth. MLPA analysis confirmed previous PCR results that had suggested complete, homozygous loss of *PROP1*.

Case 4. Four affected children (one girl, three boys) were born to a consanguineous Turkish marriage (parents are first cousins;

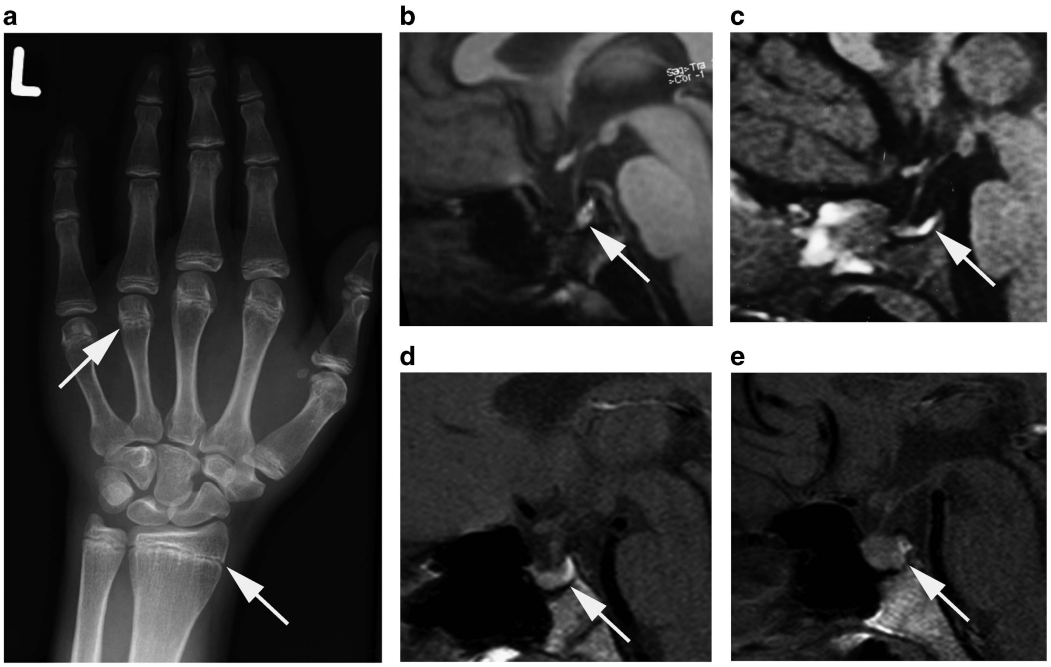

Figure 2 Imaging-based diagnostics of cases 2–4. (**a**) X-ray of the left hand of case 2 performed at the age of 40 years, demonstrating pronounced delay of skeletal maturation; arrow, epiphyseal cartilage with incomplete endochondral ossification. (**b**–**e**) Magnetic resonance imaging of the sella turcica of case 2 (**b**) and case 3 (**c**) demonstrate severe hypoplasia, whereas sagittal scans of individual IV/1 (**d**) and IV/6 of family 4 (**e**) show a nearly normal-sized and a slightly enlarged anterior lobe of the pituitary (arrows), respectively, but normal pituitary stalk and posterior lobe.

Figure 5). At 11 years of age the male index patient presented with a growth retardation of −4.68 SDS (114 cm). At 17 years he did not show any signs of puberty onset. Laboratory tests revealed deficiency of GH, TSH, gonadotropins and PRL, whereas ACTH secretion was normal (Table 1). His affected siblings also had—to a variable extent—somatotrope, thyrotrope and gonadotrope insufficiency but none of them, including those who had already reached adulthood, have developed adrenal insufficiency. Available MR images disclosed nearly normal-sized anterior pituitaries in the index patient and his sister (Figure 2d) but a slightly enlarged adenohypophysis in brother IV/6 (Figure 2e). MLPA analyses performed in parents and all but one sibling revealed complete loss of *PROP1* in the affected individuals and hemizygous *PROP1* deletion in four unaffected brothers, in a healthy sister and the parents. A further sister and brother were biallelic carriers of the wild-type gene.

POU1F1 deletion analysis

To define the extent of the deletion identified in the index patient (case 1) and to ascertain adjacent regions affected by the deletion various PCR markers down- and upstream of the two failing MLPA probes were analyzed (Figure 3a). All PCR markers were detectable in the sample of the index patient. Long-range PCR using forward and reverse primers of markers D3 and D4, respectively, yielded the expected fragment of ~6.1 kb in a WT sample and a 1.2 kb product in the patient's DNA predicting a deletion of ~4.9 kb. Direct sequencing revealed the deleted sequence to encompass 4958 bp including exon 1 and 2, and ~1.3 kb of the *POU1F1* upstream region (c.-1387_214+455del; Figure 3b). The deletion breakpoint within intron 2 resides within a MER3 (MEdium Reiteration frequency interspersed repeat) subfamily region of repetitive elements (RepeatMasker through UCSC Genome Browser: http://genome.ucsc.edu/; accessed December 2014), whereas the upstream breakpoint is apparently not part of a repeating element. Although neither breakpoint is part of a typical recombination hot spot, it appears possible that the AG dinucleotide found at both sites and/or the MER3 interspersed repeat located within the intron 2 breakpoint may have contributed to the DNA excision event. No additional genes were affected by the deletion.

PROP1 deletion analysis

To study the extent of the deletion found in the index patient of family 4, various PCR and STS markers around the *PROP1* locus were tested (Figure 4a). The PCR probes closest to *PROP1* that were unambiguously amenable to PCR amplification were R4 located ~2.2 kb downstream the *PROP1* locus and WI-16216 that maps ~60 kb upstream. Due to the presence of the pseudogene *FAM153C*, which is highly homologous to two distinct chromosomal loci (*FAM153A*: 5q35.3, *FAM153B*: 5q35.2), additional upstream located PCR probes were not informative. By employing inverse PCR and subsequent long-range PCRs that attained specificity through the forward primer located within the R4 region, we were eventually able to define the range of the deletion (Figure 4b). Direct sequencing disclosed a deleted fragment of 15 892 bp [chr5:177419141_177435032del (GRCh37/hg19)] (Figure 4c). The breakpoints of the deletion map to an *Alu*Y repetitive element that resides ~11.5 kb upstream of the *PROP1* transcription start site within the first intron of the *FAM153C* pseudogene and an *Alu*Y element immediately juxtaposed to the 3′-end of *PROP1* (569–587 bp downstream of the stop codon). The two *Alu*Y elements have the same direction on the chromosome and an overall identity of 88%. The fusion site of the deletion could be refined to an 18 bp sequence that is completely homologous between both *Alu*Y (Figure 4c). No other coding or non-coding genes were affected by the deletion. Breakpoint spanning PCR revealed that all index

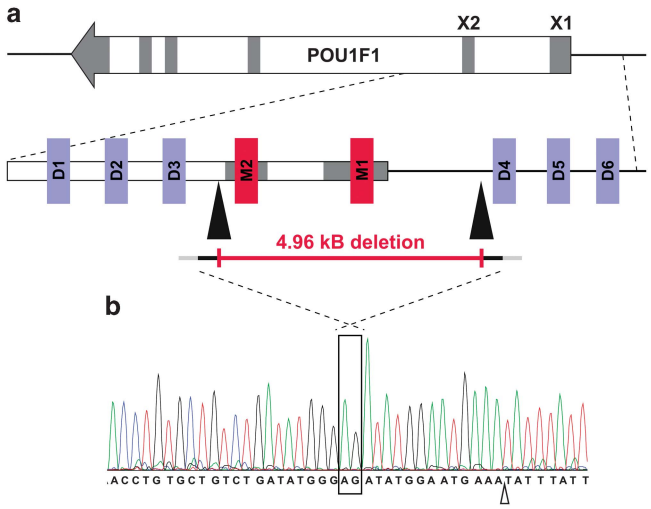

Figure 3 Mapping of *POU1F1* deletion. (**a**) Schematic of the *POU1F1* genomic locus and sequence markers applied to define the deletion boundaries (not drawn to scale). Arrow indicates the orientation of the gene and position of exons (gray bars, exon 1 and 2 designated by X1 and X2). Below the arrow location of the multiplex ligation-dependent probe amplification (MLPA) markers M1 and M2 (red boxes) as well PCR markers (D1–D6, blue boxes) is shown. All markers up- and downstream of the two MLPA probes yielded a positive signal. PCR using forward and reverse primers of markers D3 and D4, respectively, yielded a product ~5 kb shorter than expected. Direct sequencing defined the deletion breakpoints (arrowheads). (**b**) Electropherogram depicts the fusion site of the deletion (open box) that contains an AG dinucleotide, which cannot be unambiguously assigned to the 5′- or 3′-deletion breakpoint due to its occurrence at both sites. Of the 14 nucleotides downstream of the AG dinucleotide fusion site an additional single adenosine was found to be deleted (open arrow head).

patients of case 2 through 4 are carriers of the same deletion, whereas DNA of a previously reported patient from India[18] did not yield a PCR product indicating either a larger deletion and loss of the primer binding site(s) or a substantially smaller deletion that would result in a large PCR product not amplifiable under the conditions applied (Figure 4d).

Haplotype analysis

As all carriers of the *PROP1* deletion bear the same (or a highly similar) deletion and are reported to share a common geographic and/or ethnic origin, we investigated whether the mutation can be ascribed to a common ancestor (founder effect). Haplotypes were determined by analysis of three proximal and four distal STR markers, their phase was derived from the relatives of case 4 (Figure 5). Only the nearest STR marker (D5S2008) that maps ~142 kb distal from *PROP1* was shared by all affected patients from the three independent families (Figure 5). Subsequent STR markers in either direction (D5S2034, 2.49 Mb proximally, and D5S2030, 0.38 Mb distally of *PROP1* located) differed in at least one patient. Typing of SNPs revealed a shared haplotype spanning ~0.55 Mb from rs4507507 to rs4976788 (the latter one mapping 0.15 Mb distally from the non-uniform D5S2030; not shown in Figure 5) thus confirming STR mapping results in distal direction and further delimiting the shared region in proximal direction. Taking both approaches together the maximal shared region encompasses ~0.42 Mb from markers rs11249784 to D5S2030. Of note, both allelic haplotypes of case 2, whose familial relationship was not reported, differed substantially arguing against close kinship among the paternal and maternal lineage (Figure 5; data not shown).

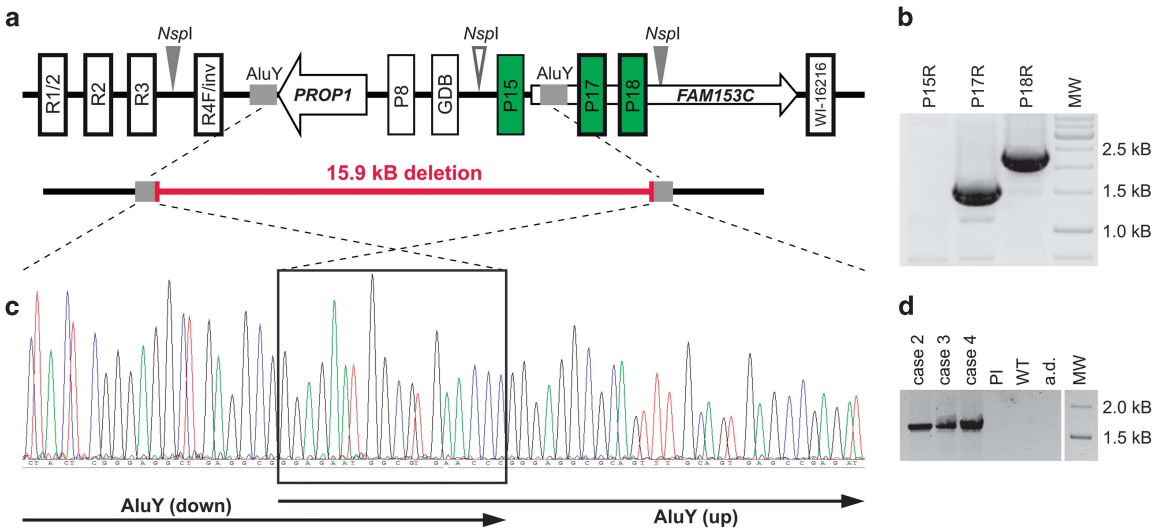

Figure 4 Mapping of *PROP1* deletion. (**a**) Schematic of the *PROP1* genomic organization (not drawn to scale). PCR/STS markers (open boxes) that were amenable to PCR amplification are boxed in bold. Upstream of *PROP1* the only previously established STS marker that yielded a PCR product and that without any doubt could be assigned to the extended *PROP1* locus was WI-16216. Inverse PCR of *Nsp*I restriction endonuclease digested and self-circulated DNA fragments revealed preservation of a *Nsp*I site 18.2 kb distal of the inverse primer pair R4inv (right-hand gray arrow head). Thus, the upstream breakpoint was narrowed down to a range of 3.8 kb (defined by the lack of the neighboring *Nsp*I site, open gray arrow head). (**b**) Long-range PCR with R4F and three additional PCR reverse probes (green boxes) yielded a product for P17R and P18R, but not for P15R. (**c**) Direct sequencing of the shortest product (R4F/P17R) disclosed the range of recombination (shown as frame in the electropherogram) within two *AluY* elements (gray boxes in panel **a**). (**d**) PCR products of the same molecular weight were obtained from genomic DNA of case 2, 3 and 4 using R4F and P17R primers suggesting equal deletion boundaries for these patients. PCR of the DNA of a previously published patient from India (PI) as well wild-type DNA (WT) did not result in the 1576 bp fragment.

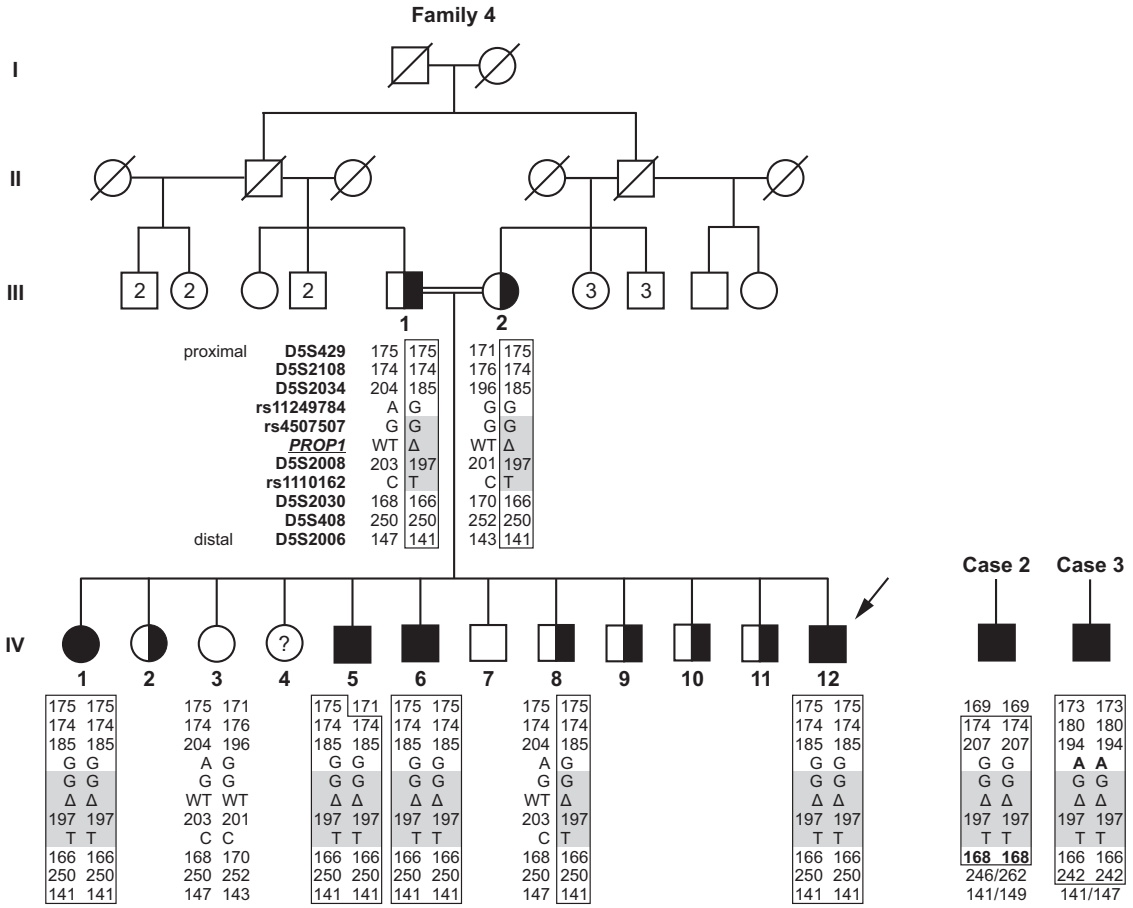

Figure 5 Pedigree of the family of case 4 and haplotypes of all *PROP1* deletion carriers. Index patient of family 4 is indicated by an arrow. Individuals with *PROP1* deletion are depicted with filled symbols, half-filled symbols represent hemizygous carriers. Individuals of generation IV are arranged arbitrarily due to incomplete birth data. The question mark (IV/4) indicates a sibling for whom DNA was not available. Haplotyping results of STR and SNP (only rs11249784, rs4507507 and rs1110162 shown) markers are shown below symbols (designation left hand to generation III results). Results are presented as PCR product length (bp) for STR markers, the specific nucleotide found at the position of SNP markers, and wild-type (WT) or deleted (Δ) genotype for the *PROP1* locus. Shared haplotypes within families are boxed, the conserved region among all deletion carriers is highlighted blue. Closest divergent markers are shown in bold. Markers that could not be phased are separated by a slash. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

DISCUSSION

MLPA analyses of 70 CPHD patients from 64 families revealed causative CNVs in seven patients belonging to four families. Index patients of three families were found to have a complete *PROP1* deletion. One patient carries a *POU1F1* gross deletion comprising exons 1 and 2 of the gene and none of the patients harbored a CNV in *GH1*, *GHRHR*, *HESX1*, *LHX3* or *LHX4*.

Mutations in *PROP1* are the most common genetic cause of CPHD accounting for 11.2% of all so far analyzed CPHD patients (meta-analysis performed in De Rienzo *et al*.[30]). However, depending on the applied screening methodology and the investigated population the prevalence of *PROP1* mutations differs markedly ranging from 0 (refs 31–33) to 64.8% in a Lithuanian population.[34] These differences are in some populations closely linked to the occurrence of a specific genetic aberration; for example, all of the 47 affected Lithuanian patients carried at least 1 c.296delGA allele (also referred to as c.301_302delGA). Until recently gross deletions affecting the complete *PROP1* gene have only been sporadically reported in single patients from Brazil spanning ~18 kb,[16] from the United Kingdom (Kurdish origin, at least 8 kb deleted),[19] Turkey (Kurdish origin, at least 11.7 kb deleted),[21] two unrelated Chinese families (at least 53.2 kb deleted)[20] and from India (no breakpoint data, see below).[18] In 2014 a comprehensive study performed in Turkish CPHD patients disclosed that a majority of mutational carriers harbored a complete deletion of *PROP1* (8/12 unrelated *PROP1* mutation carriers; no breakpoint data).[17]

Previous attempts to define the exact deletion breakpoints failed due to the presence of a duplicated region comprising the non-coding *FAM153C* gene upstream of *PROP1* (Figure 4). We applied an inverse PCR approach that requires only one end of the deletion to be defined roughly to determine both deletion boundaries. All six patients from three families were found to harbor a 15.9 kb deletion comprising the *PROP1* gene. Sequencing showed that the breakpoints result from a fusion between two *Alu*Y elements down- and upstream of *PROP1*, which share 88% identity to each other. *Alu* elements are often described as hot spots of mutations.[35,36] Non-allelic homologous recombination of two *Alu* elements, located on the same chromosome, leads to deletion or duplication of the sequence between the *Alu* elements.[35]

Our mapping results are in line with the assumption of Abrão *et al*.,[16] who, for the first time, suggested an *Alu* sequence-mediated mechanism for *PROP1* excisions. Furthermore, our study

confirms the 3′ deletion breakpoint determined by Akcay *et al.*[21] for a Kurdish CPHD girl. Comparison of size and location of published deletions with the breakpoint coordinates identified in our study discloses that deletion carriers of Kurdish descent may harbor the same or a highly similar mutation, whereas deletions identified in patients from Brazil, China and India (the latter one assessed in this study; Figure 4d) differ in size and/or chromosomal position. However, *Alu* element-driven recombination may have been the underlying mechanism in these deletions as well.

To investigate a possible founder effect we performed haplotype analyses of our three PROP1 index patients and seven relatives of case 4. Only one STR marker (D5S2008) was common to the mutated allele. Together with the SNP mapping data the putative shared haplotype comprises a region of at least 350 kb (from rs4507507 to rs1110162) and at the maximum 423 kb (defined by the closest divergent markers rs11249784 and D5S2030; Figure 5). Demonstration of a shared haplotype along with the accumulation of *PROP1* deletions within the regionally/ethnically confined Kurdish population backs the hypothesis of a founder effect. The finding of a common ancestral origin of the deletion in our patients is reminiscent of findings for the c.301_302_delAG *PROP1* mutation, which was recently shown to originate in the majority of European carriers from a common ancestor, who might have lived ~101 generations ago.[29]

Contrary to *PROP1* deletions that occur with high prevalence in the Kurdish population, only three single cases of *POU1F1* deletions have been reported to date; one of them as compound hemizygous complete gene deletion in one of the two, first ever reported *POU1F1* Dutch families.[23] A second case was identified in an Israeli–Arab girl as a homozygous microdeletion comprising the neighboring *CHMP2B* and *VGLL3* genes in addition to *POU1F1* leading to a contiguous gene syndrome with clinical features closely related to CPHD and, beyond that, signs of GH insensitivity.[27] A third patient carrying a homozygous deletion of *POU1F1* exons 1 and 2 was recently described in the Turkish CPHD mutational screening.[17] Although no refinement of the deletion boundaries was performed for this patient, it appears possible that he harbors the same or a similar mutation as our Turkish patient 1 (c.-1387_214+455del).

Clinical manifestations in our patients confirm the phenotype classically associated with biallelic *PROP1* or *POU1F1* mutations, in line with our finding that neither the *PROP1* nor the *POU1F1* deletion affects additional genes. As observed in the majority of previously reported POU1F1 patients, our patient with *POU1F1* gross deletion presented with GH, TSH and PRL deficits.[17,25] MRI, as often described for POU1F1 defects, displayed a hypoplastic anterior pituitary but normal pituitary stalk and posterior lobe. Also our patients carrying *PROP1* deletions confirmed findings from previous reports, in that they had impaired function of somatotropes and gonadotropes as well as—with some variability despite the same mutation—of thyrotropes and lactotropes.[37–45] Corticotrope insufficiency evolved in one patient at the age of 13 years that made cortisol substitution necessary and underlines the need for follow-up monitoring of the adrenal axis in patients with PROP1 defects.[9] MRI of our affected subjects showed hypoplastic anterior pituitary in two families, whereas the adenohypophysis of the deletion carriers of family 4 was rather normal, emphasizing morphological variability and lack of correlation between pituitary size and pituitary function in PROP1 patients.[13,17]

In conclusion, in this study we show by means of MLPA, deletion mapping and haplotype analyses that patients with CPHD may carry large deletions comprising several exons or the entire gene. A *PROP1* deletion that starts several kilobases upstream of *PROP1* and ends close to the translation stop probably occurs with relatively high frequency within populations of Kurdish descent. This deletion originates most likely from a common ancestor and can be assumed to be the prevailing CPHD causing aberration in these regions. In contrast, *POU1F1* deletions are rare (likewise point mutations) without any apparent regional preponderance. Clinical characteristics of deletion carriers manifest only in the homozygous but not in the hemizygous state and are indistinguishable from clinical features typically observed in carriers of *POU1F1* or *PROP1* point mutations. MLPA analysis should be considered in the routine diagnostics of CPHD patients in specific populations or if conventional or next-generation sequencing techniques yield inconclusive results (heterozygotes), at least as long as cost-effective, copy number-sensitive next-generation sequencing methodologies are not available. Particularly in cases with a dominant familial inheritance pattern MLPA analysis of samples with negative sequencing results might be advisable. In Kurdish patients—or if ethnic background is unknown in patients from Turkey, Syria, Iraq or Iran—long-range PCR as established in our study may even represent an appropriate complement to classical Sanger or next-generation sequencing as the first analytical choice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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