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Novel Mutations within the *POU1F1* Gene Associated with Variable Combined Pituitary Hormone Deficiency

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Context: Mutations within the gene encoding the pituitary-specific transcription factor *POU1F1* are associated with combined pituitary hormone deficiency (CPHD). Most of the affected individuals manifest GH, prolactin, and TSH deficiency.

Objective: We have now screened 129 individuals with CPHD and isolated GH deficiency for mutations within *POU1F1*.

Results: Causative mutations were identified in 10 of 129 individuals (7.8%). Of these, five patients harbored the dominant negative R271W mutation, which is a well-recognized mutational hot spot. We have also identified a second frequently occurring mutation, E230K, which appears to be common in Maltese patients. Additionally, we describe two novel mutations within *POU1F1*, an insertion of a single base pair (ins778A) and a missense mutation (R172Q). Functional studies have revealed that *POU1F1* (E230K) is associated with a reduction in transactivation, although DNA-binding affinity is similar to the wild-type protein. On the other hand, *POU1F1* (R172Q) is associated with a reduction in DNA binding and transactivation, whereas *POU1F1* (ins778A) is associated with loss of DNA binding and a reduction in transactivation.

Conclusions: Our data suggest that the phenotype associated with *POU1F1* mutations may be more variable, with the occasional preservation of TSH secretion. Additionally, our data revealed *POU1F1* mutations in three patients who were diagnosed as having ACTH deficiency but who, on further evaluation, were found to have normal cortisol secretion. Hence, elucidation of the genotype led to further evaluation of the phenotype, with the cessation of cortisol replacement that had been commenced unnecessarily. These data reflect the importance of mutational analysis in patients with CPHD. **(*J Clin Endocrinol Metab* 90: 4762–4770, 2005)**

NORMAL DEVELOPMENT OF the anterior pituitary in both the rodent and humans is critically dependent upon the precise spatial and temporal expression and interaction of signaling molecules in combination with a cascade of transcriptional factors, *e.g. Hesx1*, *Lhx3*, *Lhx4*, *Prop1*, and *Pit-1* (1).

Pit-1 (murine ortholog of human *POU1F1*) was the first pituitary-specific transcription factor to be identified in the human and mouse. It belongs to the POU family of transcription factors, and its expression is restricted to the anterior pituitary lobe (2). It regulates the expression of a number of target genes by binding to multiple sites on these targets (3–7). Pit-1 is a 291-amino-acid protein that contains three domains: an N-terminal transcriptional activation domain, a POU-specific domain (POU-S), and a POU-homeodomain (POU-H). The POU-S and POU-H are required for high-affinity DNA binding. The POU-S contains four α-helices, and the POU-H contains three α-helices.

Pit-1 is not only essential for cell-specific gene expression and regulation but is also essential for the development of certain anterior pituitary cells, namely somatotrophs, lactotrophs, and thyrotrophs (8). Thyrotrophs arise from two independent cell populations in mice. The first population appears on embryonic d 12 in the rostral tip of the developing anterior pituitary and is Pit-1 independent and transient, disappearing from birth. The second population is Pit-1 dependent and arises in the caudomedial region of the developing pituitary on embryonic d 15.5.

Two naturally occurring mouse models shed light on the role of Pit-1 in pituitary development. A naturally occurring point mutation within the *Pit-1* gene (W261C) is responsible for the phenotype associated with the Snell dwarf mouse (9). The phenotype is characterized by anterior pituitary hypoplasia and deficiencies of GH, prolactin (PRL), and TSH, with a low level of *Pit-1* gene expression. The Jackson dwarf

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Abbreviations: CPHD, Combined pituitary hormone deficiency; IGHD, isolated GH deficiency; MRI, magnetic resonance imaging; POU-H, POU-homeodomain; POU-S, POU-specific domain; PRL, prolactin.

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mouse has a similar phenotype, but with no *Pit-1* expression, and is a result of either an inversion or insertion of a greater than 4-kB segment of DNA disrupting the *Pit-1* gene completely.

In humans, mutations within *POU1F1* were first described in 1992 by four independent groups (10–13) and are associated with GH, PRL, and TSH deficiency, with variable pituitary hypoplasia. Deficiencies of GH and PRL are generally complete, but the TSH deficiency is more variable. In the majority of patients, hypothyroidism is early and profound, necessitating the early use of T_4. In a smaller proportion of cases, hypothyroidism is a later event, occurring between the ages of 9 and 15 yr (14). TSH deficiency has always been a feature in children with *POU1F1* mutations (15, 16). A total of 21 different mutations (five dominant, 16 recessive) have been described to date (Fig. 1). Of these, the dominant R271W mutation is by far the most frequent, having been identified in 14 of 46 patients from a variety of ethnic backgrounds (10, 13, 17–23). This mutation lies at the carboxy terminus of the homeodomain, and the substitution of tryptophan for arginine leads to a reduction in the positive charge in a basic amino acid region. The mutant R271W protein binds to DNA and acts as a dominant inhibitor of transcriptional activation by the wild-type protein (13). The only other mutations reported in more than one pedigree are the recessively inherited R172X (three pedigrees) (11, 24, 25), A158P (two pedigrees) (12), and P239S (three pedigrees) (26) mutations in the POU-S and POU-H of *POU1F1*.

Few studies have investigated the incidence of *POU1F1* mutations in cohorts of patients with sporadic combined pituitary hormone deficiency (CPHD). McLennan *et al.* (27) identified two individuals with mutations within *POU1F1* in a series of 33 patients with CPHD. We now report the results of screening 129 patients with isolated GH deficiency (IGHD) and CPHD for mutations within *POU1F1*.

Patients and Methods

Patients

Patients with various hypothalamo-pituitary disorders were recruited into the study from both national and international pediatric and adult endocrinology centers. A total of 129 probands (male:female, 1.9:1) were screened for mutations within the *POU1F1* gene. The vast majority of patients screened had sporadic CPHD, although the cohort included 24 familial cases belonging to 17 unrelated families. Given the variable phenotype such as late-onset central hypothyroidism in some patients with *POU1F1* mutations, those screened included patients with both CPHD and IGHD.

Fifty-seven patients were referred to the London Centre for Pediatric Endocrinology based at Great Ormond Street Children's Hospital and the University College London Hospitals. Ethical committee approval was obtained from the Institute of Child Health/Great Ormond Street Children's Hospital Research and Ethics Committee. Informed consent was obtained before collection of samples and genomic analysis from the parents and, where applicable, the patients. Probands were also recruited from various other national (n = 24) and international (n = 48) endocrine centers from nine different countries. These samples were sent for screening for mutations in pituitary development genes in general, or in some cases, *POU1F1* specifically. Full informed consent was obtained from parents and patients as appropriate.

Clinical evaluation

Retrospective clinical details obtained from patients included birth details, perinatal complications, history of consanguinity, family history, and parental heights. Pituitary function was assessed using standard dynamic tests (28). Hormonal assays were performed using several commercial RIA kits, and normal values for each center were taken into account. Magnetic resonance imaging (MRI) (1.5 Tesla Siemens Magnetom Symphony, Bracknell, UK) included T1 and T2 weighted high-resolution pituitary imaging through the hypothalamo-pituitary axis (T1 sagittal 3-mm slices, T1 and T2 coronal 3-mm slices). Details noted included the size of the anterior pituitary, position of the posterior pituitary signal, presence and morphology of the optic nerves, optic chiasm, pituitary stalk, septum pellucidum, and corpus callosum.

Genomic and mutation/single-nucleotide polymorphism analysis of the POU1F1 gene

Genomic analysis was conducted by initial amplification of *POU1F1* exons using previously described primers (10) and analyzed by single-stranded conformational polymorphism analysis (28). The g515a/R172Q mutation was confirmed by amplification-created restriction site, whereas the g688a/E230K was confirmed by restriction by *Ear*I enzyme. The two intronic single-nucleotide polymorphisms (IVS5-5nts g→a and IVS5-6nts c→t) could be detected by a combination of two restriction digests. *Aci*I and *Cac*8I cut the wild-type alleles; the *Aci*I site is ablated by both mutations whereas the *Cac*8I site is only ablated by the IVS5-5a allele. Therefore, by using both of these digests, it was possible to screen for the putative polymorphisms in patients and control subjects.

Plasmids

Wild-type human *POU1F1* cDNA was inserted into the effector plasmid pcDNA3. The various reporter constructs that contained Pit-1 binding sites within the context of different gene-regulatory regions were fused to a firefly luciferase gene. We used a PRL reporter construct from the proximal promoter regions of the human PRL gene −250 (134 bp; PRL 250) containing three Pit-1 binding sites (gift of J. A. Martial, Liege, Belgium). The proximal promoter of the human GH gene (Pa3-Ghp-Luc) contained two Pit-1 response elements (gift of N. L. Eberhardt, Rochester, MN). A reporter construct containing the positive autoregulatory site of the human *POU1F1* promoter gene was also used (gift of M. Delhase, San Diego, CA).

Site-directed mutagenesis

In vitro site-directed mutagenesis was achieved using the QuickChange kit (Stratagene Cloning Systems, La Jolla, CA) according to the

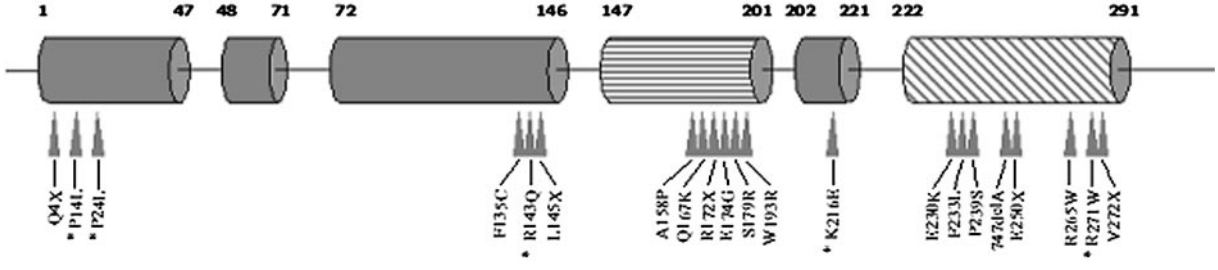

FIG. 1. Mutations to date within the *POU1F1* gene. To date, 21 mutations and one complete deletion have been described in the *POU1F1* gene. Of these, five are dominant (denoted by *). POU-S is patterned with *horizontal stripes*, and POU-H is patterned with *diagonal stripes*.

manufacturer's instructions and using mutagenetic primers as follows: sense, CAG TCA AAC AAC AAT CTG CC**A** ATT TGA AAA TCT CGA GC; antisense, GCT CGA GAT TTT CAA AT**T** GGC AGA TTG TTG TTT GAC TG(R172Q); sense, CTG CTA AAG ATG CTC TG**A** AGA GAC ACT TTG GAG AAC; antisense, GTT CTC CAA AGT GTC TCT **T**CA GAG CAT CTT TAG CAG (E230K); sense, TGG AGA AAG AAG TAG TAA GAA**G**TT TGG TTT TGC AAC CGG; antisense, CCG GTT GCA AAA CCA AAC **T**TC TTA CTA CTT CTT TCT CCA (Ins778A). *Bold type* represents mutated residues. Introduction of mutation was confirmed by direct sequencing.

Cotransfection in eukaryotic cells

Briefly, HeLa cells were maintained in DMEM supplemented with 10% fetal bovine serum, ampicillin, and amphotericin B and grown to 80% confluence in six-well plates. Transfection of 0.6 μg per well of reporter (PRL-250, GH, or *POU1F1* promoter) and 0.6 μg per well effector (empty vector or wild-type or mutant *POU1F1*) constructs was achieved using the liposome technique (Polyfect transfection reagent; QIAGEN, Hilden, Germany). Total DNA was kept constant with pcDNA3 empty vector, which also acted as a control. Transfection efficiency was determined using 0.1 μg pCMVβ-*gal* (Clontech Laboratories, Inc., Palo Alto, CA), and luciferase values were normalized to it. Cells were harvested 48 h after transfection for luciferase assays. Transfections were performed in triplicate within a single experiment, and experiments were repeated three times.

EMSA analysis

EMSAs were performed with recombinant *POU1F1* proteins synthesized by TNT-coupled transcription-translation reticulocyte lysate system, according to the manufacturer's protocol (Promega Corp., Madison, WI). Efficiency of synthesis of each protein was determined by incorporation of [^{35}S]Met (10 mCi/ml; Perkin-Elmer, Boston, MA) and assayed by autoradiograph. *POU1F1* binding was tested using a high-affinity *POU1F1* DNA binding site from the human PRL gene (PRL-P1), 5′-AATGCCTGAATCAT, TATATTCATGAAGATATC-3′, labeled with α^{32}P and binding specificity confirmed by addition of excess unlabeled oligonucleotide. Supershifts were achieved using a *POU1F1* monoclonal antibody (BD Biosciences, San Jose, CA).

Results

Patient details

Of the 129 probands, CPHD was documented in 80 patients and isolated pituitary hormone deficiencies (GH, n = 48; TSH deficiency, n = 1) in the remaining 49 patients. Detailed endocrine phenotype was available in all of the 80 CPHD patients (Table 1). Results of the MR scans were available in 29 of 48 patients with IGHD and in 55 of 80 patients with CPHD. Details regarding the structural abnormalities of the hypothalamo-pituitary axis on neuroimaging in the probands are shown in Table 2.

Genomic analysis of POU1F1

Genomic screening of the coding regions of *POUIF1* yielded causative mutations in 10 of 129 screened (7.8%) (Fig. 2) and two probable novel intronic polymorphisms. Sequence changes were analyzed using a splice site-predicting program GrailEXP to ascertain potential changes in exon definition.

Mutations within POU1F1

Ten patients (M:F 1:1) from seven pedigrees were identified to have mutations within *POU1F1* (Tables 3 and 4; Figs. 2 and 3). Patients 1.I and 1.II, siblings born to Maltese parents, were found to be compound heterozygotes for two missense mutations: a novel g515a change within exon 4 that results in the substitution of arginine by glutamine (R172Q) in the POU-S (Fig. 2, A and B) and a g688a change within exon 6 resulting in the substitution of a glutamate residue by lysine at position 230 (E230K) in the first α-helix of the POU-H (Fig. 2, C and D) that has previously been described in the homozygous state in two siblings from a consanguineous Israeli-Arab pedigree (29). Patients 2 and 3 (born to second-degree consanguineous parents), also of Maltese origin, were homozygous for the E230K substitution (Fig. 2E), whereas patients 4, 5.II and her daughter 5.I, and 6.II and her son 6.I were all found to harbor the heterozygous c811t point mutation in exon 6 resulting in the substitution of a highly conserved arginine residue by tryptophan in the homeodomain (R271W) (Fig. 2F). This mutation represents a known mutational hot spot and is believed to act as a dominant negative mutation (13), although this has been disputed in a recent publication (30). Mutational analysis of *POU1F1* in patient 7 revealed compound heterozygosity for two mutations: E230K and a novel insertion of an adenine at position 778 (ins778A) in exon 6 of the gene (Fig. 2G). The ins778A would be predicted to result in a frameshift with a truncated protein of 284 amino acids instead of the 291-amino-acid wild-type protein.

Phenotypes of patients with POU1F1 mutations

With the exception of patient 2, all of the affected patients manifested profound GH, TSH, and PRL deficiency (Table 3). Patient 2, who is now aged 20.5 yr, was found to have a free T_4 that has remained within the normal range [1.16 ng/dl (15pmol/liter)] off T_4 treatment. Although the serum cortisol concentration was normal in patient 3, he was empirically commenced on hydrocortisone treatment when he presented with symptoms of fatigue at the age of 13.1 yr. He underwent spontaneous puberty, and after the identification of a mutation within *POU1F1*, he has been successfully weaned off hydrocortisone replacement.

Patient 4 presented at the age of 6 months with short stature, a poor growth velocity, and bilaterally undescended testes. Investigations performed at that stage revealed central hypothyroidism. He was commenced on T_4 treatment but continued to demonstrate poor growth with recurrent epi-

TABLE 1. Endocrine phenotype of probands screened for *POU1F1* mutations

	No. (%) with deficiencies of				
	GH	TSH	ACTH	Gonadotropins	PRL
Isolated pituitary hormone deficiency (n = 49)	48 (98)	1 (2.0)	0	0	0
CPHD (n = 80)	80 (100)	77 (96.3)	44 (55)	34 (42.5)	40 (50)

TABLE 2. Results of MR scans of probands screened for *POU1F1* mutations

	Morphology of								
	Anterior pituitary			Posterior pituitary			Stalk		
	Normal	Small	Enlarged	Normal	Undescended	Absent	Normal	Absent	Thick
IGHD (n = 28)	7	21	0	14	13	1	20	8	0
ITSHD (n = 1)	1	0	0	1	0	0	1	0	0
CPHD (n = 55)	18	37	0	26	28	1	18	37	0
Total (n = 84)	26	58	0	41	41	2	39	45	0

ITSHD, Isolated TSH deficiency.

sodes of hypoglycemia. Full investigation of the hypothalamo-pituitary axis at the age of 2 yr confirmed CPHD with borderline hypocortisolemia [basal, 12.1 μg/dl (334 nmol/liter); peak, 15.4 μg/dl (425 nmol/liter)] and severe GH and PRL deficiencies. Treatment with recombinant human GH was commenced at the age of 2 yr, and hydrocortisone replacement was commenced at the age of 6.5 yr in view of symptoms of fatigue in conjunction with the previously documented borderline cortisol insufficiency. In view of the undescended testes, it was assumed that the patient was gonadotropin deficient, and puberty was induced at 11 yr of age with depot testosterone treatment, resulting in a final height of −3.2 SD. After the identification of a mutation within *POU1F1*, and given that mutations within this gene are not associated with ACTH and gonadotropin deficiencies, he was reinvestigated off all replacement treatment at the end of his statural growth. This reconfirmed GH [peak GH, <0.1 ng/ml (<0.3 mU/liter)], PRL [0.8 ng/ml (16 mU/liter)], and TSH [basal TSH, 0.8 μU/ml (0.8 mU/liter); peak, 1.2 μU/ml (1.2 mU/liter); free T_4, 0.4 ng/dl (5.2 pmol/liter)] deficiencies. He mounted a satisfactory serum cortisol response to insulin-induced hypoglycemia [22.6 μg/dl (624 nmol/liter)], and his gonadotropin response to LHRH was also satisfactory [LH, 15.1 mU/ml (15.1U/liter); FSH, 5.8 mU/ml [5.8 U/liter)] with a serum testosterone concentration of 4.3 ng/ml (15 nmol/liter). Hence, as with patient 3, evaluation of his genotype with confirmation of a mutation within *POU1F1* led to a revision of his endocrine phenotype, with subsequent cessation of hydrocortisone and testosterone replacement.

Patient 5.II presented with early growth failure and was confirmed to have GH deficiency. Despite GH replacement treatment, poor growth persisted, and additional tests confirmed secondary hypothyroidism and an absent TSH response to TRH stimulation. She was commenced on T_4 replacement at the age of 5 yr. She demonstrated a partial cortisol response to metyrapone at 11 yr of age that resulted in substitution with cortisone acetate treatment. Spontaneous menarche was achieved at 13 yr of age, and she has reached a final height of −3.7 SD. Neuroimaging showed a small pituitary gland and a normal infundibulum. She has since undergone full dynamic pituitary testing as an adult, off all replacement treatment. This has confirmed GH (peak GH, 0.3 ng/ml (0.9 mU/liter)] and TSH [TSH, 1 μU/ml (1 mU/liter); total T_4, <1.5 ng/dl (19.4 pmol/liter)] deficiencies but with normal cortisol secretion. PRL deficiency (serum concentration, 6.2 ng/ml (124 mU/liter), absent response to TRH stimulation) was diagnosed because of failure of lactation. Cortisol replacement has subsequently been stopped.

Of eight patients in whom MRI scans have been performed, seven had a hypoplastic anterior pituitary (Fig. 4), although one patient had a normal anterior pituitary (Table 4). There were no abnormalities of the infundibulum and posterior pituitary.

Polymorphisms within POU1F1

Two novel heterozygous intronic changes were identified within *POU1F1*; IVS5-5nt g→a and IVS5-6nt g→a, both lie in a pyrimidine tract upstream of the acceptor splice site of intron 5. The former was identified in a patient with panhypopituitarism and anterior pituitary hypoplasia, an absent infundibulum, and an undescended/ectopic posterior pituitary on MRI. Additionally, the change was identified in his unaffected mother. The IVS5-6nt g→a change was identified in a female with GH deficiency and an intermittently low free T_4 concentration. These sequence changes were not present in 228 Caucasian control alleles and are likely to represent rare polymorphisms, although one cannot exclude possible splicing defects.

Functional studies of POUIFI (E230K), POU1F1 (ins778A), and POU1F1 (R172Q)

Transient transfection assays using POU-binding sites in the *GH-1*, *PRL*, and *POU1F1* promoters showed reduced transactivation by all three mutant proteins, and this was most pronounced on the PRL promoter. *POU1F1* (E230K) was associated with less severe impairment of transactivation as compared with *POU1F1* (R172Q) and *POU1F1* (Ins778A) (Fig. 5A). EMSA revealed that *POU1F1* (E230K) had a similar binding affinity to the wild-type protein, whereas that of *POU1F1* (R172Q) was greatly reduced (Fig. 5B). *POU1F1* (ins778A) would be predicted to lack the terminal 33 amino acid residues of the third α-helix of the POU-H, and in keeping with this, the mutant protein led to complete loss of DNA binding (Fig. 5B). All three mutant proteins were equally expressed in ^{35}S *in vitro* translation studies (Fig. 5C).

Discussion

The original description of mutations within *POU1F1* suggested that mutations within the gene are associated with the classical phenotype of GH, TSH, and PRL deficiency (11–13). Subsequently, a number of reports have described a mutation within the gene in individuals from a single pedigree, and to date, *POU1F1* mutations have been described in a total of 46 patients from 34 families originating in 17 different

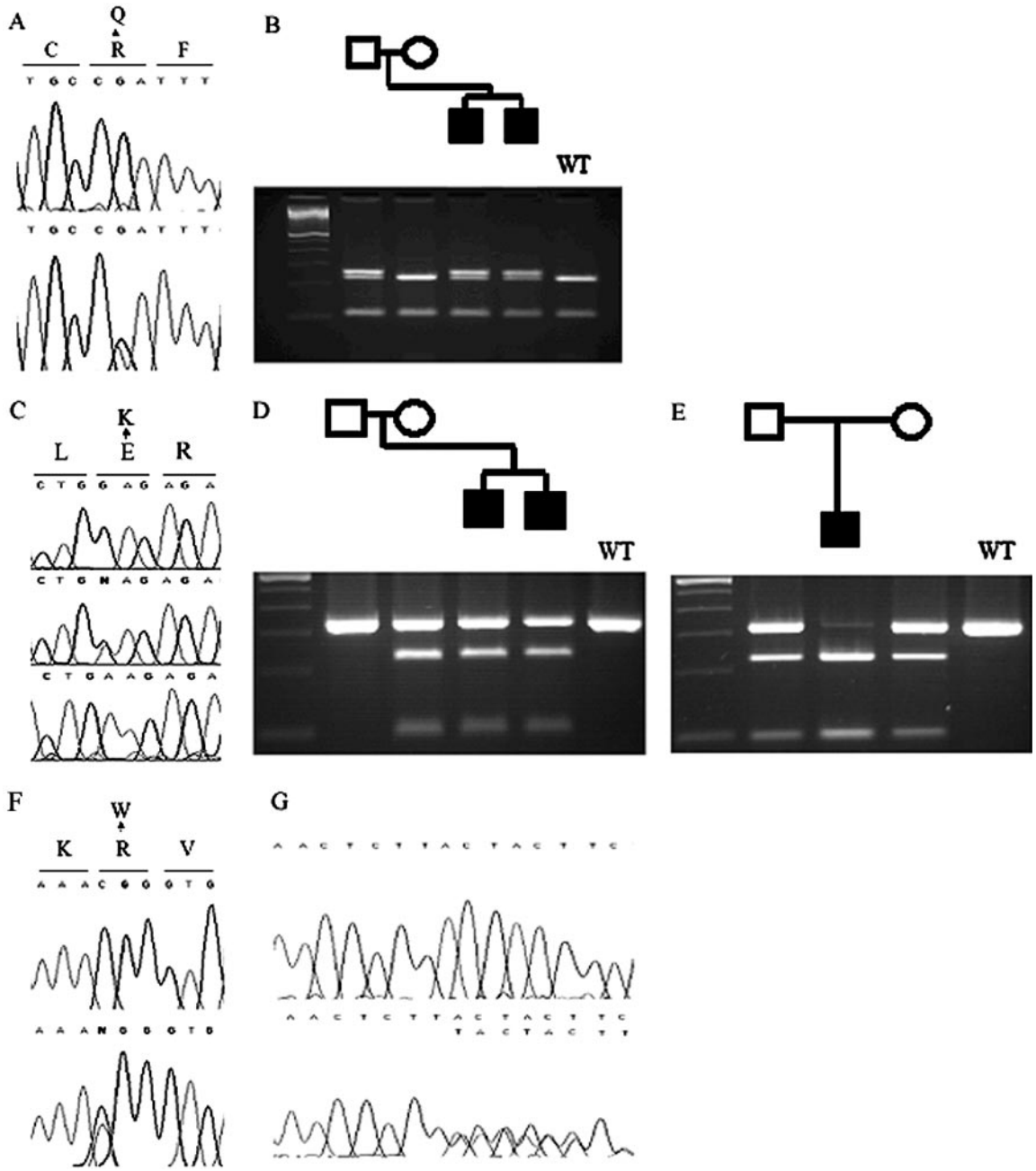

FIG. 2. Mutations identified in a cohort of hypopituitary patients. A, Electropherogram showing the heterozygous g515a/R172Q mutation in patients 1.I and 1.II. B, *Hin*f1 restriction digest of an amplification-created restriction site product confirming heterozygosity for the g515a/R172Q mutation in patients 1.I and 1.II. Wild-type DNA yielded 256- and 118-bp products, whereas heterozygotes yielded 256-, 226-, 118-, and 30-bp products, although the latter could not be visualized on the gel. The heterozygous mutation was inherited from the unaffected father. C, Electropherogram showing the heterozygous g688a/E230K mutation in patients 1.I and 1.II. *Top*, Control subject; *middle*, heterozygous change; *bottom*, homozygous for mutation. D, *Ear*I restriction digest confirming heterozygosity for g688a/E230K mutation in patients 1.I and 1.II. Restriction assay of exon 6 PCR product yielded bands of 333, 224, and 109 bp in the heterozygous mutant and a single undigested band of 333 bp in the wild type. The heterozygous mutation was inherited from the unaffected mother. E, *Ear*I restriction digest confirming homozygosity for g688a/E230K mutation in patient 3. The homozygous mutation led to two bands of 224 and 109 bp as compared with a single band of 333 bp in the wild type and three bands (333, 224, and 109 bp) in the heterozygous parents. F, Electropherogram showing the heterozygous c811t (reverse complement sequence) change in exon 6 of *POU1F1*, leading to the R271W substitution in patient 4. *Top*, Wild-type sequence; *bottom*, heterozygous c811t mutation. G, Electropherogram showing heterozygous insertion of an A residue at position 778. *Top*, Wild-type sequence; *bottom*, heterozygous insA778 mutation.

countries (for review, see Ref. 31). Of these, 28 individuals demonstrate a recessive mode of inheritance, whereas 18 demonstrate a dominant mode of inheritance. Brown *et al.* (24) suggest that the incidence of *POU1F1* mutations in patients with GH, TSH, and PRL deficiencies may be as great as 50%. We have now screened a cohort of patients with sporadic (n = 105) and familial (n = 24) hypopituitarism for mutations within *POU1F1*. Given that PRL concentrations

TABLE 3. Phenotypic details of patients with *POU1F1* mutations

Patient no.	Sex	Country of origin	Presentation		Peak GH (ng/ml)	Free T_4 (ng/dl)	Basal TSH (μU/ml)	Basal PRL (ng/ml)	Cortisol (μg/dl)		Gonadotropins		Final height (SD)	Mutation
			Height (SD)	Age (yr)					Basal	Peak	LH (IU/liter)	FSH (IU/liter)		
1.I	M	Malta	−3.6	0.35	1.3	0.4	0.06	0.7	24.4	49.8	29.8	3.9	NA	E230K/R172Q
1.II	M	Malta	−2.3	0.08	NT	0.2	0.1	0.5	NT	NT	NT	NT	NA	E230K/R172Q
2	F	Malta	−5.8	2.38	0.1	1.2	0.3	0.7	20.9	26.9	40.1	6.1	−0.2	E230K
3	M	Malta	−5.6	1.45	0.7	0.6	2.7	5.9	19.6	32.3	NT	NT	−1	E230K
4	M	UK	−7.8	1.5	0.3	2.9[a]	1	2.2	16.7	22.5	15.1	5.8	−3.7	R271W
5.I	F	UK		0.33	0.3	0.7	0.9	2.5	11	30	12	30	NA	R271W
5.II	F	UK		0.16	0.3	1.58[a]	1	6.2		26.2	30	5	−3.7	R271W
6.I	F	UK	−4.6	6	0.3	0.3	1.9	<0.6	11.3	35	106.2	14.6	NA	R271W
6.II	M	UK	−4.5	0.25	0.4	0.75	1.9	<0.4	3	35	13.5	1.4	−2.3	R271W
7	F	Russia	−5.9	2.4	0.25	Low	0.08	0.6	15.6	NA	NA	NA	NA	E230K/insA778

To convert to Systeme International units, multiply metric units by 3 [GH (mU/liter)], 12.9 [free T_4 (pmol/liter)], 1 [TSH (mU/liter)], 20 [PRL (mU/liter)], 27.6 [cortisol (nmol/liter)], or 1 [LH and FSH (U/liter)]. NT, Not tested; NA, not available; M, male; F, female.

[a] Total T_4.

are not always measured, and given the variability of the timing of onset of the TSH deficiency associated with *POU1F1*, we screened patients with IGHD for mutations in addition to those with CPHD. We also included patients with infundibular abnormalities and/or an ectopic/undescended posterior pituitary in our study, given that this category of patients has not been previously studied with respect to the presence of *POU1F1* mutations. Mutations were identified in 10 patients (7.8%), and 60% of these occurred in familial cases of CPHD.

Only one mutation (patient 2) was identified in the group of patients with IGHD (n = 48), and no mutations were identified in patients with an absent or undescended posterior pituitary (n = 43) and/or stalk abnormalities (n = 45). The latter abnormalities may represent a cohort of patients with an early developmental defect in the hypothalamus, as opposed to the pituitary (32–35).

Of the 10 patients in whom mutations were identified, six patients originated from three pedigrees and so would be classified as familial cases. Hence, the true incidence of *POU1F1* mutations in an unselected cohort of patients with sporadic CPHD is very low (approximately 3.8%), whereas that in a carefully selected population with familial hypopituitarism is greater (25%). Our findings are consistent with those of McLennan *et al.* (27), although four of these patients also had optic nerve hypoplasia and an additional three had evidence of hypothalamic dysfunction. One has to bear in mind that the sensitivity of single-stranded conformational polymorphism, used as a screening technique in our studies, is of the order of approximately 80–90% (36) and so may have resulted in a small number of false negative results, although each sample was processed at two different temperatures to increase sensitivity.

TABLE 4. Neuroimaging in patients with *POU1F1* mutations

Patient no.	Anterior pituitary	Posterior pituitary	Stalk
1.I	Small	Normal	Normal
1.II	NA	NA	NA
2	Small	Normal	Normal
3	Small	Normal	Normal
4	Small	Normal	Normal
5.I	Small	Normal	Normal
5.II	Normal	Normal	Normal
6.I	NA	NA	NA
6.II	Small	Normal	Normal
7	Small	Normal	Normal

NA, Not available.

Our study also confirms phenotypic variability in patients with *POU1F1* mutations, mainly with respect to the onset of central hypothyroidism. Pellegrini-Bouiller *et al.* (14) reported a variable onset of TSH deficiency (9–15 yr) in four members of a single pedigree who had the homozygous F135C mutation within *POU1F1*. All of the patients with *POU1F1* mutations in our study showed complete GH and PRL deficiency. Additionally, nine of 10 patients showed evidence of profound secondary hypothyroidism. Patient 2 had an identical genotype to patient 3 (E230K) but had preserved T_4 secretion at the age of 20.5 yr, unlike patient 3 who developed central hypothyroidism at the age of 1.45 yr. This, to our knowledge, is the first report of preserved T_4 secretion into the third decade in a patient with *POU1F1* deficiency. Previous reports have suggested that TSH deficiency is invariably associated with mutations within *POU1F1* (15, 16). Interestingly, two siblings who have previously been shown to have the homozygous E230K mutation in the POU-H presented with GH deficiency but had normal PRL secretion. Additionally, central hypothyroidism was diagnosed in one of the siblings at the age of 10 months, whereas the second sibling had normal thyroid function at the age of 4 yr (29).

Patients 3, 4, and 5.II were treated with hydrocortisone initially, given suboptimal cortisol concentrations and symptoms of fatigue and lethargy. Patient 4 was also commenced on testosterone supplementation, because he had bilaterally undescended testes. Once the results of mutational analysis were available, hydrocortisone treatment was stopped in patients 3 and 4. Testosterone was also stopped in patient 4. Hydrocortisone was stopped in patient 5.II once the results of retesting became available. These three cases illustrate the importance of careful phenotypic characterization and genetic analysis in patients with CPHD and IGHD. Given the vagaries of endocrine testing, it is important to consider the possibility of *POU1F1* mutations in patients with cortisol insufficiency, particularly if the MRI scan shows isolated anterior pituitary hypoplasia or even a normal anterior pituitary (12). In contrast to patients with mutations within the

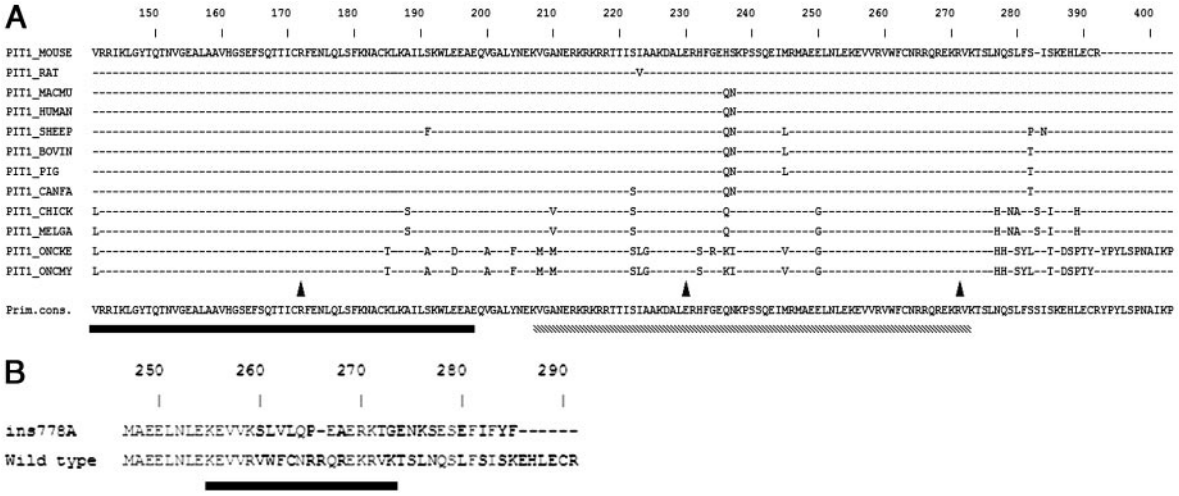

FIG. 3. Novel mutations within *POU1F1* identified in this study. A, Cross-species amino acid sequence Clustal alignment of distal end of PIT1/POU1F1 protein. *Bars* represent distal end of POU-S and POU-H, in *black* and *gray hatching*, respectively. *Arrowheads* indicate positions of R172Q, E230K, and R271W mutations. Note the total conservation of these residues. B, Clustal alignment of wild-type *POU1F1* (accession number NP_000297) and predicted amino acid sequence resulting from the ins778a mutation. *Bar* represents third α-helix of the POU-H.

homeobox gene *Prophet of Pit-1* (*PROP1*), patients with mutations within *POU1F1* do not manifest gonadotropin and cortisol deficiency. Additionally, whereas MRI scanning may reveal a small, normal, or enlarged anterior pituitary in patients with mutations within *PROP1* (37, 38), the anterior pituitary is either hypoplastic or of a normal size in patients with mutations within *POU1F1* (12, 18).

We identified the R271W mutation in five of 10 patients including two pedigrees where both the mother and a child (patients 5.II and 5.I and 6.II and 6.I) manifested GH, PRL, and TSH deficiency. Okamoto *et al.* (18) suggested that the mutation could be variably penetrant, possibly because of monoallelic expression. Our data and those of de Zegher *et al.* (19) do not support this hypothesis.

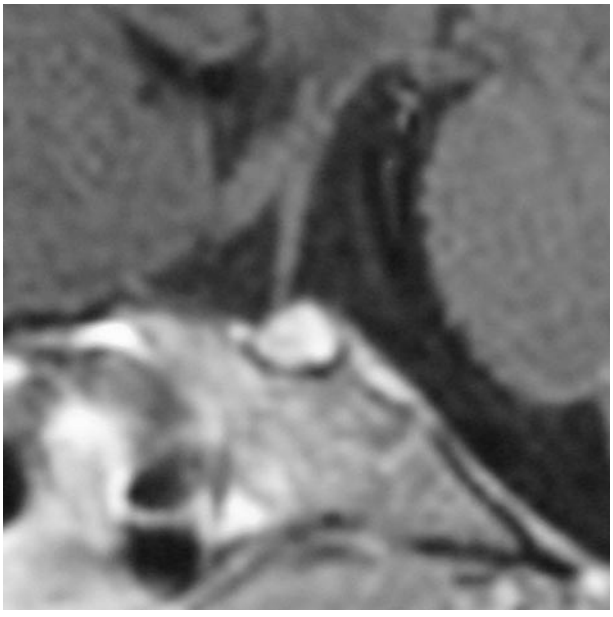

FIG. 4. Sagittal MRI scan in patient 1.I. The scan shows hypoplasia of the anterior pituitary gland with a normal posterior pituitary and infundibulum.

Our data also suggest the presence of a novel mutational hot spot within *POUIF1* (E230K), with the identification of the mutation in seven individuals from five different pedigrees (this study and Ref. 29). Of these, three pedigrees originated from Malta, suggesting that a founder effect cannot be excluded. Glutamate at position 230 is highly conserved within a number of species (chicken, turkey, mouse, rat, sheep, cow, pig, salmon, and trout) and is located within the first α-helix of the POU-H. The E230K mutation results in the substitution of an acidic residue by a basic residue and has been experimentally induced in murine *Pit-1*. Interestingly, lysine is present at this position in the homeodomain of the engrailed protein of *Drosophila*. Thus, the effects of lysine at this position are probably dependent on context and/or differences in specific binding site selectivity. This nonconservative substitution reduced the DNA binding to 35% of that of wild type (39). Our data are at variance with these data, because in our studies the mutation was not associated with a reduction in DNA binding, although it was associated with a reduction in transactivation, which probably represented a partial loss of function. The phenotype associated with this mutation shows considerable variability, and the partial loss of function might be associated with a milder phenotype.

We have also identified two novel polymorphisms within *POU1F1*. Both of these polymorphisms lie in a pyrimidine tract upstream of the acceptor splice site of intron 5. These polymorphisms were not identified in 228 Caucasian control alleles. Because the polymorphisms are present in the heterozygous state, it is highly likely that these variations reflect benign changes of no functional consequence, although a possible dominant negative effect because of aberrant splicing cannot be excluded.

To conclude, we have screened a cohort of patients with sporadic and familial IGHD/CPHD for mutations within *POU1F1* and identified a number of mutations in patients with GH, TSH, and PRL deficiencies, including two novel mutations. This study describes the largest series of patients

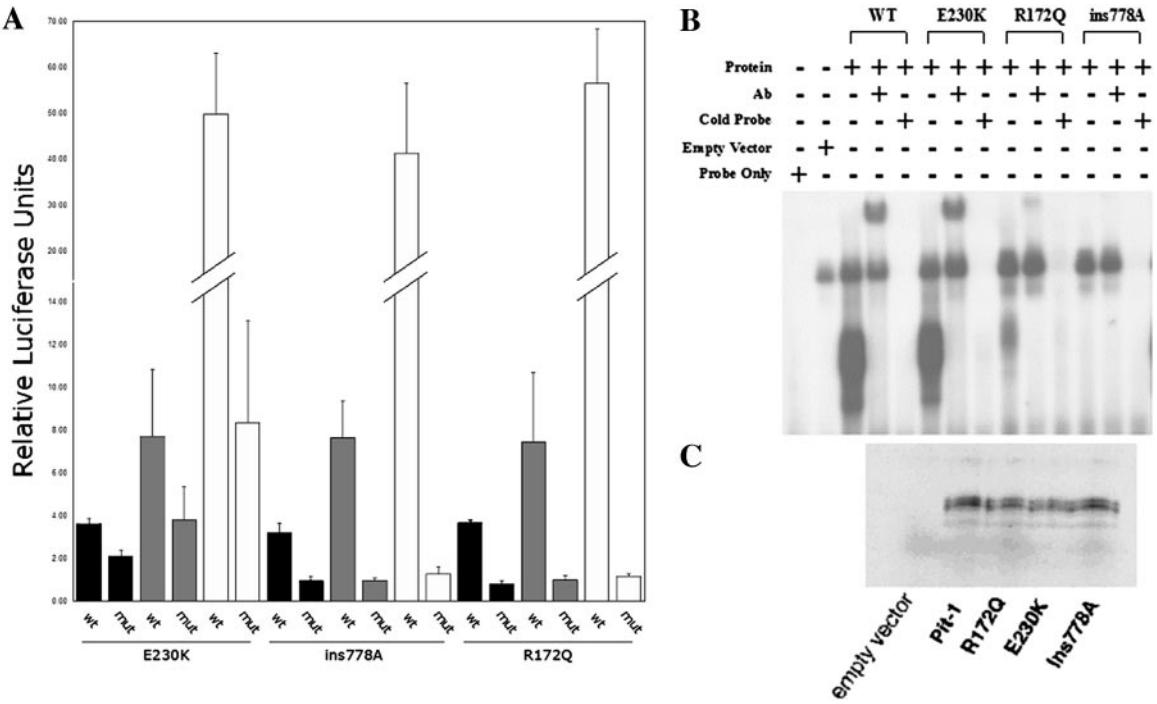

FIG. 5. Functional analyses of *POU1F1* mutants E230K, R172Q, and Ins 778A. A, Transient transfection assays. Wild-type or mutant *POU1F1* was cotransfected with a reporter construct (GH-Luc reporter, *black bars*; Pit1-Luc, *gray bars*; PRL-Luc, *white bars*). B, EMSA was performed with POU1F1 recombinant proteins and ^{32}P-labeled DNA fragments. *POU1F1* and mutant proteins (Protein) were tested at a high-affinity *POU1F1* DNA binding site from the human PRL gene (PRL-P1) (5′-AATGCCTGAATCAT, TATATTCATGAAGATATC-3′) labeled with α^{32}P (Probe). The *POU1F1* complexes were supershifted with a *POU1F1* monoclonal antibody (Ab). To confirm specificity, the binding was displaced by addition of the unlabeled probe in excess (Cold Probe). C, Autoradiograph of *in vitro* translation with [^{35}S]methionine illustrating both wild-type and mutant proteins are translated to the same level.

with *POU1F1* mutations to date. We have also identified a novel mutational hot spot (E230K), although a founder effect cannot be excluded. The patients showed some variability in phenotype, particularly with respect to the onset of TSH deficiency. We report the presence of a *POU1F1* mutation in a 21-yr-old woman who does not manifest either clinical or biochemical hypothyroidism and therefore had apparent IGHD. Finally, we suggest that the possibility of *POU1F1* mutations should be considered in patients with CPHD with either a small or normal anterior pituitary in the presence of a normal posterior pituitary and infundibulum on MRI, given the vagaries of endocrine testing in children, particularly with respect to cortisol secretion (40).

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