Molecular Analysis of *PROP1*, *PIT1*, *HESX1*, *LHX3*, and *LHX4* Shows High Frequency of *PROP1* Mutations in Patients with Familial Forms of Combined Pituitary Hormone Deficiency

ABSTRACT

Combined Pituitary Hormone Deficiency (CPHD) is a prevalent disease in Neuroendocrinology services. The genetic form of CPHD may originate from mutations in pituitary transcription factor (PTF) genes and the pituitary image in these cases may give a clue of what PTF is most probably mutated: defects in *LHX4* are usually associated with ectopic posterior pituitary (EPP); defects in *LHX3*, *PIT1*, and *PROP1*, with normally placed posterior pituitary (NPPP); *HESX1* mutations are associated with both. **Objective:** To identify mutations in PTF genes in patients with idiopathic hypopituitarism followed in our service, based on the presence or absence of EPP on sellar MRI. **Methods:** Forty patients with idiopathic hypopituitarism (36 families, 9 consanguineous), followed in the Neuroendocrinology Outpatient Clinic of UNIFESP, Brazil, were submitted to sequencing analyses of PTF genes as follows: *LHX3*, *HESX1*, *PIT1*, and *PROP1* were sequenced in patients with NPPP (26/40) and *HESX1* and *LHX4* in patients with EPP (14/40). **Results:** We identified only *PROP1* mutations in 9 out of 26 patients with CPHD and NPPP (35%). Since eight of them came from 4 consanguineous families, the prevalence of *PROP1* mutations was higher when only consanguineous families were considered (44%, 4/9). At the end of the study, we decided to sequence *PROP1* in patients with EPP, just to confirm that they were not candidates for *PROP1* mutations. No patients with EPP had *PROP1* or other PTF mutations. **Conclusions:** Patients with idiopathic CPHD and NPPP, born from consanguineous parents, are the strong candidates for *PROP1* mutations. Other developmental gene(s) may be involved in the genesis of idiopathic hypopituitarism associated with EPP. **(Arq Bras Endocrinol Metab 2007;51/7:1097-1103)**

Keywords: Pituitary transcription factor; PROP1; Hypopituitarism; Combined pituitary hormone deficiency

RESUMO

Análise Molecular de *PROP1*, *PIT1*, *HESX1*, *LHX3* e *LHX4* Mostra Alta Freqüência de Mutações no *PROP1* em Pacientes com Formas Familiares de Deficiência Combinada de Hormônios Hipofisários.

Deficiência Combinada de Hormônios Hipofisários (DCHH) é uma doença prevalente em todos os serviços de Neuroendocrinologia. A DCHH de origem genética pode resultar de mutações nos genes de fatores de transcrição hipofisários (FTH), e a ressonância magnética (RM) de sela desses pacientes pode indicar qual FTH tem maior probabilidade de estar mutado: mutações no *LHX4* estão geralmente associadas a neuro-hipófise ectópica (NHE); mutações no *LHX3*, *PIT1* e *PROP1*, a neuro-hipófise tópica (NHT); mutações no *HESX1* podem estar associadas a NHE e NHT. **Objetivo:** Identificar mutações nos FTH em pacientes acompanhados em nosso serviço, portadores de hipopituitarismo idiopático, selecionando os genes a serem estudados de acordo com a presença ou ausência de NHE à RM sela. **Métodos:** Os genes dos FTH foram seqüenciados em 40 pacientes com hipopituitarismo idiopático (36 famílias, 9 consangüíneas), acompanhados na unidade de Neuroendocrinologia da UNIFESP, SP, Brasil: *LHX3*, *HESX1*, *PIT1* e *PROP1* foram seqüenciados nos pacientes com NHT (26/40) e *HESX1* e *LHX4*, nos pacientes com NHE (14/40). **Resultados:** Somente mutações *PROP1* foram identificadas em 9 de 26 pacientes (35%) com NHT, 8 deles provenientes de 4 famílias consangüíneas (4/9, 44%). Uma vez que mutações no *PROP1* foram tão freqüentes, decidimos, ao final do estudo, seqüenciá-lo também nos pacientes com NHE. Nenhum paciente com NHE apresentou mutações no *PROP1* ou em outro FTH. **Conclusão:** Mutações no gene *PROP1* foram encontradas em 22,5% (9/40) de todos os pacientes, em 35% (9/26) dos pacientes com NHT e em 44% (4/9) se considerarmos somente as famílias consangüíneas. Portanto, pacientes com DCHH idiopática e NHT, provenientes de famílias de pais consangüíneos, são os melhores candidatos a mutações *PROP1*. **(Arq Bras Endocrinol Metab 2007;51/7:1097-1103)**

Descritores: Fator de transcrição hipofisário; PROP1; Hipopituitarismo; Deficiência combinada de hormônios hipofisários

artigo original

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Combined Pituitary Hormone Deficiency (CPHD) includes a heterogeneous group of disorders characterized by impaired production of growth hormone (GH) and one or more of the other anterior pituitary hormones, and it is a prevalent disease in all Neuroendocrinology services. Clinically, CPHD is characterized by a combination of the following findings: short stature, hypothyroidism, impaired sexual development and hypocortisolism. CPHD may result from acquired lesions in the hypothalamic-pituitary region (tumor, trauma, surgery, irradiation), from genetically defined conditions, or may be idiopathic. CPHD from genetic cause has an incidence of approximately 1:8,000 births and is usually sporadic, but nearly 5–30% of cases are familial (1-3).

During pituitary embryogenesis, homeobox genes (*HESX1*, *PITX1* and 2, *LHX3*, *LHX4*, *SIX6*, *PROP1*, *PIT1*, *TPIT*, and *SOX3*) become activated in the region of the future anterior pituitary gland, following a specific temporal and spatial pattern of activation. They encode pituitary transcription factors (PTF), which are nuclear proteins that bind to regulatory DNA sequences of target genes, activating or inhibiting their transcription. The PTF are involved in the development of the anterior pituitary gland, from the thickening and invagination of the roof of the primitive oral cavity, to the terminal differentiation of the five different pituitary cell types (somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticothophs) (4). PTF may be pituitary-specific (PIT1, PROP1, TPIT) or pituitary non-specific (HESX1, PITX1 and 2, LHX3, LHX4, SIX6, SOX3). The latter are also expressed in other regions such as the anterior central nervous system (HESX1, SOX3), the eyes (PITX2, SIX6), the teeth, the heart (PITX2), the cervical spine (LHX3), the cerebellum (LHX4), and the spinal cord (SOX3) (5,6).

Mutations in *HESX1*, *LHX3*, *LHX4*, *PROP1*, *PIT1* genes lead to the genetic form of CPHD. Clinically, the patients may present two or more deficiencies of the anterior pituitary hormones. At times, the hypopituitarism may present as an isolated anterior pituitary hormone deficiency (usually GH deficiency), which gradually evolves to CPHD (5,7,8). *HESX1* mutations cause either isolated GH deficiency or CPHD, associated or not with other anomalies such as septo-optic dysplasia and agenesis of the corpus callosum (5,9,10); *LHX3* mutations lead to GH, TSH, PRL, and gonadotrophin deficiencies, associated with short cervical spine and limited neck rotation (11-14); *LHX4* mutations cause GH, TSH, and ACTH deficiencies and cerebellar abnormalities (15). Defects in *PIT1* gene cause GH, TSH, and PRL deficiencies, and in *PROP1* cause deficiencies in pituitary hormones produced by *PIT1* dependent cell lineages (somatotrophs, thyrotrophs, lactotrophs), as well as evolving gonadotrophin and corticotrophin deficiencies (3,8,16-19).

The sellar magnetic resonance image (MRI) findings in patients with PTF mutations are variable and may give a clue of what PTF is most probably mutated: *LHX4* mutations are usually associated with an EPP, whereas *PIT1*, *PROP1*, and *LHX3* mutations are associated with NPPP. *HESX1* mutations may be associated with EPP or NPPP (5,20-22). With regard to the image of the anterior pituitary, the majority of the patients with PTF mutations have a hypoplastic or normal anterior pituitary gland, but an enlarged pituitary mass resembling hyperplasia of the adenohypophysis may be detected less frequently in some patients with *PROP1* mutations.

In this study, we searched for mutations in *HESX1*, *LHX3*, *LHX4*, *PIT1*, and *PROP1* genes in a cohort of patients with idiopathic hypopituitarism who are followed in our neuroendocrine service. The objective of this search was to identify the prevalence of PTF gene mutations in our patients and to correlate the mutations identified with the different CPHD phenotypes, improving our understanding about some genetically identified cases of CPHD.

PATIENTS AND METHODS

Patients

Patients with idiopathic hypopituitarism who are followed in the Neuroendocrinology Outpatient Clinic of the Universidade Federal de São Paulo (UNIFESP), in São Paulo, Brazil, were recruited into the study. After obtaining approval for the study protocol by the Institutional Ethics Committee, informed written consent was obtained from the patients who agreed to participate of the study.

Forty patients (30 males, 10 females) with a mean age at the diagnosis of hypopituitarism of 11.4 years (range 0.5–37 yr) participated in the study. Thirteen patients were born from consanguineous marriages (9 consanguineous families) and all patients had GH deficiency. The different pituitary hormonal deficiency profiles, the imaging findings and the characteristics of the patients are listed in tables 1 and 2. The patients were divided in two groups: Group 1– idiopathic hypopituitarism and ectopic posterior pituitary (EPP): 14 patients (10 males : 4 females). All patients from this group had initially *HESX1* and *LHX4* genes sequenced. However, since *PROP1* mutations were so common, we decided to sequence *PROP1* in these patients later on in the study. Group 2– idiopathic hypopituitarism and normally

Table 1. Clinical, hormonal and radiological features of the 14 patients with ectopic posterior pituitary.

Patients	Sex	Age at Dx of Hipopit. (years)	Z Stature at Dx of Hipopit	Current Age (years)	CONS	Pituitary Hormone Deficiencies GH	Pituitary Hormone Deficiencies TSH	Pituitary Hormone Deficiencies PRL	Pituitary Hormone Deficiencies GNT	Pituitary Hormone Deficiencies ACTH	Sellar MRI: EPP associated with:	GH peak after ITT (ng/ml)	Mutation
1	F	7.9	-4.1	27	N	+	+	+	+	+	small sella	0.57	—
2	F	0.5	-2.5	8.1	N	+	+	+		+	SP agenesis	0.8	—
3	F	2	-2.3	8	N	+	+	+		+	stalk agenesis	1.7	—
4	M	8.3	-3.8	9.9	N	+	+		+	+		0.4	—
5	M	10.3	-5.2	24		+	+		+	+	APH	1.7	—
6	M	2.5	-2.8	13.9	N	+	+			+	thin stalk	0.8	—
7	M	7.1	-1.9	8.1	N	+	+			+	APH	0.05	—
8	M	9.9	-4.9	21		+	+		+		APH		—
9	F	18	-3.3	24	N	+			+			0.8	—
10	M	8.7	-4.5	17	N	+	+					1.75	—
11	M	11.5	-4.7	11.8	N	+	+					0.2	—
12	M	4.5	-3.9	8.9	N	+					small sella	1.0	—
13	M	14	-4.9	18.2	N	+						0.5	—
14	M	11.1	-2.5	12.3	N	+						2.7	—

Cons = consanguinity, N = No, EPP = Ectopic posterior pituitary, SP = septum pellucidum, APH = anterior pituitary hypoplasia.

Table 2. Clinical, hormonal, and radiological features of the 26 patients with normally placed posterior pituitary.

Patients	Sex	Age at Dx of Hipopit. (years)	Z Stature at Dx of Hipopit	Current Age (years)	CONS	Pituitary Hormone Deficiencies GH	Pituitary Hormone Deficiencies TSH	Pituitary Hormone Deficiencies PRL	Pituitary Hormone Deficiencies GNT	Pituitary Hormone Deficiencies ACTH	Sellar MRI	GH peak after ITT (ng/ml)	Mutation
15* (family I)	F	11	-6.0	24	Y	+	+	+	+	+	Normal pit.	1.2	*PROP1* 301302delAG
16* (family I)	F	10	-4.9	22	Y	+	+	+	+	+	Norma pit.	0.2	*PROP1* 301302delAG
17 (family II)	M	5	-4.2	8	Y	+	+	+		+	Nomal pit.	0.9	*PROP1* 301302delAG)
18 (family II)	M	8	-4.9	11	Y	+	+	+		+	Normal pit.	0.4	*PROP1* (301302delAG
19* (family III)	F	10	-6.0	24	N	+	+	+	+	+	APH	< 0.5	*PROP1* 301302delAG
20* (family IV)	F	15	-2.6	26	Y	+	+	+	+		APH	0.2	*PROP1* R99Q
21* (family IV)	M	17	-4.0	25	Y	+	+	+	+		APH	0.45	*PROP1* R99Q
22* (family V)	M	29	-1.0	41	Y	+	+	+	+	+	APH	1.4	*PROP1* R120C
23* (family V)	M	37	-3.4	39	Y	+	+	+	+	+	APH	IGFI: 21 ng/mL	*PROP1* R120C
24	M	8.2	-3.9	25	N	+	+	+	+	+	APH	1.1	—
25	M	12.9	-5.8	29	N	+	+	+	+	+	APH	0.27	—
26	F	19	n.a.	22	Y	+	+	+	+		APH	0.05	—
27	M	14	-5.1	31	N	+	+		+	+	APH	3.1	—
28	M	12.5	-4.4	23	Y	+	+		+	+	APH	n.a.	—
29	M	12.8	-4.3	26	N	+	+	+			APH	2.4	—
30	M	8.6	-4.1	20	N	+	+	+			APH	n.a.	—
31	M	17	-8.38	22	Y	+	+		+		APH	0.7	—
32	M	4	-2.9	7	Y	+	+				C.C. Agenesis	3.2	—
33	M	3.8	-4.4	21	N	+	+				Pit Stalk Agenesis	3.0	—
34	M	15	-3.9	20	N	+	+		+		APH; CIL	1.7	—
35	F	6.1	-3.2	8	Y	+	+				APH	0.6	—
36	M	13	-4.7	25	N	+	+		+		APH	2.9	—
37	M	9	-5.2	12	N	+					APH	0.8 P58P	*PROP1* polymorphism
38	M	11	-3.2	13	N	+					APH	0.2	—
39	M	6.8	-3.9	11	N	+					APH	0.1	—
40	M	7.5	-3.7	12	N	+					Normal pit.	0.6	—

* families reported before, Cons = consanguinity, Y = Yes, N = No, APH = anterior pituitary hypoplasia, C.C. = Corpus Calosum, CIL = cyst intermidiate lobe, n.a. = not available.

placed posterior pituitary (NPPP): 26 patients (20 males : 6 females). All the patients of this group had *HESX1*, *LHX3*, *PROP1*, and *PIT1* genes sequenced, but only the patients with GH, TSH, and PRL or GH and TSH deficiencies had the *PIT1* gene sequenced (5 patients).

Clinical, hormonal and radiological evaluation

Clinical data from some of the patients were obtained retrospectively from the patients' records and included history of consanguinity, family history, auxological data, parental heights and anterior pituitary hormone evaluation. Other patients were included in the study as soon as the diagnosis of hypopituitarism was made. To assess anterior pituitary function, hormone levels were measured at baseline (TSH, free T4, PRL, LH, FSH, IGF-I, cortisol, estradiol/testosterone) and a combined pituitary stimulation test was performed when necessary. For the combined test, glucose, GH, cortisol, TSH, PRL, LH, and FSH were measured before and at 15, 30, 45 and 60 min after iv administration of 0.1 U/kg insulin, 200 µg TRH, and 100 µg GnRH.

The following were the diagnostic methods used for hormonal measurements: i) Free T4, TSH, PRL, GH, LH, and FSH: flurometric assay (Delfia, Wallac Oy, Turku, Finland); ii) cortisol: RIA (Diagnostic Products, Los Angeles, CA, USA); iii) IGF-I: Immunoflorimetric assay after ethanol extraction using specific monoclonal antibodies (DSL kit).

The criteria used for the diagnosis of GH deficiency were auxological, hormonal, and radiological: poor growth velocity (more than 1 SD below the mean for age), IGF-I low for age, abnormal GH peak response (< 3 ng/ml) to hypoglycemia on Insulin Tolerance Test (ITT) and anatomic pituitary abnormalities on MRI. Some patients with abnormal sellar MRIs had only one GH stimulation test performed (ITT). All patients with normal sellar MRIs were submitted to a second GH stimulation test with clonidine. Not all patients had IGF-I measured.

Radiological studies of the sella turcica were performed in a Philips Gyroscan ACS-NT 1,5 tesla. The coronal images were obtained using 2-mm slices. The maximal height of the pituitary was measured perpendicularly to the sella turcica. The classification of the size of the anterior pituitary (normal, small or increased) was made accordingly to the normal controls from the studies of Tsunoda and Argyroupoulous (9,30).

Genomic analysis

DNA was extracted from peripheral lymphocytes of the patients using a Quiagen Midi Kit (Quiagen), following the manufacturer's protocol.

All coding exons of *HESX1*, *LHX3*, *LHX4*, *PIT1*, *PROP1* genes were amplified by Polymerase Chain Reaction (PCR). One hundred nanograms of human genomic DNA were used as template in a 100 uL PCR mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM deoxy-NTPs, 2.5 U Taq polymerase (PCR Reagent System, Life-Technologies) and 0.1 nM of upstream and downstream specific primers. The sequence of the PCR primers and the PCR thermal cycling program have been described elsewhere (9,14,15,19,31). PCR products were analyzed in 1.8% agarose gel and purified using a PCR Product Purification Kit (Life Technologies, USA). Direct sequencing of the PCR products was carried out in both directions, using the ABI Prism Big Dye terminator cycle sequencing ready reaction version 3.0 (Applied Biosystems) in an ABI Prism 3100 DNA Sequencer (Perkin Elmer Corporation).

RESULTS

Genomic sequencing of the homeobox genes

Three different *PROP1* homozygous gene mutations [301-302delAG (exon 2), 358C>T (exon 3), and 296G>A (exon2)], were found in 9 patients, all of them with normally placed posterior pituitary (table 2). They were four pairs of siblings from 4 families of consanguineous parents, and one patient from a non-consanguineous family (table 2). The 301-302delAG mutation leads to a frame shift and a truncated PROP1 protein with null function. The 358C>T missense mutation predicts an amino acid change at codon 120 replacing a much conserved arginine by a cysteine (R120C) in the third helix of the DNA-binding domain of the PROP1, and the mutant protein has an eight-fold reduction in DNA binding affinity and impaired trans-activation activity (17). The mutation 296G>A predicts an amino acid change at codon 99 replacing a highly conserved arginine by a glutamine (R99Q) in the second helix of the paired DNA-binding homeodomain of PROP1. The mutant protein presents a weak binding to its target DNA sequence but still preserves some functional activity (32).

We also identified in one patient a *PROP1* heterozygous polymorphism CCG/CCA, in exon 2 of *PROP1* (nucleotide position 1965). This polymorphism does not change the amino acid in the PROP1 protein (P58P) and has been found in normal populations (OMIM SNP rs2233784).

No mutations were found in *HESX1*, *LHX3*, *LHX4* or *PIT1* genes in all the patients tested. No mutations were found in patients with EPP.

DISCUSSION

In the present study, we performed mutational analysis of the *HESX1*, *LHX3*, *LHX4*, *PROP1*, and *PIT1* genes in a cohort of 40 patients with idiopathic

hypopituitarism followed in one large neuroendocrinology service of the southeast region of Brazil, the Neuroendocrinology Clinic / Medicine Department of Escola Paulista de Medicina, Universidade Federal de São Paulo. Since *LHX4* and *HESX1* are more likely to be associated with EPP, and *LHX3*, *PIT1*, *PROP1*, and *HESX1* with NPPP (5), we divided the patients in two groups, according to the presence or not of EPP on sellar MRI, and searched the candidate genes in each group.

We identified 3 different *PROP1* mutations (301-302delAG, R99Q, and R120C) in 9 patients, all of them with CPHD and NPPP. The patients with EPP showed no mutations in the genes studied (*HESX1*, *LHX4*, and *PROP1*) and this is compatible with the rarity of PTF mutations found in patients with EPP (10,15,20,33,34). Perhaps other developmental genes yet to be identified may be involved in the etiology of EPP, or another pathogenic mechanism could be responsible for this abnormality (35,36).

The 9 patients who carry *PROP1* mutations are four pairs of siblings belonging to four independent consanguineous families, and one patient born from non-consanguineous parents. They correspond to 22.5% (9/40) of all patients in this cohort. If we consider only the consanguineous families, 44% of them (4/9) had members with *PROP1* mutations. The high prevalence of *PROP1* mutations in our patients from consanguineous families reinforces previous studies that reported *PROP1* mutations in 36 to 50% of all familial cases of CPHD (3,19,33,37,38). On the other hand, the absence of molecular abnormalities in the other PTF genes studied reinforces the scarcity of these mutations and points to the possible involvement of other developmental genes in the pathogenesis of the remaining cases of idiopathic hypopituitarism (1).

The most prevalent *PROP1* mutation in our cohort was the 301-302delAG in exon 2, found in 5 patients from 3 families. Two of them have been reported before (28,39). This mutation is so common that it is considered by several authors as a mutational hotspot (19,25). All five patients with the 301-302del AG presented severe short stature and signs of GH and TSH deficiencies before the age of 10 years. All the adult patients bearing this mutation also presented gonadotrophin deficiency. Because this mutation codifies a truncated protein with null function, it leads to a severe CPHD phenotype, affecting all the anterior pituitary hormones. The ACTH deficiency usually occurs in the third decade of life (17-19,23,25,39). In our cohort of patients, the older brother from family II presented a slightly compromised cortisol response to hypoglycemia (15.4 μg/dl) at the age of 11 years, and the patients from families I and III presented ACTH deficiency at the ages of 16 and 17 years (table 3). Hypocortisolaemia occurring during the second decade of life is an uncommon finding but has been described before (37,40).

The other two mutations found (R99Q and the R120C) are rarer. The *PROP1* 358C>T (R120C) missense mutation identified in family V has never been described in Brazilian families, and only a few families of European, Mexican-American, and Jewish-Moroccan origins have been reported before (7,17,41-43). The two brothers, born from a consanguineous marriage, developed progressive CPHD but were only diagnosed in adulthood (at 29 and 37 years of age). The central hypothyroidism was mild in both cases, GH deficiency was very mild in one case, hypogonadism was equally severe in both cases, and ACTH deficiency appeared at different ages (table 3). This illustrates the heterogeneity of this mutation.

The other mutation present in this cohort of patients, R99Q, in family IV, is also responsible for a progressive CPHD phenotype and was reported by our group three years ago. In this family, the younger brother had been diagnosed in childhood with familial short stature and constitutional delay of growth, and only in the second decade of life the diagnosis of GH deficiency associated with other pituitary deficiencies was established. The same happened with his older sister who looked for medical attention only at the age of 15 years for lack of puberty, while her stature was compatible with her target height and she had no symptoms of other pituitary hormone deficiencies (table 3). The phenotype of these siblings is also compatible with a mild PROP1 deficiency (32).

One last remark is the predominance of male patients with CPHD in our cohort (3:1, M:F). Mutations in *SOX3*, another pituitary transcription factor gene located at Xq26, have recently been described in males with congenital X-linked hypopituitarism. These patients may present mental retardation and/or anatomical abnormalities in the corpus callosum and pituitary (44,45). The molecular study of this gene in our male patients is planned for the near future.

In summary, our cohort of patients shows that PTF gene mutations such as *HESX1*, *LHX3*, *LHX4*, and *PIT1* are rare in patients with idiopathic hypopituitarism. On the other hand, *PROP1* mutations are common among patients with CPHD and normally placed posterior pituitary that are born from consanguineous parents. Therefore, patients with these features are the strong candidates for *PROP1* mutations.

Table 3. Hormonal profile of the patients with *PROP1* mutations.

Patient / Mutation	Sex	Age at diagnosis (years)	Height (cm)	FT4 (ng/dl) / TSH (U/L)	PRL (ng/ml)	IGF-I ng/ml	Peak GH (ng/ml) after ITT	Cortisol (ug/dl) Basal and Peak (ITT)	LH / FSH (U/L) after GnRH	Testo ng/dl
15 (family I) 301-302delAG	F	11	103.5 (-6.0 SD)	0.3/1.8	3.9	—	1.2	P > 18 (11 yr) B: 4.5 (16 yr)	0.2/0.2	—
16 (family I) 301-302delAG	F	10	103 (-4.9 SD)	0.6/2.6	4.5	—	0.2	P > 18 (10 yr) B: 2.2 (16 yr)	0.1/0.3	—
17 (family II) 301-302delAG	M	5	90 (-4.2 SD)	0.6/2.0	11.2	< 6	0.9	P: 18.3 (8 yrs)	n.d.	n.d.
18 (family II) 301-302delAG	M	8	101 (-4.9 SD)	0.7/1.1	9.4	< 6	0.4	P: 15.4 (11 yrs)	B: < 0.1/0.3	n.d.
19 (family III) 301-302delAG	F	10	99 (-6.0 SD)	TT4*: 3.8/2.8	1.2	41.3	< 0.5	B: 2.1 (17 yr)	< 1.5/< 1.5	—
20 (family IV) R99Q	F	15	144 (-2.6 SD)	0.7/1.5	6.9	112	0.2	B: 10 (10 yr)	1.0/5.8	—
21 (family IV) R99Q	M	17	145.5 (-4.0)	0.7/1.0	4.9	88	0.45	P: 18.1 (17 yr)	4.8/2.0	< 30
22 (family V) R120C	M	29	170 (-1.0 SD)	0.6/4.7	< 3	—	1.4	P: < 0.9 (29 yr)	8/1	22
23 (family V) R120C	M	37	154 (-3.4 SD)	0.4/3.7	< 3	21	—	B: 7.7 (42 yr) B: 2.0 (45 yr)	B: < 2/< 1	8
Normal values				0.8–2.7	3–15	**	> 3	B: 5–25 P: > 18	LHB: < 14 LHP: > 4–6x	Pre-pubertal: < 50 adult: 300–950

* TT4 = totalT4 ug/dl (normal values: 4–11 ug/dl), n.d. = not done, B = Basal.
** IGFI normal values: < 6 yr = 20–200 ng/ml, 6–12 yr: 88–450 ng/ml, 13–16 yr: 200–900 ng/ml, 17–24 yr: 180–780 ng/ml, 25–39 yr: 114–400 ng/ml.

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