Structural Pituitary Abnormalities Associated With CHARGE Syndrome

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Introduction: CHARGE syndrome is a multisystem disorder that, in addition to Kallmann syndrome/isolated hypogonadotrophic hypogonadism, has been associated with anterior pituitary hypoplasia (APH). However, structural abnormalities such as an ectopic posterior pituitary (EPP) have not yet been described in such patients.

Objective: The aims of the study were: 1) to describe the association between CHARGE syndrome and a structurally abnormal pituitary gland; and 2) to investigate whether *CHD7* variants, which are identified in 65% of CHARGE patients, are common in septo-optic dysplasia /hypopituitarism.

Methods: We describe 2 patients with features of CHARGE and EPP. *CHD7* was sequenced in these and other patients with septo-optic dysplasia/hypopituitarism.

Results: EPP, APH, and GH, TSH, and probable LH/FSH deficiency were present in 1 patient, and EPP and APH with GH, TSH, LH/FSH, and ACTH deficiency were present in another patient, both of whom had features of CHARGE syndrome. Both had variations in *CHD7* that were novel and undetected in control cohorts or in the international database of CHARGE patients, but were also present in their unaffected mothers. No *CHD7* variants were detected in the patients with septo-optic dysplasia/hypopituitarism without additional CHARGE features.

Conclusion: We report a novel association between CHARGE syndrome and structural abnormalities of the pituitary gland in 2 patients with variations in *CHD7* that are of unknown significance. However, *CHD7* mutations are an uncommon cause of septo-optic dysplasia or hypopituitarism. Our data suggest the need for evaluation of pituitary function/anatomy in patients with CHARGE syndrome. **(*J Clin Endocrinol Metab* 98: E737–E743, 2013)**

CHARGE is an autosomal-dominant syndrome with a variable combination of coloboma of the eye, heart malformations, atresia of the choanae, retardation of growth and development, and genital and ear abnormalities (1–3).

Although *SEMA3E* was the first gene to be implicated (4), chromodomain helicase DNA binding protein-7 (*CHD7*) is the predominant gene associated with CHARGE syndrome, accounting for over 65% of cases. CHD7 is composed of multiple domains (Figure 1) and acts as an ATP-dependent

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Abbreviations: ACTHD, ACTH deficiency; APH, anterior pituitary hypoplasia; CHD7, chromodomain helicase DNA binding protein-7; EPP, ectopic posterior pituitary; GHD, GH deficiency; HH, hypogonadotropic hypogonadism; IGFBP3, IGF binding protein 3; MRI, magnetic resonance imaging; rhGH, recombinant human GH; SDS, SD score.

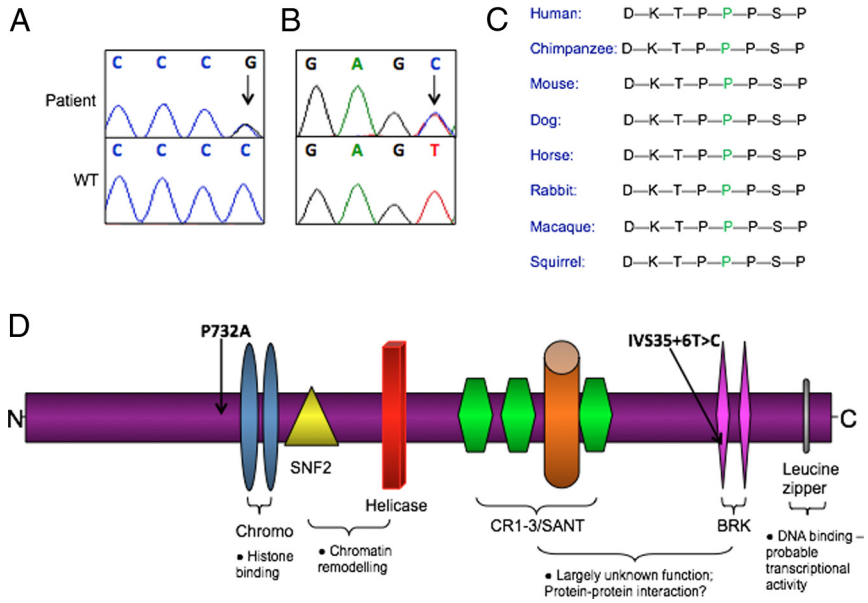

Figure 1. A, A *CHD7* mutation associated with CHARGE syndrome and hypopituitarism. A total of 102 patients with hypopituitarism including 2 with additional features of CHARGE syndrome were screened for *CHD7* mutations. A novel heterozygous missense mutation (c.2194C>G, p.P732A) was identified in exon 4 in a Caucasian male patient with probable CHARGE syndrome and hypopituitarism (shown by arrow). B, An intronic *CHD7* mutation associated with CHARGE syndrome and hypopituitarism. A heterozygous mutation (c.IVS35+6T>C) was found 6 bases 3′ of exon 35 in a Caucasian male patient with CHARGE syndrome and hypopituitarism (shown by arrow). C, The conservation of *CHD7* p.P372. The proline amino acid (represented by the green 'P') at location p.P732 is highly conserved between multiple species. In patient 1, this proline was substituted by alanine. D, Schematic diagram of the *CHD7* gene showing the location of both mutations (arrows). The P732A mutation is located before the first Chromo domain in the *CHD7* gene (indicated by the first black arrow). The c.IVS35+6T>C is located late in the first BRK domain in a proposed splicing region (indicated by the second black arrow).

chromatin remodeler that binds to methylated histones (5). CHD7 plays an important role in the regulation of gene expression for tissue development and maintenance (6, 7). In mice, it is expressed in many fetal tissues including the eye, inner ear, heart, and olfactory bulbs (8, 9).

In both mice and humans, *Chd7/CHD7* is also expressed in the pituitary and in developing and mature hypothalamus. Embryonic *CHD7*-deficient mice have reduced survival and growth and display hypoplasia of Rathke's pouch (8, 10). They have reduced *Otx2* and *GnRH1* mRNA expression in the hypothalamus and reduced *GnRHR* expression in the pituitary. Indeed, pituitary hormone deficiency is a feature of CHARGE syndrome, most notably hypogonadotropic hypogonadism (HH), but additional GH deficiency (GHD) and ACTH deficiency (ACTHD) have also been described (11). Hence, CHD7 may play a role in hypothalamo-pituitary development and function.

Kallmann syndrome and CHARGE syndrome, in which CHD7 has been implicated (12), have overlapping phenotypes such as anosmia, gonadotropin deficiencies, cleft lip/palate, sensorineural hearing loss, and autism. CHD7 interacts with SOX2 at an early stage in the control of embryonic stem cell and progenitor cell differentiation (13) and neural development (14). Mutations in *SOX2* can also result in HH and anterior pituitary hypoplasia (APH) (15, 16). Mutations in genes implicated in Kallmann syndrome can be associated with septo-optic dysplasia, further suggesting genetic overlap in Kallmann syndrome, CHARGE, and septo-optic dysplasia (17, 18).

We now report, for the first time to our knowledge, the association of CHARGE syndrome with congenital hypopituitarism due to structural abnormalities of the pituitary gland in 2 unrelated children with variations in *CHD7*, although the significance of these CHD7 variants is unclear.

Patients and Methods

Patients

Ethical approval was obtained from the University College London Institute of Child Health/Great Ormond Street Hospital for Children Joint Research Ethics Committee, and written consent was obtained from patients and/or parents.

DNA was extracted from 2 patients with hypopituitarism and features of CHARGE and their parents, if available. An additional cohort of 100 patients with septo-optic dysplasia or hypopituitarism was screened; 77 had septo-optic dysplasia with variable hormone deficiency (6 had ectopic posterior pituitary [EPP]), 21 had combined pituitary hormone deficiency without additional midline defects (6 with EPP), and 2 had isolated GHD (normal posterior pituitary). None had additional features of CHARGE syndrome.

Direct sequencing analysis

The coding region of *CHD7* consisting of 38 exons (NM_017780) was sequenced in its entirety in all patients. Exons were amplified by PCR on an Eppendorf Thermocycler over 35 cycles with exon-flanking primers, designed using Primer3 (available at http://frodo.wi.mit.edu/primer3). PCR products were treated with MicroClean reagent (catalog no. 2MCL-10; Web Scientific Ltd., Crewe, United Kingdom) according to manufacturer's instructions and then sequenced using BigDye v1.1 sequencing chemistry (Applied Biosystems, Foster City, California) and analyzed on a 3730 × 1 DNA Analyzer (catalog no. 625-0020; Applied Biosystems/Hitachi, Tokyo, Japan). Details of the PCR conditions and primers are available upon request. For sequence variants identified, we screened 190 ethnically matched control alleles at the same loci and screened the *CHD7* database describing *CHD7* mutations/variants from over 800 patients (www.chd7.org) (3), a control exome database of 1500 Caucasian/African Americans (http://snp.gs.washington.edu/EVS), and "1000 Genomes" (www.1000genomes.org). Two in

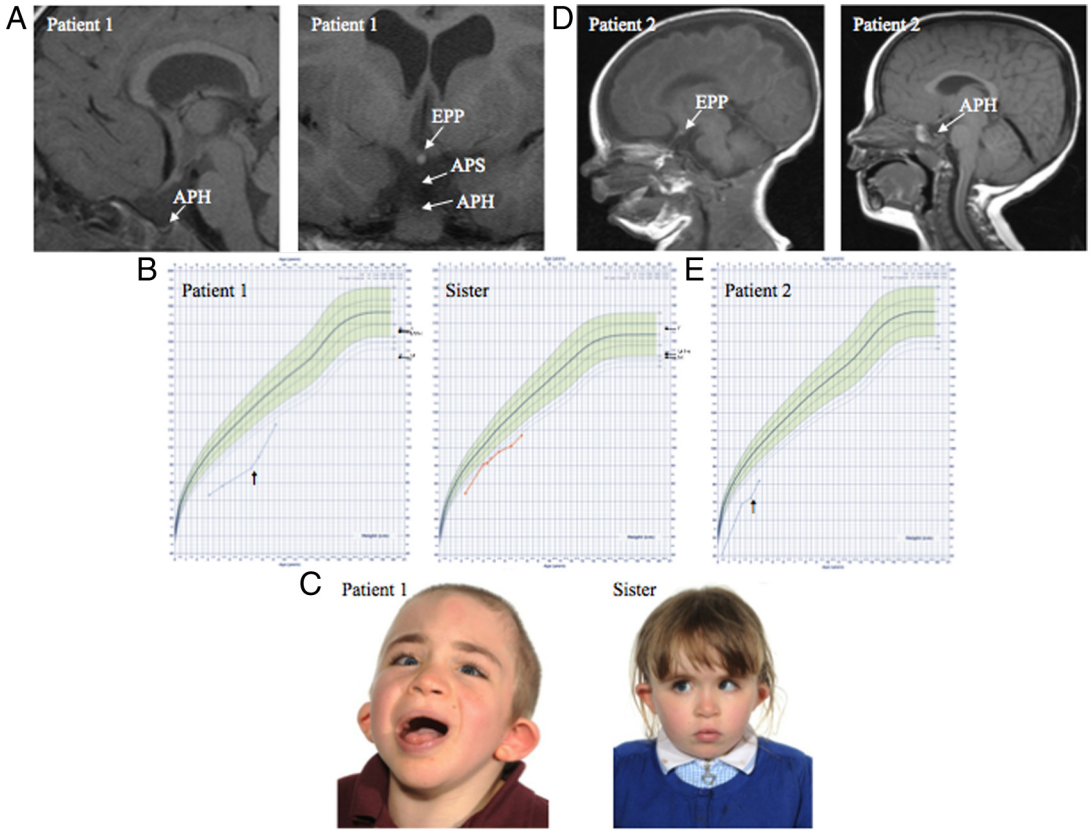

Figure 2. A, MRI in patient 1 revealed APH, an absent pituitary stalk (APS) with an undescended/EPP at the tuber cinereum and a thin corpus callosum. B, Growth chart for patient 1 and the sister of patient 1. Patient 1 presented with short stature at the age of 3 years and was commenced on GH at 7 years of age (left arrow). The sister of patient 1 was found to be GH deficient at 6 years of age. F, father; M, mother; MPH, midparental height. C, Photographs of patient 1 and his sister. Left, Patient 1 with probable/possible CHARGE syndrome according to Blake criteria. Right, The sister of patient 1 with an atrial septum defect (APS), abnormal ear, squint, developmental delay, and GHD. D, MRI of patient 2 at 2 months (left) and 20 months of age (right) revealed APH and an APS with an EPP at the tuber cinereum, bilateral colobomata, and underdeveloped frontal lobes. E, Growth chart of patient 2. Patient 2 presented with short stature at the age of 3 years and has recently been commenced on GH treatment at the age of 3 years (arrow).

silico splicing prediction models, Human Splicing Finder, version 2.4.1, and NETGENE2 (www.cbs.dtu.dk/services/NetGene2), were used to assess the effect of splicing variants.

Patients with *CHD7* variants were also screened for variants in *HESX1*, *SOX3*, *KAL1*, *PROKR2*, *FGFR1*, *FGF8*, *NELF*, *WDR11*, *SOX2*, and *OTX2*.

Hormone analysis

Hormone measurements were performed in standard assays in the biochemistry laboratories of Great Ormond Street Hospital for Children, Kent and Canterbury Hospital (UK) and Hospital Universtari Son Dureta, Palma de Mallorca (Spain).

Results

Patient 1

The first patient with features of CHARGE syndrome and a *CHD7* variant was a Caucasian male born at term with a birth weight of 3.72 kg (+0.34 SD score [SDS]). Clinical features included Tetralogy of Fallot, sensorineural hearing loss, micropenis with bilateral undescended testes, abnormally shaped ears (Figure 2C), a squint and hypermetropia, trigonocephaly, a small occipital meningocele, and severe developmental delay. He presented with short stature (height, −4.9 SDS) at 7 years of age. Developmental delay prevented sense of smell analysis. He was classified as probable/possible CHARGE using Blake criteria (1 major, 5 minor criteria), but not as CHARGE using Verloes criteria (0 major, 5 minor criteria). Endocrine testing (Table 1) revealed mild central hypothyroidism and severe GHD. He was commenced on T_4 and recombinant human GH (rhGH), and his growth velocity increased to 22 cm/y (Figure 2B). A 44K array comparative genomic hybridization analysis was normal. Magnetic resonance imaging (MRI) revealed a small anterior pituitary, an absent pituitary stalk with an undescended or EPP at the tuber cinereum, and a thin corpus callosum (Figure 2A). His lateral semicircular canals were underdeveloped, suggesting mild vestibular dysplasia. The olfactory bulbs could not be clearly identified. His mother, who carried the same CHD7 variant, was short (152 cm; −1.67 SDS) but had a normal IGF-I concentration (Table 1) and had received T_4 for primary hypothyroidism from the age of 11

Table 1. Phenotypes in Patients With *CHD7* Variations

No.	Variation	Endocrinopathy	Normal Range	MRI	CHARGE Features	Family Members
I	p. P732A (heterozygous) Possible	GHD, TSHD, possible LH/FSHD:		APH, EPP	Possible CHARGE:	
		FT4, 0.78 ng/dL (10 pmol/L)		Absent pituitary stalk	Squint and hypermetropia	
		TSH, 4.5 mU/L	<6 mU/L	Thin CC	Tetralogy of Fallot	
		GH peak, 0.7 ng/mL (0.7 μg/L)	>7 ng/mL		Retardation of growth	
		IGF-I, <25 ng/mL (<3.28 nmol/L)	64–345 ng/mL		Severe developmental delay	
		IGFBP3, <0.50 mg/L	1.6–6.5 mg/L		Genital hypoplasia (micropenis, BL undescended testes)	
		Cortisol peak, 24 μg/dL (662 nmol/L) (Synacthen test)	>20 μg/dL		Ears, abnormal shape	
		Prolactin, 5.8 ng/mL (124 mU/L)	Not available		Sensorineural hearing loss	
	Mother	FT4, 0.69 ng/dL (9 pmol/L)	0.70–1.48 ng/dL	Not available		Mother carries variant
		TSH, 20 mU/L	0.5–5 mU/L			Short Primary hypothyroidism
		IGF-I, 102 ng/mL (13.4 nmol/L)	68.5–304 ng/mL			Low-normal IGF-I
						No CHARGE features
	Sister	FT4, 1.13 ng/dL (14.5 pmol/L)	0.84–1.48 ng/dL	White matter loss	Atrial septal defect	Sister not a carrier
				Cavum SP and vergae APH	Retardation of growth	A squint
		TSH, 1.7 mU/L	<6 mU/L	A single anterior cerebral artery, thin optic chiasm	Developmental delay	GHD
		GH peak, 1.6 μg/L (1.6 ng/mL)	>7 ng/mL		Ear, abnormal shape	
		IGF-I, 30 ng/mL (3.93 nmol/L)	57–316 ng/mL			
		IGFBP3, 2.32 mg/L	1.4–6.1 mg/L			
		Cortisol, 16.31 μg/dL (450 nmol/L)	3.6–20 μg/dL			
		Prolactin, 11.2 ng/mL (237 mU/L)	Not available			

(*Continued*)

(TSH, 20 mU/L [normal 0.4–5.0 mU/L]; free T_4, 9 pmol/L [normal, 9–19 pmol/L] at diagnosis). She did not manifest features of CHARGE syndrome. The sister (Figure 2, B and C) (birth weight, 4.060 kg [+2.65 SDS] at 37 wk gestation), who did not carry the *CHD7* variant, had an atrial septal defect, an abnormally shaped ear, a squint, and developmental delay, but no hearing loss. She was short (height, 98.1 cm [−2.92 SDS] at 5.75 y) and had GHD but normal cortisol, prolactin, and free T_4 concentrations (Table 1). Her brain MRI showed generalized white matter loss and midline abnormalities (cavum septum pellucidum and vergae, small anterior pituitary, a single anterior cerebral artery, thin optic chiasm), but a normal infundibulum and posterior pituitary. She currently has a normal height velocity of 8 cm/y at the chronological age of 7.59 years and has not yet been commenced on rhGH replacement.

The proband carries a novel heterozygous missense variant, c.2194C>G (Figure 1A), in exon 4 of *CHD7*, resulting in substitution of a highly conserved proline residue by alanine, p.P732A. The variant was also detected in the unaf-

Table 1. Continued

No.	Variation	Endocrinopathy	Normal Range	MRI	CHARGE Features	Family Members
II	c. IVS35 + 6T>C (heterozygous)	GHD, LH/FSHD, TSHD, ACTHD:		Thin CC	Typical CHARGE:	Mother carries variant; clinically unaffected
				Cavum SP	Coloboma	
		FT4, 0.67 ng/dL (8.6 pmol/L)	0.82–1.48 ng/dL	APH, EPP	Atresia of choanae (mild)	
		TSH, <0.1 mU/L	<6 mU/L	Absent pituitary stalk	Retardation of growth	
		GH peak, <0.1 ng/mL (<0.1 μg/L)	>7 ng/mL	BL coloboma	Severe developmental delay	
		IGF-I, <0.25 ng/mL (<3.28 nmol/L)	64–345 nmol/L	Underdeveloped frontal lobes	Genital hypoplasia	
		IGFBP3, 0.52 mg/L	1.6–6.5 mg/L		Ear, abnormal shape	
		Cortisol peak, 2.7 μg/dL (75 nmol/L) (Synacthen test)	>20 μg/dL		Sensorineural hearing loss	
		Prolactin, 10 ng/mL (211 mU/L)			Hypoplasia of semicircular canals	
		LH/FSH, <0.2 mIU/mL (0.20 U/L)	Not available			
		Testosterone peak, <20 ng/dL (<0.69 nmol/L) (3-d hCG test)	>87 ng/dL			

Abbreviations: TSHD, TSH deficiency; FSHD, FSH deficiency; FT4, free T_4; SP, septum pellucidum; CC, corpus callosum; BL, bilateral; hCG, human chorionic gonadotropin. Endocrine deficits, CHARGE features, and results of MRI are shown in patients with *CHD7* variants.

fected mother, but not in the father or sister, in 1500 healthy Caucasian/African Americans (http://snp.gs.washington.edu/EVS), in "1000 Genomes," or in 190 control alleles. This variant is listed in the CHARGE database (http://www.chd7.org), but it represents the same English patient and his mother that we describe here (Prof. C. van Ravenswaaij, University Medical Center Groningen, personal communication). The mutated nucleotide/codon is highly conserved between species (Figure 1C).

Patient 2

The second patient is a Caucasian male (birth weight, 1.260 kg [+0.39 SDS] at 28.5 wk gestation), with a full spectrum of CHARGE features (coloboma, mild choanal atresia, deafness, abnormal ear shape, growth and developmental delay, genital hypoplasia). He fulfilled both Blake (3 major, 4 minor criteria) and Verloes criteria (2 major, 4 minor criteria) for diagnosis of CHARGE syndrome. His endocrinopathy (Table 1) included GHD, with undetectable IGF-I and a low IGF binding protein 3 (IGFBP3), gonadotropin deficiency (undetectable LH and FSH, and undetectable T on 3-day human chorionic gonadotropin test), severe cortisol deficiency, and central hypothyroidism, but with a normal prolactin concentration. MRI at 2 months of age showed a thin corpus callosum and a cavum septum pellucidum, and at 20 months a small anterior pituitary and an absent pituitary stalk with an EPP, located at the tuber cinereum (Figure 2D). Bilateral coloboma and underdeveloped frontal lobes were also present (Table 1). His inner ear structures and semicircular canals were normal; however, his olfactory bulbs appeared small. He was commenced on T_4, hydrocortisone, rhGH, and a 3-month course of im T. His growth velocity increased from a pretreatment velocity of 6.6 cm/y to 15 cm/y (Figure 2E), and there was a marked improvement in phallus size.

Genetic analysis revealed a novel heterozygous splice site variant in intron 35, c.IVS35+6T>C (Figure 1B), in a region predicted to be important for splicing. NETGENE2 showed a decrease (0.86 to 0.32) in the confidence with which the splice donor site will be detected. The mother harbors the same heterozygous *CHD7* variant and has no obvious endocrinopathy. The sequence variant was not identified in 190 control alleles, in the CHD7 database (www.chd7.org), in a control database of 1500 Caucasian/African Americans, or in "1000 Genomes."

Patients with hypopituitarism

No *CHD7* variants were identified in our septo-optic dysplasia/hypopituitarism (without CHARGE features) cohort.

Discussion

CHARGE syndrome is a continuum of multiorgan disorders of variable severity, often caused by *CHD7* muta-

tions. We describe 2 patients harboring rare sequence variants in *CHD7*, both of whom have features of CHARGE syndrome accompanied by multiple pituitary hormone deficiencies and structural abnormalities of the pituitary gland. CHARGE syndrome is associated with HH and occasional GHD; however, structural abnormalities of the pituitary gland have not previously been described.

Although CHARGE syndrome is classically associated with HH, our data suggest that in some patients, congenital hypopituitarism may be associated with structural abnormalities of the pituitary gland and variations in *CHD7*. A previous study has suggested that GHD occurs in 9% of CHARGE patients; 3 patients with GHD in this study had APH (19), showing that CHD7 might have a role in anterior pituitary development. One patient from a cohort (n = 379) with a clinical diagnosis of CHARGE has been reported to have TSH deficiency (1). ACTHD has been described in 1 patient with a clinical but not genetically confirmed diagnosis of CHARGE syndrome (20). To summarize, abnormalities of pituitary function including GHD, TSH deficiency, and ACTHD, with a small anterior pituitary size, have rarely been associated with CHARGE syndrome, but an undescended/EPP has not (19, 21), and we describe this association for the first time. Variations in other genes involved in pituitary development, for example *HESX1*, can also result in GHD with APH and either a normal or EPP. Our data support the hypothesis that *CHD7* variations may lead to a continuum of anatomical and functional hypothalamic/pituitary abnormalities.

Most *CHD7* mutations are heterozygous de novo, with a small number of inherited cases usually from an unaffected parent (9). Most mutations lead to truncated CHD7. No genotype-phenotype correlation has been identified, but missense mutations are associated with a milder phenotype (4). In both mice and humans, *Chd7*/*CHD7* is expressed in the developing anterior pituitary, at lower levels in the intermediate and posterior lobes, and in the developing and mature hypothalamus (8), suggesting a potential role of Chd7 in the development of the hypothalamo-pituitary axis. Hurd et al (8) showed that homozygous mutant mice present with an olfactory pit and Rathke's pouch hypoplasia. Chd7 was identified as a Sox2 transcriptional cofactor; both genes physically interact and have overlapping genome-wide binding sites that regulate a set of target genes, such as Gli2 (14), which when mutated, can cause defects of pituitary development including an EPP (22). CHD7 and SOX2 interaction may be further evidence of a potential role for CHD7 in hypothalamo-pituitary development, given the role of SOX2 here (23). Layman et al (11) have shown a reduction in the expression of *Fgfr1*, *Bmp4*, and *Otx2* in the olfactory placode of Chd7 mutant embryos, as well as a reduction of *Otx2* in the hypothalamus. CHD7 is also thought to be necessary for access to OTX2 target genes in the olfactory epithelium (24). Because mutations in *OTX2* in humans are associated with variable hypopituitarism, often with an undescended/EPP, this could be a further mechanism whereby *CHD7* mutations could lead to structural abnormalities of the pituitary gland.

Although *CHD7* mutations have been described in association with Kallmann syndrome, usually additional CHARGE features are present in Kallmann syndrome patients with *CHD7* variants (25). Our data parallel these findings; the patients with congenital hypopituitarism without additional features of CHARGE syndrome did not harbor *CHD7* variants, suggesting that features of CHARGE may have to accompany the hypopituitary phenotype for *CHD7* to be implicated in the etiology.

In patient 1, the p.P732A variant occurs at a highly conserved amino acid, which is not part of a domain with an established function (Figure 1, C and D). The proband's sister had abnormally shaped ears, abnormal facial features, and isolated GHD with a small anterior pituitary, but she did not carry the variant. The mother, who carries the variant, does not have features of CHARGE syndrome or hypopituitarism, although an MRI to assess her pituitary anatomy could not be performed. These data suggest that p.P732A may be a contributory factor to the etiology of the hypopituitary phenotype in our patient but is unlikely to be causative in isolation. Recent evidence suggesting that the genetic basis in many cases of Kallmann syndrome is digenic/oligogenic could be extrapolated to include overlapping syndromes such as CHARGE or congenital hypopituitarism. A second mutation has not yet been identified in either patient, although we have screened other genes involved in hypothalamo-pituitary development and in Kallmann syndrome (26).

The IVS35+6T>C variant occurs in the intron/exon boundary of the exon encoding the first BRK domain, suggesting that the final protein may be mistranslated, leading to disrupted BRK and transcriptional activity mediated through the leucine zipper. Because IVS35+6T>C is located in a proposed splicing region and is predicted to affect splicing using 2 *in silico* prediction models, it may cause transcription to proceed through into the intron, creating 63 new amino acids before an early stop codon. In this scenario, exons 36–38 would not be translated, resulting in a truncated protein without a second BRK domain or leucine zipper (refer to Figure 1D). Again, the unaffected mother carried the variant.

Familial CHARGE syndrome has been described, and in such families, offspring are often more severely affected than the parent (9). Other family members occasionally seem to have milder associated clinical features without

carrying the variant (27), as noted in the sister of the proband in pedigree 1, concluding that these features are unrelated to the variant in the proband and further supporting the possible presence of digenicity.

Conclusion

We have for the first time identified an EPP in combination with severe hypopituitarism in 2 unrelated patients with variable features of CHARGE syndrome and *CHD7* variants, which are of unknown significance. Our data suggest the need for pituitary function testing and MRI in patients with CHARGE syndrome. However, *CHD7* mutations are not common in pituitary insufficiency or septo-optic dysplasia without features of CHARGE.

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