Isolated Central Hypothyroidism in Young Siblings as a Manifestation of PROP1 Deficiency: Clinical Impact of Whole Exome Sequencing

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Established Facts
- Central hypothyroidism may be caused by a variety of genetic defects.
- In combined pituitary hormone deficiency due to mutations in PROP1, central hypothyroidism generally is preceded by other pituitary hormone deficiencies.

Novel Insights
- PROP1 deficiency may present with isolated central hypothyroidism in infants or young children.
- Whole exome sequencing may facilitate rapid genetic diagnosis and affect clinical management.

Key Words
Central hypothyroidism · PROP-1 · Whole exome sequencing · Gene diagnostics · Genetics of endocrinopathies

Abstract
Background/Aims: Central hypothyroidism (CH) in children is rare and may be due to a variety of genetic defects. Most of these defects, but not all, are associated with additional pituitary hormone deficits. In a young child presenting with CH, it is important to determine whether additional pituitary hormone deficiencies are present, but this may be difficult to establish clinically. Methods: We describe the clinical characteristics of two young siblings, aged 6 months and 2 years, presenting with isolated CH. Whole exome sequencing was performed to determine the genetic basis of isolated CH. Results: A homozygous frameshift mutation of PROP1 (296delGA) was identified in both probands. Defects in PROP1 cause progressive deficiency of multiple pituitary hormones. Based on this genetic diagnosis, further clinical testing was performed that demonstrated growth hormone deficiency in one sibling. Conclusions: PROP1 deficiency may present as isolated CH at a very young age. In disorders with multiple potential causative genes, whole exome sequencing may facilitate rapid genetic diagnosis and lead to important changes in clinical management.
Introduction

Central hypothyroidism (CH), the insufficient production of thyroid hormone due to lack of normal thyrotropin (TSH) secretion, is most often acquired through damage to the pituitary or hypothalamus. Congenital CH is rare, with an incidence traditionally estimated between 1:110,000 and 1:29,000 [1–3]; however, recent data suggest that the incidence may be as high as 1:16,000 [4] and that CH may account for up to 13% of permanent congenital hypothyroidism [5]. Because the majority of congenital CH occurs in the setting of combined pituitary hormone deficiency (CPHD), congenital CH in the absence of other pituitary hormone deficits (‘isolated CH’) is very rare, occurring in no more than 1:92,000 infants [5].

When a child presents with apparently isolated CH, a primary concern is to identify whether the defect is limited to the thyrotroph lineage or affects multiple pituitary hormones, a distinction that has significant implications for clinical management and prognosis. However, the challenge of diagnosing pituitary hormone deficiencies in young children can make it difficult to distinguish true isolated CH from early or evolving CPHD. We report a case of two siblings with isolated CH in whom whole exome sequencing provided rapid genetic diagnosis of a homozygous frameshift mutation in PROP1, resulting in important changes in clinical management.

Patients and Methods

Case 1

A female infant was born at 32 weeks’ gestation due to spontaneous preterm labor. Her birth weight (1.810 g; –0.1 SDS) and length (43.2 cm; +0.1 SDS) were normal for gestational age. Other than a brief need for nasogastric feeding, her neonatal course was unremarkable, with no hyperbilirubinemia or hypoglycemia. Newborn screening at 5 days of age showed normal TSH 3 mIU/l (normal, <20 mIU/l) and total T4 7.8 μg/dl (normal, >6.0 μg/dl). Newborn screening was repeated at 20 days of age (35 weeks’ corrected gestational age) and revealed normal TSH 2 mIU/l and slightly low total T4 5.4 μg/dl (normal, >6.0 μg/dl); nevertheless, these results were reported as normal by the newborn screening program and no follow-up was recommended.

At age 2 years 3 months, the patient was noted to have a decrease in height SDS from –1.8 to –2.5, accompanied by a lesser decline in weight SDS (–0.4 to –1.2), with normal weight-for-length (+0.6 SDS). Developmentally, she began to sit up at 8 months (6 months corrected gestational age) and to crawl at 9 months, but began walking only at age 2 years. At the time of presentation, she did not yet speak two-word phrases. She had delayed tooth eruption, with only seven teeth present, and her bone age was delayed at 18 months. Laboratory evaluation showed low free T4 0.73 ng/dl (normal, 1.00–2.10 ng/dl) and normal TSH 1.78 mIU/l (normal, 0.70–5.70 mIU/l), consistent with CH. Of note, thyrotropin-releasing hormone (TRH) is not available for stimulation testing in the United States. Treatment was begun with levothyroxine 25 μg daily. Magnetic resonance imaging (MRI) of the brain was normal, including normal pituitary size for age.

At the time of initial evaluation, insulin-like growth factor 1 (IGF-1) was low (<25 ng/ml; normal, 51–303 ng/ml), but IGF-binding protein 3 (IGFBP-3) was normal (1.4 μg/ml; normal, 0.8–3.9 μg/ml). Serum prolactin (PRL) was normal (8.3 ng/ml; normal, 3.2–25.9 ng/ml), as was random serum cortisol (12.1 μg/ml; normal, 5.0–25.0 μg/ml). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were not tested, as the gonadotropin axis is normally quiescent at this age. Once treated with levothyroxine, the patient exhibited catch-up growth with an increase in height SDS by +0.9 (height –1.6 SDS; midparental height –1.2 SDS). Because low IGF-1 is not a reliable marker of growth hormone (GH) deficiency in young children [6] and may also be caused by decreased GH secretion due to hypothyroidism [7, 8], and because the patient’s linear growth improved and then remained normal with restoration of euthyroidism, the patient was considered to have isolated CH. At age 5 years, the patient’s growth velocity remained normal (6.3 cm/year; fig. 1). Despite her normal growth, GH stimulation testing was performed following the genetic diagnosis of PROP1 deficiency. This demonstrated profound GH deficiency, with a peak GH response of 0.23 ng/ml to arginine and glucagon, and GH treatment was started (25 μg/kg/day).

Case 2

A male infant, the younger brother of case 1, was born at 40 weeks’ gestation with normal birth weight (3,510 g; –0.1 SDS) and length (48 cm; –1.4 SDS). His neonatal course was notable for mild indirect hyperbilirubinemia that resolved without treatment and was attributed to glucose-6-phosphate dehydrogenase deficiency. Newborn screening at 2 days of age showed normal TSH <2.5 mIU/l (normal, <20.0 mIU/l) and total T4 9.0 μg/dl (normal, >5.0 μg/dl). His subsequent medical and developmental history was normal.

At his 6-month well visit, the patient was noted to be severely obese (weight +3.1 SDS), despite a dietary history that was not consistent with excess caloric intake. His linear growth and physical examination were normal, including a normal-size phallus and bilaterally descended testes. Based on his obesity and his sister’s history of CH, thyroid function tests were obtained, showing low free T4 0.66 ng/dl (normal, 0.80–1.80 ng/dl) and normal TSH 3.57 mIU/l (normal, 0.70–5.70 mIU/l) consistent with CH. Treatment was begun with levothyroxine 25 μg daily. MRI of the brain was normal. Like his sister, this patient had low IGF-1 (<25 ng/ml; normal, 55–327 ng/ml) and normal IGFBP-3 (1.6 μg/ml; normal, 3.2–25.9 ng/ml), as was random serum cortisol (12.1 μg/ml; normal, 5.0–25.0 μg/ml). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were not tested, as the gonadotropin axis is normally quiescent at this age. Once treated with levothyroxine, the patient exhibited catch-up growth with an increase in height SDS by +0.9 (height –1.6 SDS; midparental height –1.2 SDS). Because low IGF-1 is not a reliable marker of growth hormone (GH) deficiency in young children [6] and may also be caused by decreased GH secretion due to hypothyroidism [7, 8], and because the patient’s linear growth improved and then remained normal with restoration of euthyroidism, the patient was considered to have isolated CH. At age 5 years, the patient’s growth velocity remained normal (6.3 cm/year; fig. 1). Despite her normal growth, GH stimulation testing was performed following the genetic diagnosis of PROP1 deficiency. This demonstrated profound GH deficiency, with a peak GH response of 0.23 ng/ml to arginine and glucagon, and GH treatment was started (25 μg/kg/day).

Fig. 1. Growth curves (Centers for Disease Control and Prevention) for case 1 (a–d) and case 2 (e, f). Measurements for case 1 are uncorrected for gestational age. Arrowheads (●) to the right of height or length curves indicate midparental height (b) or midparental height percentile (a, e). Open arrows indicate diagnosis of central hypothyroidism. Closed arrows indicate normalization of serum free T4. * indicates diagnosis of GH deficiency.
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0.7–3.6 μg/ml). Serum PRL was normal (20.7 ng/ml; normal, 3.2–25.9). Although a morning cortisol level at age 6 months was indeterminate (7.5 μg/dl), he had no symptoms of adrenal insufficiency, and a subsequent morning cortisol level was normal (16.3 μg/dl) at age 18 months. The patient’s rapid weight gain arrested after normalization of his serum free T4, and his growth velocity remained normal during subsequent follow-up (fig. 1).

Whole Exome Sequencing
The parents of these siblings were of Hispanic ethnicity, originally from the Dominican Republic, and had no known consanguinity. The father’s height was 165 cm (−1.6 SDS) and the mother’s height was 160 cm (−0.5 SDS). Both the father and mother were obese (BMI 33 and 41 kg/m^2, respectively) but were otherwise healthy and biochemically euthyroid. The occurrence of isolated CH in siblings of both genders with unaffected parents suggested an autosomal recessive genetic etiology. Whole exome sequencing of the two probands and their mother was performed as part of a research protocol approved by the Boston Children’s Hospital Institutional Review Board. Written informed consent was obtained, and DNA was extracted from peripheral blood mononuclear cells using standard techniques. Hybrid selection was performed using NimbleGen’s SeqCap EZ Exome Version 3.0 (Roche NimbleGen Inc., Madison, Wisc., USA). Samples were sequenced using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, Calif., USA). The resulting reads were aligned to the hg19 reference genome with the Burrows-Wheeler Aligner [9], Genome Analysis Toolkit [10] base quality score recalibration was applied, indel realignment was conducted, and single nucleotide polymorphism and indel discovery and genotyping were performed across all samples simultaneously using variant quality score recalibration [11]. Variants were annotated for functional effect using SnpEff 2.0.5 (http://snpeff.sourceforge.net/). As individuals with phenotypes of this severity are extremely uncommon, it was assumed that any causal genetic variants would be rare. Therefore, only variants with minor allele frequency <1% in the 1,000 Genomes project (February 2012 release) [12] and the National Heart, Lung, and Blood Institute exome variant server [13] were considered. To investigate the recessive model, a search was conducted for autosomal genes containing either a homozygous or two heterozygous rare nonsynonymous variants. This analysis yielded 13 variants in 9 genes, including a previously reported homozygous pathogenic mutation in PROP1 (296delGA). Conventional Sanger sequencing confirmed that both patients were homozygous for this mutation and that both parents were heterozygous. This mutation in PROP1 was considered the likely etiology of the patients’ CH.

Discussion
Congenital CH is a rare condition that may be caused by mutations in any of several identified genes involved in hypothalamic or pituitary development, differentiation, or function (table 1) [3, 14–16]. In a minority of cases, including inactivating mutations of the TSH β-subunit (TSHB) [17], TRH receptor (TRHR) [18, 19], or IGSF1 [16], the functional defect is isolated to pituitary thyrotrhops, although defects in TRHR or IGSF1 usually cause combined TSH and PRL deficiency. In >75% of cases, congenital CH occurs as a component of CPHD [5], and in this setting other pituitary hormone deficiencies usually precede or manifest concurrently with CH. Thus, TSH deficiency is rarely the sole presenting feature of CPHD.

In a patient presenting with CH, distinguishing an isolated thyrotroph defect from CPHD has important clinical implications, and evaluation for other pituitary hormone deficiencies is imperative. In infants or young children, however, diagnosis of pituitary hormone deficiencies can be difficult, making it challenging to differentiate true isolated CH from CPHD. Physical examination may detect midline defects (e.g. cleft palate) or neuroimaging may reveal structural abnormalities (e.g. ectopic posterior pituitary or absent septum pellucidum) that suggest a problem with hypothalamic or pituitary development that is likely to be associated with CPHD. Patients with certain genetic forms of CPHD may have additional clinical features that can assist diagnosis (table 1), but the absence of such findings in a patient with CH does not exclude CPHD.

Here, we report two siblings with the unusual presentation of isolated CH, which strongly suggested an underlying genetic abnormality. Because CH has multiple genetic causes, we used whole exome sequencing to rapidly identify the causative mutation among the many candidate genes. Both siblings were found to be homozygous for a known pathogenic mutation of PROP1. This gene encodes a transcription factor critical for the normal development of multiple anterior pituitary cell lineages, and inactivating mutations of PROP1 cause deficiency of GH, TSH, PRL, LH, FSH, and in some cases adrenocorticotropic hormone (ACTH). PROP1 deficiency is the most commonly identified genetic cause of CPHD, accounting for 30–50% of familial cases [20–22], although PROP1 mutations are found only rarely in sporadic CPHD [22–25]. The 296delGA mutation present in our patients is the most commonly identified PROP1 mutation, accounting for 50–72% of pathogenic mutant alleles [20, 26]. This two-base pair deletion causes a frameshift that leads to premature truncation of the PROP1 protein, loss of its DNA-binding homeodomain, and abrogation of its function [27].

These cases illustrate two important clinical aspects of PROP1 deficiency in children. First, children may present with isolated CH as the sole initial manifestation of PROP1 deficiency, in contrast to previous reports suggesting that loss of other anterior pituitary hormones generally precedes TSH deficiency [20]. The most common presentation of PROP1 deficiency is childhood
growth failure due to GH deficiency. Gonadotropin deficiency may be apparent at birth as microphallus or cryptorchidism in a male, but more frequently manifests as delay or failure of normal pubertal progression. ACTH deficiency is a more variable feature that is more common with advancing age but may present during childhood, including with life-threatening adrenal crisis [21, 28–30]. Of note, both siblings in these cases had normal adrenal function at last follow-up. Case 1 had a peak serum cortisol of 28.9 μg/dl after glucagon injection (during GH stimulation testing), while case 2 had a normal morning cortisol level of 16.3 μg/dl at age 18 months.

Second, these cases demonstrate that isolated CH due to PROP1 deficiency may occur at an earlier age than generally recognized. Two previous case series identified no children with PROP1 mutations who developed CH un-
nder about 3 years of age [21, 30], while another series reported CH in 11% of patients at 1 year of age and in 22% at 3 years of age; however, the prevalence of CH under 1 year and whether TSH was the only deficient hormone in these cases was not specified [20]. To our knowledge, there is only 1 well-documented case of PROP1 deficiency presenting with isolated CH under 1 year of age [31]. The current case therefore further supports the idea that isolated CH may be the presenting sign of PROP1 deficiency in infants and young children. Notably, although PRL deficiency is reported to be common in PROP1 deficiency, both patients in these cases had normal basal PRL levels. Although TRH-stimulated PRL levels could not be measured, these cases suggest that PRL deficiency may evolve over time and may not be present in very young children with PROP1 deficiency.

It is interesting to note that CH presented with severe obesity in case 2. Although hypothyroidism in infancy rarely leads to obesity, in this case the marked decrease in weight SDS after levothyroxine treatment strongly suggests a causative relationship. Although a similar growth pattern could be observed in a breastfed infant who weans from breast milk, case 2 was formula-fed from birth and had begun to eat pureed foods by age 6 months. Increased energy expenditure from walking, which began at age 14 months, cannot account for his decreased weight gain. This case serves as a reminder that evaluation for hypothyroidism should be considered in infants and young children with severe obesity, particularly if the dietary history does not suggest excess caloric intake.

The abnormalities noted on newborn screening in case 1 suggest that CH may have been present shortly after birth, although interpretation was complicated by her prematurity. More typically, as in case 2, thyroid function abnormalities are not detected in newborns with PROP1 deficiency, which has been interpreted as evidence that pituitary hormone deficiencies due to PROP1 mutations may not be present at birth but develop gradually during postnatal life. However, the fact that some individuals have microphallus and cryptorchidism at birth [21, 30] implies that elements of CPHD, such as gonadotropin deficiency, may be present at or before birth in at least a subset of patients. With this in mind, these cases raise the possibility that the incidence of neonatal CH in PROP1 deficiency has been underestimated. Such underestimation could be due in part to the limited sensitivity of newborn screening for CH, particularly in programs that measure TSH as the primary screening test, as remains the standard in many areas. Conversely, although up to 13% of congenital hypothyroidism detected in the newborn period is of central origin and the majority of these individuals have CPHD [5], it is unknown what proportion of CH detected on newborn screening is due to mutations in PROPI or other genes associated with CPHD. These cases suggest that such patients may benefit from genetic testing to clarify their underlying defect.

Making a genetic diagnosis in these cases of apparently isolated CH had several important clinical consequences. First, had an isolated thyrotroph defect been discovered, it would have obviated the need for lifelong surveillance for and management of other pituitary hormone deficits. Instead, the finding of PROP1 deficiency clarified the siblings’ prognosis for developing GH, PRL, and gonadotropin deficiency, as well as indicating the need for careful monitoring of adrenal function even in childhood. The genetic diagnosis led directly to the diagnosis and treatment of profound GH deficiency in case 1, in whom the diagnosis was otherwise obscured by her normal growth velocity. Although it is possible that GH deficiency may have been present since birth, despite the absence of neonatal hypoglycemia, this presentation reinforces that the diagnosis of GH deficiency in children may be complicated by the fact that some individuals display normal growth in childhood despite lack of normal GH secretion [32]. Nevertheless, GH therapy may be indicated to address the adverse metabolic consequences of GH deficiency, including decreased lean body mass, increased fat mass, and decreased bone density [33]. As GH replacement was recently initiated, we cannot yet comment on any response to therapy. Finally, identifying the causative mutation in these cases facilitated appropriate genetic counseling for the patients’ family and could provide the basis for pre-implantation or prenatal genetic testing in future pregnancies.

In summary, congenital CH may represent isolated TSH deficiency or the initial manifestation of CPHD, a distinction of profound clinical consequence. However, even a complete evaluation of pituitary function, which is mandatory in any child with CH, may not reliably make this distinction in a young child. We present a case of two siblings with isolated CH in whom whole exome sequencing rapidly identified a pathogenic mutation in PROPI. This genetic diagnosis, in turn, led to important changes in clinical management, including more aggressive evaluation for (and diagnosis of) associated pituitary deficiencies and the ability to provide more accurate prognostic information and genetic counseling to the family. These cases continue to broaden the clinical spectrum of...
PROP1 deficiency to include patients presenting in infancy or early childhood with CH that may not be accompanied by other pituitary hormone deficits. Furthermore, the marked benefit of securing a genetic diagnosis raises the possibility that infants and children diagnosed with CH may benefit from genetic testing for mutations in PROP1 or other genes associated with CH. In fact, the benefits of genetic diagnosis – including improved genetic counseling and directed screening for pituitary defects – may apply more generally to patients with other congenital pituitary hormone deficits such as isolated GH deficiency or hypogonadotropic hypogonadism [34–36]. This case demonstrates that whole exome sequencing may be an effective diagnostic tool in such patients, although a formal comparison of the utility of whole exome sequencing versus targeted candidate gene sequencing is necessary to evaluate the cost effectiveness of this approach. As it becomes less costly and more widely available clinically, whole exome sequencing will be a powerful tool for evaluating multiple candidate genes in these and other conditions.

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