A Mutational Hot Spot in the Prop-1 Gene in Russian Children with Combined Pituitary Hormone Deficiency


1Department of Pediatrics, 5Research Center for Medical Genetics and 6DNA-Diagnostics Laboratory, Endocrinology Research Center, Moscow, Russia; 2Department of International Health and Radiation Research and 7Department of Nature Medicine, Atomic Bomb Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan; 3Department of Pediatrics, Nagasaki University School of Medicine, Nagasaki, Japan; 4Department of Pediatrics, Division of Endocrinology, Emory University School of Medicine, Atlanta, GA, USA

Abstract. Combined pituitary hormone deficiency (CPHD), including growth hormone (GH), prolactin (Prl) and thyroid-stimulating hormone (TSH) in children is now considered a heterogeneous syndrome. Recent findings on expression of mouse pituitary-specific homeodomain factors demonstrate dependence of adenopituitary ontogeny on interactive expression of these factors, suggesting their involvement in etiology of CPHD. Prophet of Pit-1 (Prop-1) gene, a novel pituitary-specific homeodomain factor, was analyzed in 14 Russian children with CPHD, in whom Pit-1 gene was intact. We found a mutational hot spot in three patients from two families in homeodomain part of the second exon of Prop-1 gene. The common 2-base pair deletion (GA296) in the homozygous state resulted in a Serine to Stop codon (S109X) substitution and generated a truncated Prop-1 protein. Parents were phenotypically normal and heterozygous for GA296 deletion, indicating an autosomal recessive inheritance. These results demonstrate a novel type of Prop-1 gene mutation as one of the causes of CPHD in Russian patients.

Key Words. combined pituitary hormone deficiency (CPHD), prophet of Pit-1 (Prop-1), 2-base pair deletion GA296, Russia

Introduction

Combined pituitary hormone deficiency (CPHD), including growth hormone (GH), prolactin (Prl) and thyroid-stimulating hormone (TSH) deficiency is now considered to be a genetically heterogeneous state, both in animal models and in humans [1–4]. Three models of hereditary dwarf mice, e.g. Snell (dw/dw), Jackson (dwj/dwj) and Ames (df/df), although similar phenotypically, all have complete (dw, dwj) or incomplete (df) lack of somatotropes, lactotropes and thyrotropes, yet they differ in their background genetic defect. Snell and Jackson mice defects are attributed to Pit-1 (Pituitary-Specific Transcription Factor) gene, mapped on chromosome 16, whereas the Ames mice defect is attributed to Prop-1 (Prophet of Pit-1) gene, mapped on chromosome 11 [1,3,5]. The importance of Pit-1 in activating expression of GH, Prl and TSH-β genes as well as in the differentiation of three, adenopituitary cell lineages has been demonstrated by a variety of expression studies during organogenesis [6–11]. In mice, Prop-1 expresses from e10 to e10.5, reaching maximal expression at e12.0. During e10.5–e13.5 Prop-1 co-expresses with Rpx, which is believed to be one of the first genes expressed in the anterior pituitary. Undetectable Rpx expression coexists with the beginning of Pit-1 gene expression at e13.5 [1,7,10]. Thus, adenopituitary cell determination and differentiation are under the control of a cascade of tissue-specific transcription factors, including Prop-1, Pit-1, Rpx, P-Lim, OTX, Pax6, Brn4, neuronatin and others [7,11,13–18].

In humans, Pit-1 gene was the first among other POU-domain pituitary factors to be cloned. To date, 10 different types of Pit-1 gene mutations in children with CPHD have been reported [3,4,18–20], including ours. We showed that in a cohort of 15 children from Russia with CPHD Pit-1 mutation only occurred in one patient (P14L in the heterozygous state), suggesting the rarity
of Pit-1 gene mutations in dwarf children with CPHD (20). We, therefore, emphasized that negative cases of Pit-1 gene mutation require further genetic analysis for other pituitary-specific transcription factors, as reported in mice [1,2,10,11].

Prop-1, another new pituitary-specific paired-like homeodomain factor, has been cloned recently in man. To date, there have been no reports concerning Prop-1 mutational analysis in children with CPHD. We therefore focused on assessing Prop-1 gene mutations in Russian children with CPHD and without Pit-1 gene abnormality.

Materials and Methods

Subjects
14 (10 females, 4 males) children with combined pituitary hormone deficiency, including complete GH and complete or partial Prl and TSH deficiency were studied. Previous analysis of Pit-1 gene and it’s promoter region in this cohort of patients failed to reveal Pit-1 abnormalities [20]. There were 3 familial cases and 8 sporadic cases.

DNA extraction
Genomic DNA was extracted from peripheral leukocytes using standard phenol-chloroform extraction method.

Sequencing analysis
DNA fragments, covering all three Prop-1 gene exons and boundary regions were amplified using PCR (Perkin Elmer, USA). Amplified PCR products were purified using QIAEX II Agarose Gel Extraction kit (QIAGEN, USA) and SUPREC-02 columns (Takara, Japan). The further PCR with Dye Terminator was performed using Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, USA). PCR reaction mixture (20 µl) for each sense and antisense primer contained 30 ng of PCR product, 8 µl Terminator Ready Reaction Mix, 3.2 pmol primer and deionized water. PCR was performed with 25 cycles at 96 °C for 10 sec, 50 °C for 5 sec and 60 °C for 4 min. Subsequent CENTRI-SEP Column Purification (Princeton Separation Inc, USA) was performed to remove excess dye terminators. Direct DNA sequencing of both strands of PCR products was performed and analyzed using ABI PRISM 377 DNA Sequencer (Perkin Elmer, USA).

Results and Discussion

A common mutation in the Prop-1 gene, i.e., 2-base pair deletion GA296 in the homeodomain part of exon 2 in the homozygous state, resulting in a Serine to Stop codon substitution (S109X) and severely truncated Prop-1 protein (Fig. 1), was detected in 3 (21.4%) of 14 CPHD children. All patients (No.1-3) represented familial cases of the disorder. In all familial cases, however, there was no consanguinity between the parents. Family studies showed that the mother of patients No. 1 and No. 2 and both parents of patient No. 3, all of whom were phenotypically normal, were heterozygous for a GA296 deletion, indicating an autosomal recessive inheritance (Fig. 2).

Phenotypically there were some differences between the patients with identified Pit-1 gene and Prop-1 gene mutations. However, because of the rarity of Pit-1 gene mutation and lack of common clinical features of the involvement of Pit-1 gene mutations in CHPD as comparisons are limited. However, the relatively high incidence of Prop-1 compared to Pit-1 gene mutations in CPHD in Russian children is striking.

Clinical and hormonal characteristics of the children with Prop-1 gene mutation are depicted in Tables 1 and 2. Despite the fact that all affected patients had total GH-deficiency, the severity of growth retardation differed. At the time of examination, all three patients had attained pubertal chronological age, but bone age was still prepubertal. None of these patients had signs of spontaneous puberty. Later, one patient (No.2), reached pubertal bone age without spontaneous puberty and she was started on estrogens. We may therefore reconsider the phenotypical characteristics of CPHD with or without gonadotropin deficiency after reaching pubertal age.

Taking into account the fact that all cases showed the same GA296 deletion in the Prop-1 gene, it is likely to be the mutational hot spot and it would therefore be expedient to perform screening for this common type of Prop-1 gene mutation in affected children with CPHD and their relatives to elucidate genetic basis of the disorder. As a restriction enzyme (BcgI) can excise a newly created sequence of mutated Prop-1 gene (data not shown), this screening will be facilitated by restriction enzyme analysis of PCR products in patients with CHPD cases and their parents.

Compared to the modest Ames mouse defect, only complete functional loss of Prop-1 seems to trigger and develop human CHPD. Human Prop-1 gene expression profile and its functional role may be different from the mouse pituitary ontogeny. Alternatively, other types of Prop-1 gene mutations may yet be discovered.

Finally, based on these novel observations of a Prop-1 gene mutation, careful follow-up of these CPHD patients is needed and the negative cases of Prop-1 an Pit-1 gene mutations should be studied for other candidate genes of pituitary specific transcriptional factors.