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Mutation-in-Brief

A Novel Splicing Mutation of the GNAS Gene in a Patient with Pseudohypoparathyroidism Ia

Akie Nakamura[1], Tomoyuki Hostubo[2], Wakako Jo[1], Katsura Ishizu[1], and Toshihiro Tajima[1]

[1]*Department of Pediatrics, Hokkaido University School of Medicine, Sapporo, Japan*

[2]*Department of Pediatrics, NTT Higashinihon Hospital, Sapporo, Japan*

Pseudohypoparathyroidism (PHP; MIM 103580) refers to end-organ resistance that primarily impairs the renal actions of PTH (1). In addition, patients with PHP-Ia show resistance to other hormones, as well as Albright's hereditary osteodystrophy (AHO), a constellation of features including short stature, obesity, brachydactyly, ectopic ossifications and/or mental retardation. These patients have maternally-inherited heterozygous inactivating mutations in one of the 13 GNAS exons, including missense mutations, nonsense mutations, insertions and deletions (1, 2).

We describe here a Japanese patient with PHP-Ia and tuberous sclerosis. In this patient, a novel splicing mutation (c.210+1G>A) was identified.

The patient is a 5-yr-old Japanese boy. He was born at 40 wk of gestation by caesarean section due to placental abruption. Both parents were healthy. At the age of 3 mo, he developed epileptic seizures. Radiological examinations revealed calcifications in cortical tubers. At this time, laboratory examination demonstrated normal serum calcium (9.5 mg/dl). Further evaluation demonstrated retinal hamartoma and cardiac rhabdomyoma, and a diagnosis of tuberous sclerosis was made. At 3 yr of age, he was referred to our hospital with hypocalcemia (5.9 mg/dl), hyperphosphatemia (9.6 mg/dl) and an elevated intact parathyroid hormone (PTH) level (210 pg/ml). On physical examination, his height was 100 cm (+0.7 SD for normal Japanese boys), and his weight was 22 kg. He had a round face and brachydactyly. A roentgenogram of his hands revealed shortened metacarpals and subcutaneous calcifications (Fig. 1). Hypothyroidism was evident (serum free T3, 3.4 pg/ml; free T4, 0.94 ng/dl; and serum TSH, 13.09 μU/ml). A PTH infusion test showed no response of cAMP (U4/U3, 1.93; normal range >10). In addition, urinary phosphate excretion did not increase [(U4+U5)–(U2+U3), –1.5 mg/2 h, normal range 35>] after PTH infusion.

Based on these clinical and biochemical

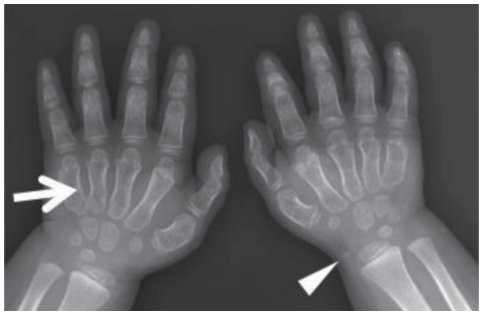

Fig. 1 Roentgenogram of hands. A roentgenogram showed shortened metacarpals on both the left and right hands (arrow). The arrowhead shows subcutaneous calcifications.

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Correspondence: Dr. Akie Nakamura, Department of Pediatrics, Hokkaido University School of Medicine, N15, W7, Sapporo 060-8638, Japan
E-mail: akieda@med.hokudai.ac.jp

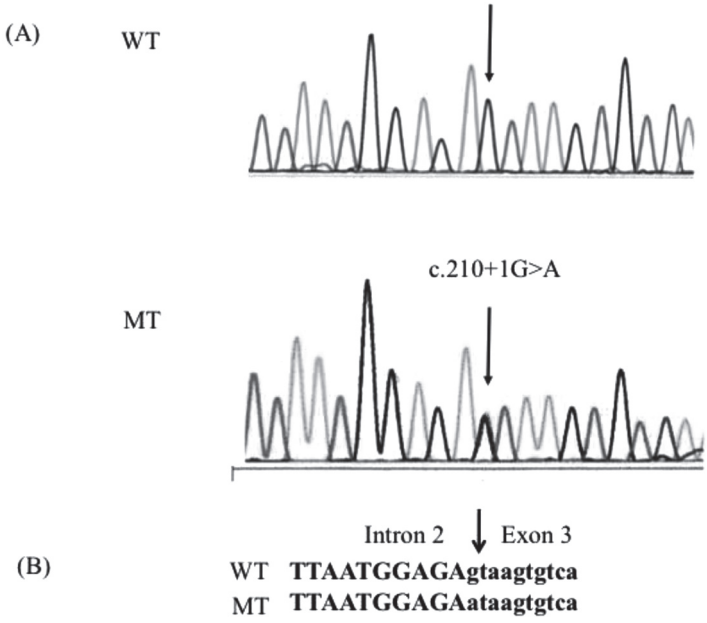

Fig. 2 Direct sequencing analysis of the intron 2/exon 2 junction in the *GNAS* gene. Mutated and normal nucleotides (A and G respectively) were present in the patient (arrow).

findings, he was diagnosed as having PHP-Ia, and treatment with 1,25 hydroxyvitamin D3 [1, 25(OH)2D3] (0.04 μg/kg/d) and L-thyroxine was initiated.

We obtained approval of this study from the institutional ethics committee of Hokkaido University School of Medicine. The 13 exons and exon-intron boundaries of the *GNAS* gene were amplified by polymerase chain reaction (PCR) using oligonucleotide primers as described in a previous report (3). Direct sequencing of the amplified genomic DNA fragments identified a previously unreported substitution of adenine for guanine at the splicing acceptor site in intron 2 (c.210+1G>A) (Fig. 2).

Since this mutation occurs at a splicing acceptor site, we surmised that it might be pathogenic. Its functional consequences were analyzed with the NNSPLICE0.9 automated splice site analysis program (http://www.fruitfly.org/seq_tools/splice.html), which predicted that this mutation generated a nonfunctional splice acceptor site, resulting in aberrant splicing of the *GNAS* mRNA.

According to the literature, although GNAS mutations have been identified in the majority of patients with PHP-Ia, as well as in their relatives with pseudo-PHP, in about 30% of PHP-Ia patients, a molecular diagnosis has yet to be ascertained (1, 2). Methylation defects in the imprinted GNAS gene cluster have been found in PHP-Ib (2). Recently, however, Mantovani *et al.* (4) reported *GNAS* cluster imprinting defects in 24 of 40 patients with sporadic AHO and multiple hormone resistance. These findings suggest that both mutational analysis of the GNAS exons as well as epigenetic defects should be considered, even in patients with PHP-Ia.

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