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NOTE

Mutations in Exon 7 of the GTP-Binding Protein $G_s\alpha$ Were Not a Common Cause of Pseudohypoparathyroidism with Albright's Hereditary Osteodystrophy in Japanese

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Abstract. The identification of unique point mutations in patients with pseudohypoparathyroidism (PHP) with Albright's hereditary osteodystrophy (AHO) in different ethinic backgrounds has proved that defects within the $G_s\alpha$ gene account for $G_s\alpha$ deficiency in those patients. To search a mutation hot spot of the $G_s\alpha$ gene in Japanese patients, we have screened exons 2–13 of the $G_s\alpha$ gene for mutations in three unrelated Japanese PHP patients with AHO. We could find no abnormalities by denaturing gradient gel electrophoresis and no mutations of sequencing of exon 7 in these subjects. This suggests that mutations in exon 7 of the $G_s\alpha$ gene may not be a common cause of PHP with AHO in Japanese.

Key words: $G_s\alpha$ mutation, Pseudohypoparathyroidism, Albright's hereditary osteodystrophy, Hormone resistance

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THE guanine nucleotide-binding (G) proteins mediate signal transduction coupling the heptahelical receptors to intracellular effector systems [1–3]. G proteins are made up of three polypeptides: an α subunit that binds and hydrolyzes GTP with high affinity and specificity, a β subunit, and a γ subunit. Over twenty different G protein α subunits from four subfamilies, five G protein β and twelve G protein γ subunits provide significant combinational signal-trunsduction options [4]. G proteins perch just inside the plasma membrane on lipidic feet of the myristoylated or palmitoylated $G\alpha$ subunit and of franesylated or geranylgeranylated $G\gamma$ of the $G\beta\gamma$ dimer subunits [4]. The crystal structure of the G protein heterotrimer indicates that the whole signalling complex acts like a nanomachine consisting of a lever (receptor), switch ($G\alpha$) and propeller ($G\beta\gamma$) [5–9].

Mutations of the $G_s\alpha$ gene lead to either constitutive activation or loss of function in human diseases [10–22]. Molecular genetic studies have shown that PHP with AHO can result from heterozygous loss-of-function mutations in the gene encoding the GTP-binding protein $G_s\alpha$.

We have previously shown the presence of a 4-base pair deletion at codon 189 in exon 7 of the $G_s\alpha$ gene in a Japanese patient with PHP with AHO and in his mother with pseudopseudohypoparathyroidism (PPHP). Here we report the results of mutational analysis of the $G_s\alpha$ gene in three additional Japanese patients with PHP with AHO.

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Materials and Methods

Subjects

Three unrelated subjects were studied, the clinical features of which are summarized in Table 1. The diagnosis of PHP was suspected on the basis of biochemical hypoparathyroidism (depressed serum calcium level and elevated serum phosphate level), elevated serum PTH level, and/or a habitus suggestive of AHO. All patients were personally examined by at least one of the authors (YH, HY and TS), and underwent a limited radiographic bone survey (including hands and feet). Renal responsiveness to PTH was determined by measuring urinary cAMP excretion after an intravenous infusion of human PTH 1–34 [23]. The noticeably blunted peak urinary cAMP (urinary cAMP responses <1 μmol/h or <10% of normal) and phosphate responses to PTH (the incremental excretion of urinary phosphate <0.35 mg/2 h) were consistent with the diagnosis of PHP type I in all the subjects. Informed consent for these studies was given by each subject.

Molecular methods

DNA was prepared from peripheral blood lymphocytes as described previously. Exons 2, 4, 5 and 7–13 of the $G_s\alpha$ gene were amplified by the polymerase chain reaction (PCR) and specific primer pairs as described before [12, 19]. The PCR products were then reamplified with the same primer pairs except that one of the primers contained a GC-clamp of the sequence: 5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGC-CCCCGCCCG-3′. Exons 3 and 6 were amplified directly with specific primers one of which included the GC-clamp. The PCR products containing the GC-clamp sequence were separated by electrophoresis at 60 °C and 80 volts for 5–9 h on denaturing gradient polyacrylamide gels as described previously [12, 19, 24–26]. Following electrophoresis, the gels were stained with ethidium bromide and photographed under ultraviolet transillumination. In addition to screening exon 7 for mutations by the technique of denaturing gradient gel electrophoresis (DGGE), the amplified PCR products of this exon (without GC-clamp) were cloned into pGEM-T (Promega) and sequenced with a Sequenase® Version 2.0 DNA Sequencing Kit (USB).

Results

Exons 2–13 of the $G_s\alpha$ gene of the three subjects with PHP described above, an unrelated normal control and a subject with PHP having a 4-bp deletion mutation in codon 189 of exon 7 [19] were screened for mutations with PCR and DGGE. The DGGE analysis showed no evidence of bands of abnormal mobility in any exon except for the known mutation in exon 7 in the one subject with PHP identified in our earlier study. Furthermore, sequencing of exon 7 in the three new subjects with PHP showed no mutations (Fig. 1).

Discussion

Eighteen different mutations in the $G_s\alpha$ gene have been reported in patients with PHP and PPHP [11–20, 27], but no mutation of the $G_s\alpha$ gene was found in "only AHO" family [28]. These are distributed throughout the gene with all but one located in exons 2–13. They include missense mutations, splicing mutations that result in the synthesis of $G_s\alpha$ mRNA of abnormal sequence, an insertion, and deletions that lead to a change in the reading frame and the synthesis of a truncated

Table 1. Subjects and their profiles

subject no.	age	sex	s-Ca (mg/dl)	s-Pi (mg/dl)	i-PTH (pg/ml)	FT4 (ng/dl)	TSH (μU/ml)
1	36	M	3.1	4.6	59.4	1.55	3.3
2	45	M	6.5	5.2	1270	–	9.5
3	38	F	5.3	5.4	246	1.4	9.7

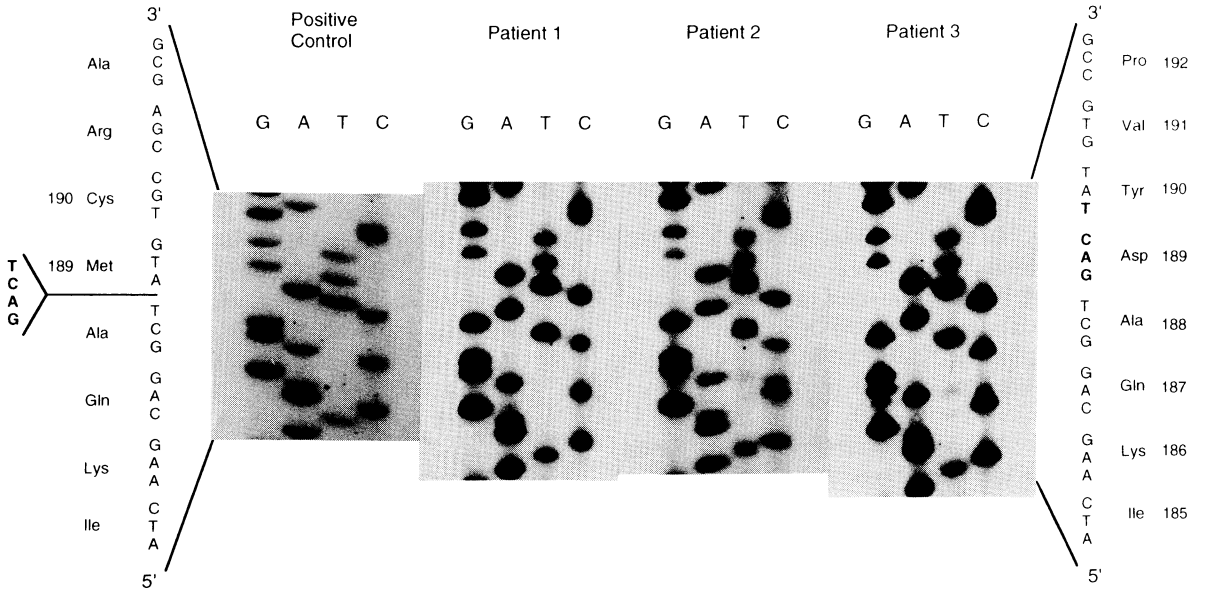

Fig. 1. Sequencing analysis on exon 7 of $G_s\alpha$ gene in three patients and positive control. Four bp (GACT) deletions were shown as a positive control (left).

protein. While most of the mutations identified to date are private in that they are found only in a single family, a 4-bp deletion which centered around codons 188–190 has been found in six unrelated families of different racial and ethnic backgrounds including Japanese, consistent with this region of exon 7 being a site of recurrent mutations or mutational hotspot [27]. Exons 1 and 10 were reported not to be common mutation sites [29] but the absence of mutations in exon 7 of the $G_s\alpha$ gene in three Japanese subjects with PHP with AHO in this report suggests that mutations in exon 7 of this gene may not be a common cause of the disorder in Japanese. While we had screened for a mutation in the $G_s\alpha$ gene by DGGE, we could not find any abnormalities in exons 2–13 of the $G_s\alpha$ gene in these subjects. Although we cannot exclude a mutation in exon 1 of $G_s\alpha$ in these three subjects, we believe it unlikely that all three would have mutations in this region of the gene. Unfortunately for technical reasons possibly relating to the base composition of exon 1, it is not possible to readily examine this exon for mutations. We have also not screened the promoter region for mutations. It is possible that mutations in this part of the gene which impair transcription and lead to reduced levels of $G_s\alpha$ could also result in PHP with AHO, although no examples of disease-associated promoter mutations have been reported. Our results presented here might support the hypothesis that cases of PHP with AHO are genetically more complexed than might have been expected and might have some mutations not only in the $G_s\alpha$ gene but also in other genes including genes encoding other proteins that comprise the intracellular signalling pathway leading from the PTH receptor to cellular sites of PTH action.

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