Pseudohypoparathyroidism type Ia: two new heterozygous frameshift mutations in exons 5 and 10 of the Gsα gene

Abstract Pseudohypoparathyroidism type Ia (PHP-Ia) is a hereditary disease characterized by resistance to PTH and other hormones that act via cAMP. Patients have deficient activity of Gsα, the α subunit of the G protein, which couples hormone receptors to stimulation of adenylate cyclase. We describe two new mutations discovered in two sporadic patients with PHP-Ia. Using genomic DNA, we have amplified exons 2–13 of the Gsα gene (GNAS1) by PCR, and sequenced the resulting products. Both patients had Albright’s hereditary osteodystrophy, resistance to multiple hormones, and deficient Gsα activity. In the first patient, a deletion of a C in exon 5 at codon 115 was found. In the second patient, an insertion of a C in exon 10 at codon 267 was detected. Both these heterozygous mutations cause frameshift, and predict decreased production of Gsα. This report adds two new Gsα mutations to the known ten mutations recently described.

Introduction Pseudohypoparathyroidism type Ia (PHP-Ia) is a hereditary disease characterized by resistance to parathyroid hormone (PTH) and other hormones that work via cAMP, such as TSH and gonadotropines (Levine and Spiegel 1995). Most patients have characteristic skeletal features, which include short stature, round head and brachydactyly, termed Albright’s hereditary osteodystrophy (AHO). Gs protein, the GTP-binding protein that couples hormone receptors to the stimulation of adenylate cyclase (Conklin and Bourne 1993; Hepler and Gilman 1992), is deficient in membranes of various cells of patients with PHP-Ia, accounting for the patients’ resistance to multiple hormones (Farfel et al. 1980, 1981; Levine et al. 1980). G proteins are heterotrimeric, composed of an α subunit and tightly associated βγ subunits (Conklin and Bourne 1993; Hepler and Gilman 1992). Patients with PHP-Ia have a reduction of 50% in the activity of the Gsα subunit, caused by decreased amounts of Gsα (Patten and Levine 1990).

A few heterozygous mutations in the gene encoding the Gsα subunit (GNAS1) were recently described (Iiri et al. 1994; Levine and Spiegel 1995; Miric et al. 1993; Oude Luttikhuis et al. 1994; Patten et al. 1990; Weinstein and Shenker 1993; Weinstein et al. 1990, 1992). Here we describe two new heterozygous frameshift mutations, in exons 5 and 10 of the Gsα gene, in two sporadic patients with PHP-Ia.

Patients and methods The first patient (BM), was previously described (Shapiro et al. 1980). He is 32 years old, of Iranian-Jewish extraction and his maternal grandfather and paternal grandmother were first cousins. His parents, four brothers and three sisters are normal; none of them have AHO, and their serum calcium and phosphorus concentrations are normal. At the age of 11 years the patient developed grand mal seizures, and 2 years later he was found to have hypocalcemia and hyperphosphatemia. Physical examination showed the typical features of AHO including brachydactyly and mental retardation. PTH concentration was high, and a blunted urinary cAMP response to PTH administration was observed. TRH and LHRH administration caused an exaggerated response of TSH and LH, respectively. Gsα activity in erythrocyte membranes was measured by in vitro complementation with S49 cyc− cell membranes as described (Farfel et al. 1980), and was reduced to 50% of the level found in normal subjects.

The second patient (SY) was previously described (Weisman et al. 1985). He is 18 years old, of Iraqi-Yemenite-Jewish extraction. His parents and brother and sister are normal. At the age of 10 days, congenital hypothyroidism was diagnosed because of hypothermia, jaundice, macroglossia and low T4 and high TSH. Thy-
roxine therapy was started. At the age of 5 years, the typical features of AHO were noticed, including brachydactyly and mental retardation. Mild hypocalcemia was found, PTH was high, and urinary cAMP response to PTH administration was blunted. Erythrocyte Gsα activity was 50% (Farfel et al. 1980). Two years later he developed grand mal seizures, his serum calcium was found to be 6.5 mg/dl, and vitamin D therapy was started.

The gene of Gsα contains 13 exons (Kozasa et al. 1988). The Gsa gene of each patient was analyzed by sequencing PCR-amplified exons 2–13, using genomic DNA extracted from peripheral leucocytes. The primers used were as follows:

Exon 2: upstream 5'-CAGCAGACCTCCTGCCCACATTGTGTTT-3'

downstream 5'-CCCCTTACTGGTGCCTCACTGCTT-3'

Exon 3: upstream 5'-GGCTGGCGGGAGCAATTGTGTTT-3'

downstream 5'-CCCGGTCTCTGGCCTCAGTTTCCC-3'

Exon 4: upstream 5'-GCAATCTCTACACTGACATGGTG-3'

downstream 5'-GTCAGGGAACAGCACTGGCCTGGA-3'

Exon 5: upstream 5'-CTCTGTGCTGTCTGTCTTGTAGCG-3'

downstream 5'-TCCTATATGGACACTGTGCTCAGG-3'

Exon 6: upstream 5'-GGACAGAGGGAACAGCTCCTGACG-3'

downstream 5'-TGATGGGTTGGGTGGCGGTTACTT-3'

Exons 7–10: upstream 5'-GCCGCTGTGACAACCCACCTGTTCT-3'

downstream 5'-CGCAGGGGGTGGCGGCTACCTCA-3'

Exon 11: upstream 5'-GACCCCTGGCCGAAAGCGCGCTTC-3'

downstream 5'-AGCCAGCAAGAGTGGAAGCCATAC-3'

Exons 12–13: upstream 5'-GGGAGCTACAGAGATGCTAGCACC-3'

downstream 5'-TTAAAGCCTTTAATAAATTTGGGGTCC-3'

Exons of genomic DNA were amplified under the following cycling conditions: denaturation at 95°C for 1 min followed by 30 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min and than further extension at 72°C for 5 min. PCR products were resolved in 1% agarose gels, and purified with a Wizard PCR Preps kit (Promega). Exons of genomic DNA were sequenced with a fmol DNA sequencing kit (Promega) according to the manufacturer’s instructions. The resulting sequence is based on complete sequencing of both sense and antisense strands.

The study was approved by the hospital’s ethics committee. Informed consent was given by all participating persons.

Results and discussion

In the first patient a heterozygous mutation was found in exon 5, consisting of a deletion of a C in codon 116 (Fig. 1). This introduces a stop codon 48 bases downstream. Exons 2–4 and 6–13 were normal. In the second patient a heterozygous mutation was found in exon 10, consisting of an insertion of a C in codon 267 (Fig. 2). This introduces a stop codon 96 bases downstream. Exons 2–9 and 11–13 were normal. In the patient’s mother, no mutation was found in exon 10, and her erythrocyte Gsα activity was normal (102%). The coding frameshift mutations in both patients would prevent the generation of a normal full-length Gsα from the abnormal allele. Indeed, Gsα functional activity in both of them was reduced to 50%.

The mutations described here add to the spectrum of the recently described Gsα mutations in PHP-Ia. One mutation was detected on the basis of lack of recognition of the patient’s Gsα by an antibody directed against the Gsα amino-terminus. Another mutation was detected by finding a large 43-bp deletion of PCR-amplified Gsα exon 4.

The existence of all the other mutations was heralded by finding an abnormal migration pattern of amplified exons by screening with denaturing gradient gel electrophoresis (DGGE). In our study, we PCR-amplified and directly sequenced exons 2–13 of the Gsα gene in both patients, without the use of a screening technique.

Of the ten previously described mutations, only one was observed in two unrelated patients (Iiri et al. 1994); all the other mutations were unique to each kindred. These mutations were scattered throughout the gene in exons 1,4,6,7,8,10 and 13 (Iiri et al. 1994; Levine and Spiegel 1995; Miric et al. 1993; Oude Luttikhuis et al. 1994; Patton et al. 1990; Weinstein and Shenker 1993; Weinstein et al. 1990, 1992). They include single base substitutions predicting single amino acid replacement (Iiri et al. 1994; Levine and Spiegel 1995; Miric et al. 1993) or abnormal mRNA splicing (Weinstein et al. 1990), and base deletions causing frameshift mutations (Miric et al. 1993; Oude Luttikhuis et al. 1994; Weinstein et al. 1990, 1992). This shows that the molecular defect of PHP-Ia is hetero-