Parental origin of $Gs\alpha$ gene mutations in Albright's hereditary osteodystrophy

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Abstract

Heterozygous mutations of the $Gs\alpha$ gene leading to reduced $Gs\alpha$ activity have been identified in patients with Albright's hereditary osteodystrophy (AHO). However, AHO may be associated with hormone resistance (pseudohypoparathyroidism type Ia, PHPIa) or a normal response (pseudopseudohypoparathyroidism, PPHP). As both disorders may occur within the same family, the relationship between $Gs\alpha$ genotype and phenotype remains unresolved. The AHO phenotype may be dependent upon the sex of the parent transmitting the $Gs\alpha$ mutation, perhaps through a gene imprinting mechanism. We have used an intragenic $Gs\alpha$ *FokI* polymorphism to determine the parental origin of $Gs\alpha$ gene mutations in sporadic and familial AHO. We now show that a de novo G→A substitution at the exon 5 donor splice junction in a child with PPHP was paternally derived. Furthermore, in a female with PPHP, the $Gs\alpha$ abnormality was shown to be of paternal origin, while subsequent maternal processing and transmission resulted in PHPIa in two offspring. As transmission of PPHP has rarely been reported, determining parental origin of the disease allele in sporadic cases may provide insight into the mechanism of hormone resistance in AHO.

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Albright's hereditary osteodystrophy (AHO) is an autosomal dominantly inherited syndrome of short stature, obesity, round face, brachymetaphalangism, subcutaneous ossification, and variable mental retardation. Many subjects with AHO have associated pseudohypoparathyroidism type I (PHPI), failing to increase urinary cAMP and phosphate excretion after exogenous PTH.¹² Resistance to other adenylyl cyclase stimulating hormones including TSH, LH, and FSH is frequently also observed.³ The remaining subjects, who respond normally to exogenous PTH, have pseudopseudohypoparathyroidism (PPHP).⁴

Upon extracellular binding of many hormones to their specific receptors, an intracellular heterotrimeric G protein is activated. G proteins comprising α , β , and γ subunits can either stimulate (Gs) or inhibit (Gi) the formation of cAMP through adenylyl cyclase.⁵ The majority of patients with PHPI have a 50% reduction in $Gs\alpha$ levels in membranes

from various tissues including erythrocytes and renal cortex and these patients are classified as PHPIa. Interestingly, levels of $Gs\alpha$ activity are similarly reduced in patients with PPHP.⁶

The human $Gs\alpha$ gene has been mapped to chromosome 20q13,⁷ is 20 kb in length, and has 13 exons.⁸ Heterozygous $Gs\alpha$ gene mutations, either single base pair substitutions or small deletions of up to 43 bp,^{9,10} have been identified in patients with AHO. Within five separate kindreds, related persons with PHPIa and PPHP have been observed to cosegregate with the same $Gs\alpha$ mutation.¹¹⁻¹³ Hence, molecular heterogeneity of $Gs\alpha$ mutations is not sufficient to explain the phenotypic difference in hormone responsiveness seen in PHPIa and PPHP.

A recent review of published familial cases of AHO¹⁴ suggests that parental origin of the mutated allele may influence phenotype since 66/66 offspring with PHPI resulted from maternal transmission and 6/6 cases with PPHP resulted from paternal transmission. Such effects may be the result of genomic imprinting. Extensive studies in the mouse have led to identification of chromosome regions involved in imprinting.¹⁵ The mouse homologue of $Gs\alpha$ (GNAS) has been localised to distal chromosome 2 (2E1-2H3),¹⁶ a region known to show imprinting effects. In a further series of four families, we have noted phenotype concordance in all nine sibs, as predicted by an imprinting hypothesis (L Wilson, unpublished observations). A single instance of paternal transmission to an offspring with PHPIa has recently been described,¹⁷ but the $Gs\alpha$ mutation in this family has not been characterised.

In view of the paucity of reported male transmissions of AHO, we sought to test these observations in two persons with sporadic PPHP using a coding sequence polymorphism⁷ in order to determine the parental origin of the $Gs\alpha$ disease alleles.

Subjects and methods

PATIENTS

AHO 6 (II-1) (fig 1), is a 14 year old girl with short stocky build (height < 3rd centile, weight 50th centile), a round face, subcutaneous calcification, marked brachymetaphalangism, developmental delay, and grand mal fits. Her only medication is Carbamazepine. She was born after an uneventful pregnancy at 37 weeks' gestation by emergency caesarian section for fetal distress and footling breech presentation and weighed 2100g. The neonatal course was complicated by bilateral pneumothoraces. At 2 weeks she was noted to have

abnormal renal function (urea 6.7 mmol/l, NR 2.8–6; creatinine 122 µmol/l, NR 42–74; glomerular filtration rate 16 ml/min/1.73 m²). Renal imaging by ultrasound, ^{99m}Tc diethylene triamine penta-acetic acid (DTPA) scan, in-

travenous urogram, and cystogram was normal. At 14 years of age, all serum calcium levels have been normal but her renal function remains impaired complicating assessment of her endocrine status (urea 7.7 mmol/l (NR 2.8–6), creatinine 121 µmol/l (NR <102), Ca 2.35 mmol/l (NR 2.22–2.58), Pi 1.01 mmol/l (NR 0.9–1.38), Mg 0.52 mmol/l (NR 0.73–0.99), basal intact PTH 53 ng/l (NR 5–45)). However, thyroid function is normal (T4 78 nmol/l (NR 65–165), TSH 2.9 mU/l (NR 0.5–5)) and intravenous PTH infusion testing at the age of 4 years produced a normal urinary cAMP and phosphaturic response (table). Her four younger sibs are clinically normal. Erythrocyte Gsα bioactivity was reduced to 76% of the normal control compared with 126% and 127% respectively in her clinically normal father and mother. These findings are compatible with a diagnosis of PPHP.

AHO 2 (II.2) (fig 2) is a 22 year old female with short stature (<3rd centile), marked shortening of the fourth and fifth metacarpals and distal first phalanges, but no ectopic calcification. She is normocalcaemic (Ca 2.45 mmol/l (NR 2.22–2.58), Pi 1.08 mmol/l (0.9–1.38), basal intact PTH 10 ng/l (NR 5–45)) with normal urinary cAMP and phosphaturic responses to PTH (table). Currently she is treated with Carbimazole for hyperthyroidism (free T3 16.5 pmol/l (NR <8.6), positive antithyroid microsomal antibody titre 1:100 000). Her erythrocyte Gsα activity was

Figure 1 The upper photograph shows the DGGE analysis of exon 5, identifying the FokI genotype and the sequence variation in subject II.1 from AHO 6. The lower photograph shows the FokI digests on PCR products of the same persons on a 2% agarose gel. The genotypes of the subjects are shown between the photographs. The sequences of the oligonucleotide primers used for amplification of exon 5 are: 5'CGCCCGCCGCGCCCGCGCCCGGC-CCGCGCCCGCCCGAGCCAGTGTCTGTTCCCT-GACCG 3' (forward primer) and 5'CCTATATGGACA-CTGTGCTCAG 3' (reverse primer). Comparison of the two figures showed that homoduplex band 2 arises from the FokI (-) allele and homoduplex band 1 from the FokI (+) allele. The DGGE analysis also showed that the variant pattern in II.1 is present on the paternally derived allele.

Results of PTH infusion tests

Patient	Basal urinary cAMP/Cr	Peak urinary cAMP/Cr	Basal urinary Pi/Cr	Peak urinary Pi/Cr
AHO 6, II.1	0.4	6.4	0.90	1.82
AHO 2, II.2	0.3	21.9	0.65	2.9
AHO 2, III.1	0.67	1.1	-	-

PTH infusion testing was performed using a bolus of synthetic PTH-(1–34) (Rorer pharmaceuticals) at 3 U/kg in 10 ml of diluent (Rorer pharmaceuticals). Basal levels are the mean urinary cAMP (µmol/l) to urinary creatinine (mmol/l) ratios and urinary phosphate (mmol/l) to creatinine ratios for two 30 minute urine samples taken in the hour before administration of PTH. Following PTH infusion urine samples were collected at 30 minute intervals for three hours and the maximum cAMP and phosphate to creatinine ratios achieved are recorded. Normal responses are a greater than six fold rise in cAMP/Cr and three fold rise in Pi/Cr.

Figure 2 Results of DGGE analysis of exon 5 in family AHO 2. Bands 1 and 2 represent the homoduplexes of the (+) and (-) alleles respectively and bands 3 and 4 the heteroduplexes from the (+) and (-) alleles. Filled symbol denotes the PPH1a phenotype and the shaded symbol denotes the PPHP phenotype

reduced to 54%. These data support a diagnosis of PPHP.

Her mother, I-2, is clinically normal with *Gsα* activity of 110%. Her dead father was of normal stature with no known endocrine disorder.

The proband's daughter, III-2, was induced at term. The neonatal period was complicated by the development of necrotising enterocolitis on the second day. Bradycardia was noted soon after birth and proved secondary to hypothyroidism (T4 47 mmol/l (NR 90–195), TSH > 30 mU/l (NR 0.5–6)). Thyroid microsomal antibodies were weakly positive. Transient hypocalcaemia (Ca 1.83 mmol/l (NR 2.2–2.6)) was documented. Now aged 4 years she has a round face, obesity, developmental delay, and brachymetaphalangism but no ectopic calcification. She has subsequently remained normocalcaemic but had a markedly blunted urinary cAMP and phosphate response to intravenous PTH (table). Erythrocyte *Gsα* levels are reduced to 60% of a normal control. These clinical and metabolic features are compatible with a diagnosis of PHPIa. The proband's son, III-1, also presented with hypothyroidism in the neonatal period. At 1 year he has a round face, stocky build, and developmental delay. He has remained normocalcaemic and has not been assessed by PTH infusion testing. His *Gsα* levels are reduced to 64% of a control.

MEASUREMENT OF *GSα* BIOACTIVITY

Two-five ml samples of venous blood in citrate anticoagulant were frozen on dry ice and stored at -70°C . Measurement of erythrocyte *Gsα* bioactivity was by cyc-reconstitution assay as described by Bourne *et al*¹⁸ and membranes were kindly provided by Dr C Van Dop. Purification of [^{32}P]cAMP was as described by Salomon *et al*¹⁹ using Dowex Alumina chromatography and [^3H]cAMP to monitor recovery. Sample eluates were counted in 8 ml of scintillation fluid (Universol ES) in a dual channel scintillation counter. Values are the mean of duplicates corrected for cAMP recovery and expressed as a percentage of the [^{32}P]cAMP production in a concurrent sample from an unrelated healthy control.

PCR AMPLIFICATION OF GENOMIC DNA

Genomic DNA isolated from peripheral leucocytes was used for amplification in a 50 μl reaction mixture containing deoxynucleotides (200 $\mu\text{mol/l}$ each), forward and reverse oligonucleotide primer (1 $\mu\text{mol/l}$ each), 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mmol/l MgCl_2 , and 1 U *Taq* DNA polymerase (Applied Biotechnologies). Amplification consisted of denaturation at 93°C followed by 35 cycles of 93°C for one minute and 60°C for 30 seconds and a final primer extension of two minutes at 70°C . The PCR products were analysed on 2% agarose gels, stained with ethidium bromide, and visualised by UV light. Sequences for the oligonucleotide primers were obtained from L S Weinstein and are mentioned in the legend to fig 1.

DGGE ANALYSIS OF PCR AMPLIFIED DNA FRAGMENTS

PCR amplified DNA fragments were analysed by DGGE as described previously.²⁰ The samples were subjected to electrophoresis for 5.5 hours at 150 V and 60°C in an 8% polyacrylamide gel containing a gradient linearly increasing from 40 to 70% (100% denaturant is 7 mol/l urea and 40% (v/v) formamide). After electrophoresis, the DNA fragments were visualised by ethidium bromide staining.

FokI CODING SEQUENCE POLYMORPHISM ANALYSIS

A *FokI* polymorphism present within exon 5⁷ was confirmed as follows: a 10 μl aliquot of exon 5 amplified product was digested with 0.8 U *FokI* (New England Biolabs) in the recommended buffer for one hour at 37°C and analysed on 2% agarose gels.

DNA SEQUENCING

PCR amplified DNA fragments were sequenced using a PCR based assay. Gel purified fragments were dissolved in 10 μl H_2O . Asymmetrical PCR was performed using 1 μl of the purified fragment, 75 $\mu\text{mol/l}$ deoxynucleotides, and 1 $\mu\text{mol/l}$ forward or reverse primer for 15

Figure 3 Sequence analysis of exon 5 and flanking regions in family AHO 6(A) in I.1, the unaffected father, (B) in II.1, and (C) of the paternally derived allele in II.1. This identified that a G $\rightarrow$ A substitution has arisen at position +1 of intron 5 on the paternally derived allele.

cycles as described, followed by another purification, after which the DNA was dissolved in 5 µl H₂O. The generated template was subsequently used in cycle sequencing, using primers end labelled with [γ -³²P]dATP (Amersham) and DNA *Taq* polymerase.²¹ The products were mixed with an equal volume of formamide loading dye (95% formamide and 20 mmol/l EDTA), heat denatured, and run on an 8% denaturing polyacrylamide gel at 50 W for two to four hours, followed by *x* ray autoradiography.

Results

PARENTAL ORIGIN OF A G→A SUBSTITUTION IN AHO 6

Analysis of PCR amplified products encompassing exon 5 by DGGE detected sequence variation within this exon and genotypes were confirmed by *FokI* restriction analysis. Comparison of DGGE and restriction fragment analyses allowed assignment of homoduplex bands (fig 1). The father (I·1) and mother (I·2) of the proband (II·1) are homozygous negative and positive respectively for the diallelic *FokI* polymorphism. The proband was confirmed by *FokI* restriction fragment analysis to be heterozygous. However, on DGGE analysis she was found to have an altered banding pattern compared with all other heterozygotes indicating the presence of a DNA sequence alteration within this amplified fragment. Furthermore, the absence of a paternally derived homoduplex confirmed that the mutation had arisen on the paternal allele. Direct sequencing of amplified genomic DNA encompassing exon 5 from the patient II·1 showed a heterozygous G→A substitution (fig 3B) which was not present in either parent.

To confirm the parental origin of this Gsα mutation, the PCR amplified exon 5 fragment in II·1 was digested with *FokI*. The minus (−) allele was purified from an agarose gel and sequenced to show the presence of the mutation (fig 3C). The de novo origin of the mutation was confirmed through multilocus DNA fingerprinting for paternity (data not shown). The G→A substitution was not observed in 78 normal chromosomes.

PARENTAL ORIGIN OF THE MUTANT Gsα IN AHO 2

PCR products encompassing exon 5 were subjected to DGGE. The analysis (fig 2) showed the family to be informative for the *FokI* polymorphism. Genotyping of affected subjects II·2, III·1, and III·2 showed cosegregation of the (−) allele with the reduced Gsα activity and the AHO phenotypes indicating that this allele carries the Gsα mutation. The grandmother I·2 is homozygous for the (+) allele hence the mutant allele is grandpaternally derived. Thus within one family a paternal Gsα mutation has resulted in the PPHP phenotype while subsequent maternal transmission has resulted in offspring with PHPIa.

Discussion

This is the first report showing the parental origin of a Gsα gene mutation in sporadic

AHO. This is of particular interest in the light of the proposal that genomic imprinting may explain the alternative phenotypes, PHPIa or PPHP that result from identical Gsα mutations. Only six cases of paternal transmission have previously been reported. This may be because of reduced male fertility or alternatively ascertainment bias should these offspring have normal endocrine function. Under these circumstances, identifying the parental origin of Gsα mutations in sporadic PPHP cases is a valuable approach to testing this apparent parental effect on phenotype.

The proband in family AHO 6 has a classical AHO phenotype. The diagnosis of PPHP is based on her normal response to PTH infusion testing, normocalcaemia, normal thyroid function and reduced Gsα levels. While the presence of renal impairment in this subject would have caused difficulty in interpreting a blunted cAMP and phosphaturic response to exogenous PTH, it does not alter the significance of a normal response. We have identified a heterozygous G→A substitution within the consensus donor splice sequence at position +1 of intron 5 which is likely to result in loss of the donor splice site. In addition, we have shown that this mutation is paternally derived and has arisen de novo since paternity has been confirmed by DNA fingerprinting (data not shown). The *FokI* polymorphism results exclude the possibility of uniparental disomy.

In family AHO 2, subject II·2 has unequivocal PPHP based on her AHO phenotype, normal response to exogenous PTH, and reduction in erythrocyte Gsα levels. While the de novo nature of the AHO cannot be confirmed since her father is dead, an imprinting hypothesis predicts equivalent outcomes from a paternally derived de novo mutation and a paternal transmission. Hyperthyroidism is not a typical feature of AHO and strongly positive antithyroid microsomal antibody titres in II·2 supports a coincidental autoimmune aetiology. Interestingly both her offspring have presented with neonatal hypothyroidism, a feature which has been described before in PHPIa.^{22,23} The diagnosis has been confirmed in III·2 by the blunted response to exogenous PTH and the reduced erythrocyte Gsα levels. Our data show the "switch" in phenotype is dependent on the sex of the transmitting parent across two generations.

There is accumulating evidence that genomic imprinting plays a role in human disease.²⁴ Genomic imprinting of the Gsα gene alone would not explain the clinical observations in AHO as equivalent reductions in Gsα bioactivity are found in PHPIa and PPHP in all tissues tested to date.^{25–30} However, imprinting at a tissue or even cellular specific level could account for these findings. A simple model would require that cells such as renal tubular cells express only the maternal Gsα allele while other cells such as erythrocytes express both alleles. A mutation in the maternal allele would result in renal resistance to PTH. Elsewhere a mutation in either parental allele would result in a 50% reduction in measured Gsα activity, giving rise to the AHO phenotype. A paternal

Gsα mutation would not be expressed in the imprinted cells resulting in no observed PTH resistance and hence PPHP. Of note, the finding of Downs *et al.*³⁰ that *Gsα* levels in the total renal cortical membranes of a patient with PHP1 were 30 to 40% of controls is compatible with a cellular specific imprinting model. Tissue specific imprinting has been reported in the mouse *IGF2* gene where only the paternal allele is expressed in all embryonic tissues except the choroid and leptomeninges where both parental alleles are expressed.³¹

PTH responsive cells suitable for direct testing of a cell specific imprinting hypothesis are not readily accessible and no animal model of AHO has been identified. At present, evidence for imprinting is derived solely from clinical observation of parental effects on phenotype. We have used a molecular genetic strategy to identify two additional instances of paternally derived *Gsα* abnormalities causing PPHP and shown in one of these that subsequent maternal transmission has resulted in PPH1a. Clarification of the nature of any parental effect on phenotype will be of particular significance for accurate genetic counselling in AHO as well as understanding the pathological mechanisms responsible for development of the disorder.

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- Albright F, Burnett CH. Pseudohypoparathyroidism—an example of "Seabright-Bantam syndrome". *Endocrinology* 1942;30:922–32.
- Chase LR, Melson GL, Aurbach GD. Pseudohypoparathyroidism: defective excretion of 3',5'-AMP in response to parathyroid hormone. *J Clin Invest* 1969;48:1832–43.
- Levine MA, Downs RW, Moses AM, *et al.* Resistance to multiple hormones in patients with pseudohypoparathyroidism—association with deficient activity of guanine-nucleotide regulatory protein. *Am J Med* 1983;74:545–56.
- Albright F, Forbes AP, Henneman PH. Pseudo-pseudohypoparathyroidism. *Trans Assoc Am Physicians* 1952;65:337–50.
- Spiegel AM, Shenker A, Weinstein LS. Receptor-effector coupling by G-proteins—implications for normal and abnormal signal transduction. *Endocr Rev* 1992;13:536–65.
- Levine MA, Jap TS, Mauseth RS, Downs RW, Spiegel AM. Activity of the stimulatory guanine nucleotide-binding protein is reduced in erythrocytes from patients with pseudohypoparathyroidism and pseudopseudohypoparathyroidism—biochemical, endocrine, and genetic analysis of Albright hereditary osteodystrophy in 6 kindreds. *J Clin Endocrinol Metab* 1986;62:497–502.
- Gejman PV, Weinstein LS, Martinez M, *et al.* Genetic mapping of the *Gsα* subunit gene (*GNAS1*) to the distal long arm of chromosome 20 using a polymorphism detected by denaturing gradient gel electrophoresis. *Genomics* 1991;9:782–3.
- Kozasa T, Itoh H, Tsukamoto T, Kaziro Y. Isolation and characterization of the human *Gα* gene. *Proc Natl Acad Sci USA* 1988;85:2081–5.
- Weinstein LS, Shenker A. G protein mutations in human disease. *Clin Biochem* 1993;26:333–8.
- Oude Luttikhuis MEM, Wilson LC, Leonard JV, Trembath RC. Characterization of a de novo 43 base pair deletion of the *Gsα* gene in Albright hereditary osteodystrophy. *Genomics* 1994;21:455–7.
- Patten JL, Johns DR, Valle D, *et al.* Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy. *N Engl J Med* 1990;322:1412–19.
- Weinstein LS, Gejman PV, Friedman E, *et al.* Mutations of the *Gα*-subunit gene in Albright hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. *Proc Natl Acad Sci USA* 1990;87:8287–90.
- Miric A, Vechio JD, Levine MA. Heterogeneous mutations in the gene encoding the α -subunit of the stimulatory G protein of adenylate cyclase in Albright hereditary osteodystrophy. *J Clin Endocrinol Metab* 1993;76:1560–8.
- Davies AJ, Hughes HE. Imprinting in Albright's hereditary osteodystrophy. *J Med Genet* 1993;30:101–3.
- Cattanach BM, Kirk M. Differential activity of maternally and paternally derived chromosome regions in mice. *Nature* 1985;315:496–8.
- Ashley PL, Ellison J, Sullivan KA, Bourne HR, Cox DR. Chromosomal assignment of the murine *Gi* and *Gs* genes. *Am J Hum Genet* 1987;41:A155.
- Schuster V, Kress W, Kruse K. Paternal and maternal transmission of pseudohypoparathyroidism type Ia in a family with Albright hereditary osteodystrophy: no evidence of genomic imprinting. *J Med Genet* 1994;31:84.
- Bourne HR, Steinberg F, Golbus MS, Farfel Z. A convenient method for measuring receptor-cyclase coupling activity in whole blood: application to Duchenne muscular dystrophy. *Am J Med Genet* 1983;15:469–74.
- Salomon Y, Londos C, Rodbell M. A highly sensitive adenylate cyclase assay. *Anal Biochem* 1974;58:541–8.
- Myers RM, Maniatis T, Lerman LS. Detection and localization of single base changes by denaturing gradient gel electrophoresis. *Methods Enzymol* 1987;155:501–27.
- Innis MA, Myambo K, Gelfand DH, Brow MAD. DNA sequencing with *thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. *Proc Natl Acad Sci USA* 1988;85:9436–40.
- Weisman Y, Golaner A, Spirer Z, Farfel Z. Pseudohypoparathyroidism type Ia presenting as congenital hypothyroidism. *J Pediatr* 1985;107:413–15.
- Levine MA, Jap TS, Hung W. Infantile hypothyroidism in two sibs: an unusual presentation of pseudohypoparathyroidism type Ia. *J Pediatr* 1985;107:919–22.
- Hall JG. Genomic imprinting—review and relevance to human-diseases. *Am J Hum Genet* 1990;46:857–73.
- Farfel Z, Brickman AS, Kaslow HR, Brothers VM, Bourne HR. Defect of receptor-cyclase coupling protein in pseudohypoparathyroidism. *N Engl J Med* 1980;303:237–42.
- Levine MA, Downs RW Jr, Singer M, Marx SJ, Aurbach GD, Spiegel AM. Deficient activity of guanine nucleotide regulatory protein in erythrocytes from patients with pseudohypoparathyroidism. *Biochem Biophys Res Commun* 1980;94:1319–24.
- Farfel Z, Bourne HR. Deficient activity of receptor-cyclase coupling protein in platelets of patients with pseudohypoparathyroidism. *J Clin Endocrinol Metab* 1980;51:1202–4.
- Bourne HR, Kaslow HR, Brickman AS, Farfel Z. Fibroblast defect in pseudohypoparathyroidism, type-I-reduced activity of receptor-cyclase coupling protein. *J Clin Endocrinol Metab* 1981;53:636–40.
- Farfel Z, Abood ME, Brickman AS, Bourne HR. Deficient activity of receptor-cyclase coupling protein in transformed lymphoblasts of patients with pseudohypoparathyroidism type-I. *J Clin Endocrinol Metab* 1982;55:113–17.
- Downs RW, Levine MA, Drezner MK, Burch WM, Spiegel AM. Deficient adenylate-cyclase regulatory protein in renal membranes from a patient with pseudohypoparathyroidism. *J Clin Invest* 1983;71:231–35.
- DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 1991;64:849–59.