Primary hyperparathyroidism-associated polyostotic fibrous dysplasia: Absence of McCune-Albright syndrome mutations

M.M. Hammami*, A. Al-Zahrani*, AI Butt**, L.J. Vencer**, and S.S. Hussain**

Departments of *Medicine and **Biological and Medical Research, King Faisal Specialist Hospital and Research Centre, Kingdom of Saudi Arabia

ABSTRACT. Several cases of sporadic primary hyperparathyroidism in association with fibrous dysplasia of the bone have been reported in the English literature. Since fibrous dysplasia is a major feature and hyperparathyroidism is occasionally found in the McCune-Albright syndrome, we hypothesized that such cases may represent a variant of this syndrome. A 28-year-old male had primary hyperparathyroidism associated with polyostotic fibrous dysplasia but no other manifestations of the McCune-Albright syndrome. Genomic DNA samples from his parathyroid adenoma, dysplastic bone sample, and peripheral leukocytes were analyzed for the presence of activating mutations of the stimulating G protein α subunit gene (gsp). Allele-specific hybridization revealed the presence of normal sequences only, coding for arginine and glutamine at codons 201 (exon 8) and 227 (exon 9), respectively. Further, single strand conformational analysis of a 224 base pair fragment of exon 8 revealed no conformational aberrations. Furthermore, the sequences of a 164 base pair fragment of exon 8 and a 170 base pair fragment of exon 9 were normal. The results strongly suggest that gsp mutation is absent in affected and normal tissues in this patient and that the association of hyperparathyroidism and fibrous dysplasia may not represent a variant of the McCune-Albright syndrome.

(J. Endocrinol. Invest. *20:* 552-558, 1997)

INTRODUCTION

Fibrous dysplasia is a major feature of the McCune-Albright syndrome (MAS), where it is typically associated with café-au-lait skin lesions, sexual precocity, and hyperfunctioning of any of several endocrine glands including the parathyroid (1-3). Isolated fibrous dysplasia has also been described in association with sporadic primary hyperparathyroidism. Although only up to ten such cases have been reported in the English literature (4-8), it has been suggested that the association is more than expected by chance and may represent a syndrome (6-8).

Recently, MAS has been shown to be associated with early postzygotic mosaic activating mutations in exon 8 (arginine 201 to histidine or cysteine, gsp) of the gene for the stimulatory α subunit of the G protein ($G_s\alpha$) (9-10). The identification of these mutations in affected tissues has permitted the diagnosis of MAS when the overall clinical picture is not typical (11-13).

We hypothesized that the association between sporadic primary hyperparathyroidism and fibrous dysplasia may represent a variant of MAS that may be due to a lower abundance of mutated $G_s\alpha$. We examined the genomic DNA of a new case with such association for activating mutations of $G_s\alpha$ using polymerase chain reaction (PCR)-based techniques.

MATERIALS AND METHODS

Case Report

A 28-year-old Saudi male was referred to King Faisal Specialist Hospital and Research Centre (KFSH & RC) with the complaint of a painless slowly growing left mandibular swelling of eight months' duration. Previously, he had two similar swellings in the right (13 years before) and left (six years before) mandibles. The swellings were partially removed in other hospitals and were reportedly found to be benign bone lesions. Seven years prior to admission he had primary hyperparathyroidism with a calcium level of 4.5 mmol/l, and a PTH level of > 1000 pmol/l (mid region assay; normal range (nl),

Key-words: McCune-Albright syndrome, hyperparathyroidism, fibrous dysplasia, G protein.

Correspondence: Muhammad M. Hammami, M.D., Ph.D., FACP, FACE, Head, Section of Endocrinology, Department of Medicine (MBC-46), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia.

Accepted July 31, 1997.

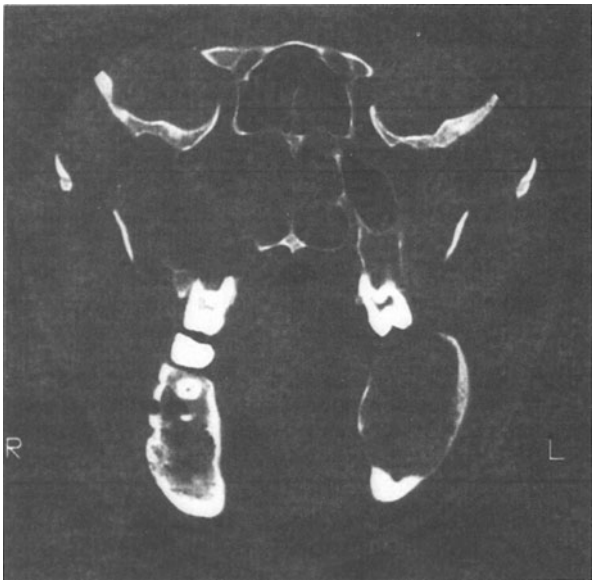

Fig. 1 - *Computed tomography scan of the facial skeleton. There are three bony lesions, one in the right maxilla obliterating the maxillary antrum and reaching the orbital floor and two in the right and left mandibles, respectively. The appearance of the lesions is characteristic of fibrous dysplasia.*

29-85), and he underwent neck exploration and excision of a single parathyroid adenoma. On admission, he had no complaints apart from the left mandibular swelling. He had normal puberty at the age of 14 years. He had no family history of hyperparathyroidism or bone swelling. The physical examination was unremarkable except for a 2 x 2 cm hard non-tender swelling in the left mandible and a similar lesion in the right maxilla. His height was 165 cm and his weight was 80.5 kg. There were no café-au-lait or any other skin lesions.

Laboratory investigations (on two occasions) showed: calcium 2.37 and 2.51 mmol/l (nl, 2.1-2.6); phosphate 1.35 and 1.29 mmol/l (nl, 0.80-1.45); alkaline phosphatase 184 and 174 U/l (nl, 40-135); PTH 50 and 44 pg/ml (nl, 10-65); urinary calcium 3.5 mmol/24 hour (nl, 2.5-6.3); creatinine 95 mmol/l (nl, 40-105); albumin 39 g/l (nl, 35-50); 25 hydroxy vitamin D_3 40 ng/ml (nl, 50-312), TSH 0.99 IU/l (nl, 0.2-5); free T_4 23 pmol/l (nl, 12-28); LH 2.6 IU/l (nl, 0-8); FSH 1.7 IU/l (nl, 0-6); prolactin 21 mg/l (nl, 5.7-25.1); testosterone 28 ng/ml (nl, 10-40); fasting GH <0.5 mU/l (nl, 0-13.5); 8 am cortisol 400 nmol/l (nl, 220-880); and 8 am ACTH 20 pg/ml (nl, 9-52). All laboratory investigations were performed in the clinical laboratory at KFSH&RC using standard methods.

X-rays of the mandibles showed multiple radiolucent lesions in the body and ramus of the right and left mandibles; the largest measuring 2 x 3 x 3 cm with slight expansion of the bone and thinning of the cortex. A skeletal survey showed in addition, a lytic area in the proximal right femur with ground glass appearance, no sclerotic margin, no cortical break, no periosteal reaction and no calcification. There was also similar smaller lesions in left femur, right humerus, left 7-9 ribs and left and right tibia. A CT scan of the facial skeleton (Fig. 1) revealed a large bone lesion coming off the alveolar ridge of the right maxilla, obliterating the maxillary antrum and reaching the orbital floor. A similar expansion was noted in the body of the left mandible extending into the ramus in the molar area and a third smaller expansion was seen in the ramus of the left mandible. The findings were consistent with polyostotic fibrous dysplasia.

Biopsies of the left mandibular and right maxillary lesions were also consistent with fibrous dysplasia showing fibrous tissue stroma with spindle shaped cells; variably sized, irregularly shaped bone spicules; no osteoblast rimming; sparse osteoclasts; and no inflammatory cells or hemosiderin deposits.

He continued to have normal calcium, phosphate and PTH levels seven and a half years after surgery. However, the swellings continued to grow slowly and he underwent resection and reconstruction of the left mandibular body as well as debulking of the right maxillary lesion. The histology was again consistent with fibrous dysplasia. The patient gave informed consent for the following studies.

DNA Preparation

Leukocytes from the patient and from normal controls were isolated by the Ficoll-Hypaque method.

Table 1 - *Sequence of the primers and probes used in the current study.*

Primer and Probes	Sequence 5' ® 3'
Primers of exon 8	
201A	TCGGTTGGCTTTGGTGAATCCA
201B	AGAAACCATGATCTCTGTTATA
201C	CTCTGAGCCCTCTTTCCAAACTAC
Primers for exon 9	
227A	CCAGTCCCTCTGGAATAACCAG
227B	AGCGACCCTGATCCCTAACAAC
Probes	
201Arg	TTCGCTGCCGTGTCCTGACTT
201Cys	TTCGCTGCTGTGTCCTGACTT
201His	TTCGCTGCCATGTCCTGACTT
227Gln	TGGGTGGCCAGCGCGATGAA
227Leu	TGGGTGGCCTGCGCGATGAA
227Arg	TGGGTGGCCGGCGCGATGAA

High-molecular-weight DNA was prepared from leukocytes as previously described (14). Genomic DNA was isolated from tissues embedded in paraffin (15), after localizing the fibrous dysplasia region (left mandible surgical specimen) and the parathyroid adenoma region by microscopic observation of hematoxylin and eosin stained samples.

PCR amplification of genomic DNA

Oligonucleotide primers were synthesized by the phosphoramidite method using an applied Biosystem 380B DNA synthesizer. The sequences of the wild type primers (201A, 201B, 201C, 227A, 227B) have been previously reported (9-10) and are shown in Table 1. Amplification of exons 8 and 9 was carried out separately in 100 ml reaction mixture of the following composition: 1mg DNA, 0.1 mmol/l dNTPs, 50 pmol of each of the primers, 50 mmol/l potassium chloride, 10 mmol/l Tris-HCl pH 8.3, 2.5 mmol/l MgCL2, 0.01 percent gelatin and 2.5 U Taq polymerase. The thermal cycler (Perkin Elemer 480) was programmed as follows: denaturation for one cycle at 94 C for 5 min; 40 cycles of denaturation at 94 C for 1 min, annealing at 58 C for 45 seconds and extension at 72 C for 1 min; and final extension at 72 C for 2 min.

Allele specific hybridization

Slot-blotting was done on Hybond N^+ after denaturing PCR-amplified DNA in 0.4 N NaOH and 25 mmol/l ethylenediamine tetraacetic acid (EDTA). The transferred DNA was cross-linked to the membrane by exposure to UV. Hybridization to sequence specific oligonucleotides (Table 1) to detect arginine, cysteine, or histidine at codon 201 in exon 8; and glutamine, arginine, or leucine at codon 227 in exon 9, was done as previously reported (16).

Single strand conformational analysis

Exon 8 was amplified using primers 201B and 201C (Table 1). The amplified products were migrated on 2% low melting point agarose gel and the band of 224 base pair was excised, purified, and resuspended in 10 mmol/l Tris, 1 mmol/l EDTA (TE). An equal volume of denaturation solution (95% formamide, 0.05% bromophenol and 0.05% xylene cyanole, and 20 mmol/l EDTA) was added to an aliquot of amplified DNA solution and the denaturation was carried out at 100 C for 3 min after which the solution was rapidly chilled. 1 ml of the solution was loaded on a pre-run 8-25% native gradient polyacrylamide gel (Phast Gel, Pharmacia,

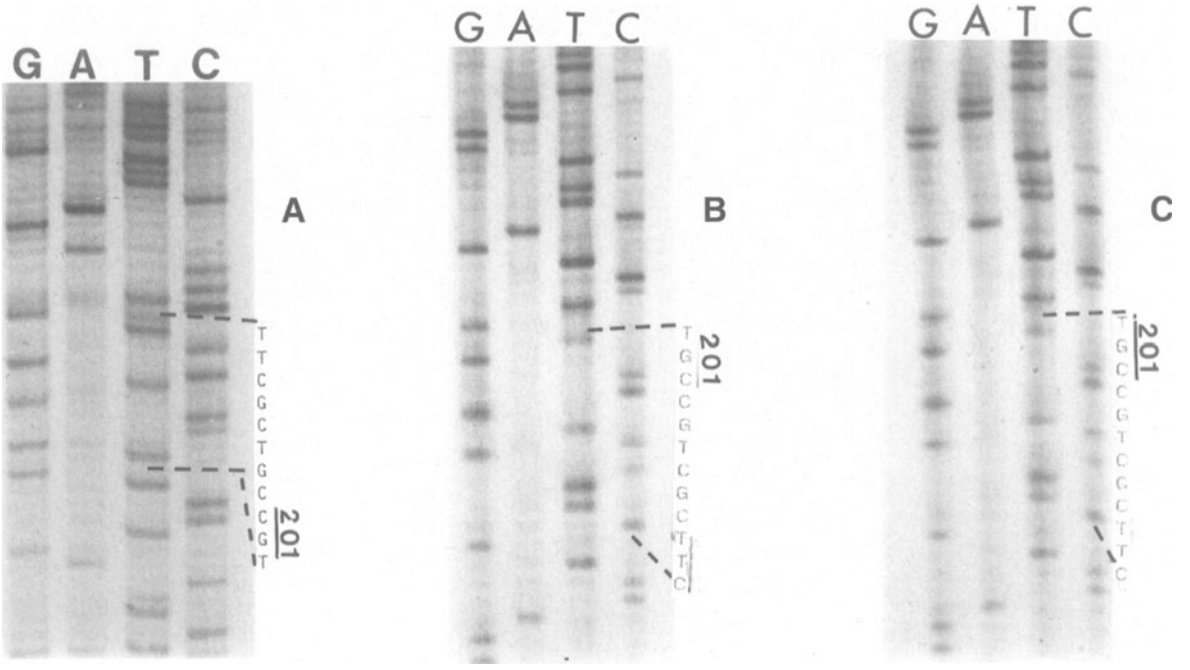

Fig. 2 - *Sequencing of a fragment of exon 8 of $G_s\alpha$ gene. A, B, and C represent the patient's leukocytes (upper strand), parathyroid adenoma (lower strand) and dysplastic bone (lower strand) samples, respectively. There is a normal coding sequence of codon 201 (CGT) in the three samples.*

Uppsala, Sweden). The electrophoresis was done at 280 V, 8 mA, 1.8 W and 10 C for 140 Vh.

Sequencing of amplified DNA

PCR-amplification of exons 8 and 9 was done after phosphorylating one primer of each primer set. The bands corresponding to the amplified products were purified after migrating in 2 percent low melting point agarose gel. The single-stranded templates were prepared by digestion of the phosphorylated strand by lambda endonuclease using a PCR Template Prep kit (Pharmacia, Uppsala). The sequencing reaction was carried out using Sequenase kit (US Biochemicals, Cleveland) and the same primer (0.5 pmol) as was phosphorylated. Thereafter, the sequence was resolved in 8 percent polyacrylamide gel. Both strands were sequenced.

RESULTS

Analysis of codon 201 and exon 8

Codon 201 in exon 8 of $G_s\alpha$ codes for arginine in the wild state and for cysteine or histidine in the reported mutated states in MAS (9-13,17). Using primers 201A and 201B (Table 1), a 164 base pair fragment of exon 8 containing codon 201 was PCR-amplified from genomic DNA obtained from the parathyroid adenoma, dysplastic bone sample, and peripheral leukocytes of the patient (but not from a blank control). The amplified DNA as well as positive controls for cysteine 201 and histidine 201 mutations were analyzed by allele specific hybridization technique using probes (Table 1) specific for arginine (201Arg), cysteine (201Cys), or histidine (201His) at codon 201, respectively. Slot blots revealed hybridization of the three patient's samples to the arginine probe only (data not shown). The 164 base pair fragment of exon 8 (from the three samples) was also sequenced and found to be normal (Fig. 2). An overlapping 224 base pair fragment of exon 8 was PCR-amplified using primers 201B and 201C (Table 1) and subjected to single strand conformational analysis. This also showed no conformational aberrations to indicate base changes (data not shown).

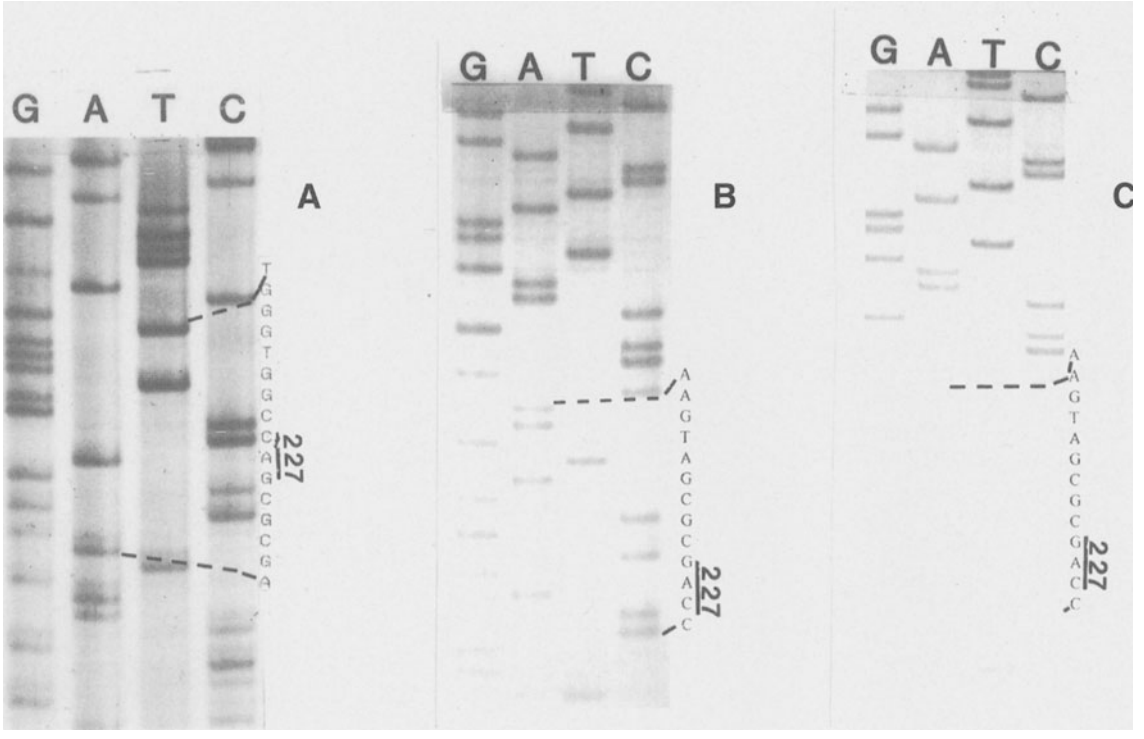

Fig. 3 - *Sequencing of a fragment of exon 9 of $G_s\alpha$ gene. A, B, and C represent the patient's leukocytes (upper strand), parathyroid adenoma (lower strand) and dysplastic bone (lower strand) samples, respectively. There is a normal coding sequence of codon 227 (CAG) in the three samples.*

Analysis of codon 227 and exon 9

Since mutations of codon 227 can also cause constitutive activation of $G_s\alpha$ (18), that theoretically may result in MAS like phenotype, we also analyzed codon 227 for such mutations. Using primers 227A and 227B (Table 1), a 170 base pair fragment of exon 9 containing codon 227 was PCR-amplified from genomic DNA obtained from the three tissue samples of the patient (but not from a blank control). Allele specific hybridization analysis of the three patient's samples as well as positive controls for wild type glutamine 227, arginine 227 mutation, or leucine 227 mutation, using probes (Table 1) specific for glutamine (227Gln), arginine (227Arg), or leucine (227Leu) at codon 227 revealed the presence of only glutamine 227 in all patient's samples (data not shown). The 170 base pair fragment of exon 9 (from the three samples) was also sequenced and found to be normal (Fig. 3).

DISCUSSION

Although it is difficult to differentiate the bone lesions of fibrous dysplasia from those of primary hyperparathyroidism (7, 8), it is clear that the bone lesions in our patient represent fibrous dysplasia and are not secondary to primary hyperparathyroidism. They developed years before the diagnosis of hyperparathyroidism and continued to occur years after curative parathyroidectomy. Further, bone biopsies showed no osteoblast rimming or hemosiderin deposits and only sparse osteoclasts, features that are rather atypical for brown tumors (7). To our knowledge, only 8-10 cases (2 cases may have been reported twice) of sporadic primary hyperparathyroidism in association with fibrous dysplasia have been previously reported (4-8). However, the prevalence of primary hyperparathyroidism in patients with fibrous dysplasia has been estimated to be 5% to 10% (6). On the other hand, in a retrospective review of 350 cases of primary hyperparathyroidism, five patients were found to have fibrous dysplasia (8). These and other studies suggested that this association is more than expected by chance and that it may represent a syndrome (6-8).

The McCune-Albright syndrome (MAS), classically the trio of polyostotic fibrous dysplasia, café-au-lait pigmentation and precocious puberty, has been estimated to be present in one of 30 to 40 cases of polyostotic fibrous dysplasia (19). The spectrum of this syndrome has been recently expanded to include milder cases, such as nodular adrenal hyperplasia in association with ostotic fibrous dysplasia (12), and more severe cases, such as hyperactivity of up to four different endocrine glands in the same patient (3), as well as hepatobiliary disease, cardiac disease, and other non-endocrine abnormalities (11). Activating mutations in the gene encoding the α subunit of G_s have been found in variable abundance in multiple tissues from several MAS patients (9-13,17). In all but one reported cases of MAS (20), there were missense mutations encoding the substitution of arginine 201 residue in exon 8 with either cysteine or histidine (9-13,17). This substitution inhibits the intrinsic guanosine triphosphatase (GTPase) activity of $G_s\alpha$ and results in prolonged stimulation of adenylyl cyclase activity (21). Activating mutations of codon 227 of exon 9 of $G_s\alpha$ that may also inhibit the GTPase activity, and thus could theoretically result in a phenotype that resembles MAS, have been described in several endocrine tumors (22) but not in association with MAS.

We examined whether the association of hyperparathyroidism and polyostotic fibrous dysplasia in our patient may represent a variant of MAS that may be due to a low abundance of a mutation that affects GTPase activity. We were unable to detect such a mutation in normal or affected patient's tissue samples. Allele specific hybridization was negative for cysteine 201 and histidine 201 mutations. Further, the normal sequence of a 164 base pair fragment (and the normal single strand conformational analysis of a 224 base pair fragment) of exon 8 that spans codon 201 did not support the possible presence of other mutations in this fragment. Moreover, allele specific hybridization and sequencing of a 170 base pair fragment of exon 9 were negative for codon 227 (and other) mutations. Although we did not examine the full sequence of $G_s\alpha$, these results strongly suggest that the association of primary hyperparathyroidism and polyostotic fibrous dysplasia in our patient does not represent a variant of MAS. To our knowledge, the presence of gsp mutations has not been studied in the other reported cases of primary hyperparathyroidism-associated polyostotic fibrous dysplasia. Whether this association represents a syndrome that resembles MAS, an unrelated syndrome, or just a chance occurrence needs further study. In this regard, an atypical case of MAS has been recently reported in which gsp mutations are absent and $G_s\alpha$ function is normal (20). In addition, an autosomal dominant syndrome (Hereditary Hyperparathyroidism - Jaw Tumor Syndrome) of recurrent parathyroid adenomas and fibro-osseous tumors restricted to the mandible and/or maxilla with or without Wilms tumor and parathyroid carcinoma has been described in 8 families (23-24) and its candidate gene has been recently mapped to the long arm of chromosome 1 (25).

Finally, the rare association of fibrous dysplasia of the bone and primary hyperparathyroidism should be recognized as such and differentiated from recurrent/persistent hyperparathyroidism especially with the high prevalence of elevated PTH levels after apparently curative parathyroidectomy (26,27).

ACKNOWLEDGMENTS

We thank Ms. Judith Lansi Sosa for her excellent secretarial assistance in the preparation of the manuscript.

REFERENCES

1. Mauras N., Blizzard R.M.
The McCune-Albright syndrome.
Acta Endocrinol. (Copenh.) *279:* 207, 1986.
2. Levine M.A.
The McCune-Albright syndrome. The whys and wherefore of abnormal signal transduction.
N. Engl. J. Med. *325:* 1738, 1991.
3. Cavanah S.F.W., Dons R.F.
McCune-Albright syndrome: How many endocrinopathies can one patient have.
South. Med. J. *86:* 364, 1993.
4. Benedict P.H.
Endocrine features in Albright's syndrome (Fibrous dysplasia of bone).
Metabolism *11:* 30, 1962.
5. Firat D., Stutzman L.
Fibrous dysplasia of the bone.
Am. J. Med. *44:* 421, 1968.
6. Ehrig U., Wilson D.R.
Fibrous dysplasia of bone and primary hyperparathyroidism.
Ann. Intern. Med. *77:* 234, 1972.
7. Caudill R., Saltzman D., Gaum S., Granite E.
Possible relationship of primary hyperparathyroidism and fibrous dysplasia: Report of case.
J. Oral Surg. *35:* 483, 1977.
8. Rosen I.B., Palmer J.A.
Fibroosseous tumors of the facial skeleton in association with primary hyperparathyroidism: an endocrine syndrome or coincidence?
Surgery *142:* 494, 1981.
9. Weinstein L.S., Shenker A., Gejman P.V., Merino M.J., Friedman E., Spiegel A.M.
Activating mutations of the stimulatory G protein in the McCune-Albright syndrome.
N. Engl. J. Med. *325:* 1688, 1991.
10. Schwindinger W.F., Francomano C.A., Levine M.A.
Identification of a mutation in the gene encoding the alpha subunit of the stimulatory G protein of adenylyl cyclase in McCune-Albright syndrome.
Proc. Natl. Acad. Sci. USA *89:* 5152, 1992.
11. Shenker A., Weinstein L.S., Moran A., Pescovitz O.H., Charest N.J., Boney C.M.,Van Wyk J.J., Merino M.J., Feuillan P.P. and Spiegel A.M.
Severe endocrine and non-endocrine manifestations of the McCune-Albright syndrome associated with activating mutations of stimulatory G protein G_s.
J. Pediatr. *123:* 509, 1993.
12. Boston B.A., Mandel S., LaFranchi S., Bliziotes M.
Activating mutation in the stimulatory guanine nucleotide-binding protein in an infant with Cushing's syndrome and nodular adrenal hyperplasia.
J. Clin. Endocrinol. Metab. *79:* 890, 1994.
13. Malchoff C.D., Reardon G., MacGillivray D.C., Yamase H., Rogol A.D., Malchoff D.M.
An unusual presentation of McCune-Albright syndrome confirmed by an activating mutation of the G_s alpha-subunit from a bone lesion.
J. Clin. Endocrinol. Metab. *78:* 803, 1994.
14. Miller S.A., Dykes D.D., Polesky H.F.
A simple salting out procedure for extracting DNA from human nucleated cells.
Nucleic Acids Res. *16:* 1215, 1988.
15. Wright D.K., Manos M.M.
Sample preparation from paraffin-embedded tissues
In: Innis M.A. et al (Eds), PCR Protocols: A guide to methods and applications.
Academic Press, London, 1989, p. 153.
16. Lyon J., Landis C.A., Harsh G., Vallar L., Grunewald K., Feichtinger H., Duh Q.Y., Clark O.H., Kawasaki E., Bourne H.R., McCormick F.
Two G Protein Oncogenes in human endocrine tumors.
Science *249:* 655, 1990.
17. Shenker A., Weinstein L.S., Sweet D.E., Spiegel A.M.
An activating G_s alpha mutation is present in fibrous dysplasia of bone in McCune-Albright syndrome.
J. Clin. Endocrinol. Metab. *79:* 750, 1994.
18. Spiegel A.M., Shenker A., Weinstein L.S.
Receptor-effector coupling by G proteins: Implications for normal and abnormal signal transduction.
Endocr. Rev. *13:* 536, 1992.
19. Olmsted W.W.
Some skeletogenic lesions with common calvarial manifestations.
Radiol. Clin. North Am. *19:* 703, 1981.
20. Gessl A., Freissmuth M., Czech T., Matula C., Hainfellner J.A., Buchfelder M., Vierhapper H.
Growth hormone-prolactin-thyrotropin-secreting pituitary adenoma in atypical McCune-Albright syndrome with functionally normal G_s alpha protein.
J. Clin. Endocrinol. Metab. *79:* 1128, 1994.
21. Neer E.J., Clapham D.E.
Roles of G protein subunits in transmembrane signalling.
Nature *333:* 129, 1988.
22. Yoshimoto K., Iwahana H., Fukuda A., Sano T., Itakura M.
Rare mutations of the G_s alpha subunit gene in hu-

man endocrine tumors. Mutation detection by polymerase chain reaction-primer-introduced restriction analysis.
Cancer *72:* 1386,1993.

23. Jackson C.E., Norum R.A., Boyd S.B., Talpos G.B., Wilson S.D., Taggart R.T., Mallette L.E.
Hereditary hyperparathyroidism and multiple ossifying jaw fibromas: A clinically and genetically distinct syndrome
Surgery *108:* 1006, 1990.

24. Kakinuma A., Morimoto I., Nakano Y., Fujimoto R., Ishida O., Okada Y., Inokuchi N., Fujihira T., Eto S.
Familial primary hyperparathyroidism complicated with Wilms' tumor.
Intern. Med. *33:* 123, 1994.

25. Szabo J., Heath B., Hill V.M., Jackson C.E., Zarbo R.J., Mallette L.E., Chew S.L., Besser G.M.., Thakker R.V., Huff V., Leppert M.F., Heath III H.
Hereditary Hyperparathyroidism - Jaw Tumor Syndrome: The Endocrine Tumor Gene HRPT2 Maps to Chromosome I q21-q31.
Am. J. Hum. Genet. *56:* 944, 1995

26. Duh Q-Y., Arnaud C.D., Levin K.E., Clark O.H.
Parathyroid hormone: Before and after parathyroidectomy.
Surgery *100:* 1021, 1986.

27. Irvin III G.L., Newell D.J., Morgan S.D.
Parathyroid metabolism after operative treatment of hypercalcemic (primary) hyperparathyroidism.
Surgery *102:* 898, 1987.