

ORIGINAL ARTICLE

Somatic and Germ-Line Mutations of the HRPT2 Gene in Sporadic Parathyroid Carcinoma

Trisha M. Shattuck, B.S., Stiina Välimäki, M.D., Takao Obara, M.D., Randall D. Gaz, M.D., Orlo H. Clark, M.D., Dolores Shoback, M.D., Margaret E. Wierman, M.D., Katsuyoshi Tojo, M.D., Christiane M. Robbins, M.S., John D. Carpten, Ph.D., Lars-Ove Farnebo, M.D., Ph.D., Catharina Larsson, M.D., Ph.D., and Andrew Arnold, M.D.

ABSTRACT

BACKGROUND

From the Center for Molecular Medicine (T.M.S., A.A.) and the Division of Endocrinology and Metabolism (A.A.), University of Connecticut School of Medicine, Farmington; the Departments of Molecular Medicine (S.V., C.L.) and Surgical Sciences (S.V., L.-O.F.), Karolinska Hospital, Stockholm, Sweden; the Department of Endocrine Surgery, Tokyo Women's Medical University, Sinjuku-ku, Tokyo, Japan (T.O.); the Department of Surgery, Massachusetts General Hospital, Boston (R.D.G.); the Department of Surgery, University of California, San Francisco, Mt. Zion Medical Center, San Francisco (O.H.C.); the Endocrine Research Unit, Veterans Affairs Medical Center, University of California, San Francisco, San Francisco (D.S.); the Division of Endocrinology, University of Colorado, Veterans Affairs Medical Center, Denver (M.E.W.); the Division of Diabetes and Endocrinology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan (K.T.); and the Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Md. (C.M.R., J.D.C.). Address reprint requests to Dr. Arnold at the Center for Molecular Medicine, University of Connecticut School of Medicine, 263 Farmington Ave., Farmington, CT 06030-3101, or to Dr. Larsson at catharina.larsson@cmm.ki.se.

Ms. Shattuck and Dr. Välimäki contributed equally to this article.

N Engl J Med 2003;349:1722-9.

Copyright © 2003 Massachusetts Medical Society.

We looked for mutations of the *HRPT2* gene, which encodes the parafibromin protein, in sporadic parathyroid carcinoma because germ-line inactivating *HRPT2* mutations have been found in a type of familial hyperparathyroidism — hyperparathyroidism–jaw tumor (HPT-JT) syndrome — that carries an increased risk of parathyroid cancer.

METHODS

We directly sequenced the full coding and flanking splice-junctional regions of the *HRPT2* gene in 21 parathyroid carcinomas from 15 patients who had no known family history of primary hyperparathyroidism or the HPT-JT syndrome at presentation. We also sought to confirm the somatic nature of the identified mutations and tested the carcinomas for tumor-specific loss of heterozygosity at *HRPT2*.

RESULTS

Parathyroid carcinomas from 10 of the 15 patients had *HRPT2* mutations, all of which were predicted to inactivate the encoded parafibromin protein. Two distinct *HRPT2* mutations were found in tumors from five patients, and biallelic inactivation as a result of a mutation and loss of heterozygosity was found in one tumor. At least one *HRPT2* mutation was demonstrably somatic in carcinomas from six patients. Unexpectedly, *HRPT2* mutations in the parathyroid carcinomas of three patients were identified as germ-line mutations.

CONCLUSIONS

Sporadic parathyroid carcinomas frequently have *HRPT2* mutations that are likely to be of pathogenetic importance. Certain patients with apparently sporadic parathyroid carcinoma carry germ-line mutations in *HRPT2* and may have the HPT-JT syndrome or a phenotypic variant.

PARATHYROID CARCINOMAS ARE AN UNCOMMON and often devastating cause of primary hyperparathyroidism.^{1,2} These cancers characteristically result in more profound clinical manifestations of hyperparathyroidism than do parathyroid adenomas, the most frequent cause of primary hyperparathyroidism. If a parathyroid carcinoma spreads to distant sites, it can cause relentless hypercalcemia and severe metabolic complications that are notoriously difficult to control and often result in death. Affected patients may require repeated palliative surgical extirpation of metastatic nodules.¹⁻³ Early en bloc resection of the primary tumor is the only curative treatment. Because the histopathological features of parathyroid carcinoma and adenoma may overlap, a definitive diagnosis of parathyroid carcinoma requires the presence of invasion of surrounding structures by the tumor, local recurrence, or metastasis,³⁻⁹ yet these features signify a stage at which cure is usually impossible. An understanding of the molecular pathogenesis of parathyroid carcinoma could have considerable value with respect to early diagnosis, prognosis, and new approaches to treatment.

No specific gene has been established as a direct contributor to the pathogenesis of sporadic parathyroid carcinoma, although several important molecular clues have been uncovered.¹⁰⁻¹⁵ For example, a region on chromosome 13 is frequently lost in parathyroid carcinomas.¹⁰⁻¹⁴ However, molecular analysis¹⁶ has not yet established the identity of the relevant gene or genes in this region of the chromosome.^{17,18}

We investigated the HRPT2 gene, which encodes the parafibromin protein, in sporadic parathyroid carcinoma because inactivating germ-line mutations in this gene were recently identified in the majority of kindreds with the hereditary hyperparathyroidism–jaw tumor (HPT-JT) syndrome, or hyperparathyroidism 2 (Online Mendelian Inheritance in Man number #145001), a rare autosomal dominant cause of parathyroid tumors, ossifying fibromas of the mandible and maxilla, and various cystic and neoplastic renal abnormalities.¹⁹⁻²⁶ Also, somatic inactivating mutations of the gene were reported in 4 percent of cystic parathyroid adenomas (2 of 47), none of which had germ-line mutations.¹⁹ Parathyroid tumors often occur asynchronously in patients with the HPT-JT syndrome, and although most of the tumors are benign, the incidence of malignant parathyroid carcinomas is markedly in-

creased in these patients. For these reasons, it seemed plausible that inactivating somatic mutations of HRPT2 might occur in sporadic parathyroid carcinomas.

METHODS

PATIENTS AND TUMOR SPECIMENS

A total of 21 parathyroid-carcinoma samples were obtained from 15 patients who had been treated surgically for primary hyperparathyroidism in the United States and Japan. The 21 specimens included 5 primary carcinomas, 6 locally recurrent tumors, and 10 distant metastases (Table 1). For inclusion in this study, we required an unequivocal diagnosis of parathyroid carcinoma, as demonstrated by the presence of either distant metastasis or widespread invasion of contiguous structures and local recurrence.³⁻⁹ Histopathological features often associated with parathyroid carcinoma (but not stringently diagnostic), such as fibrous bands, numerous cells in mitosis, trabecular cellular architecture, nuclear atypia, and microvascular or microscopic capsular invasion, were commonly present. For 10 of the 15 patients, peripheral-blood leukocytes served as a source of germ-line DNA; for Patient 6, normal muscle was the source. Germ-line DNA was not available from four patients.

At initial parathyroidectomy, the 15 patients ranged in age from 20 to 62 years (mean, 43); 5 were women and 10 were men. None of the 15 patients had a personal or family history of the HPT-JT syndrome, multiple endocrine neoplasia type 1, or another familial form of hyperparathyroidism at presentation. After the diagnosis of parathyroid carcinoma in Patient 5, a parathyroid adenoma was diagnosed in an uncle. No patient had a history of irradiation to the head and neck or of uremic secondary or tertiary hyperparathyroidism, and no patient had been treated with radiation therapy or chemotherapy.

Immediately after surgical resection, tumor samples were frozen in liquid nitrogen for storage at -80°C until use. Genomic DNA was extracted from tumor and nontumor tissues by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. Tumor and blood samples were obtained in accordance with protocols approved by institutional review boards for human studies; patients provided informed consent as dictated by these protocols.

Table 1. HRPT2 Gene Mutation and Loss-of-Heterozygosity Analyses in Parathyroid Carcinomas from 15 Patients.*

Patient No. and Tissue Analyzed	HRPT2 Mutational Status†	Level of HRPT2 Mutation	Effect on Parafibromin‡	LOH at HRPT2§
1 Primary tumor	82del4 in exon 1 732delT in exon 8	Somatic Somatic	Frame shift at amino acid 28; stop codon at 35 Frame shift at amino acid 244; stop codon at 256	No
2 Metastasis	70G>T in exon 1 746delT in exon 8	Somatic Somatic	Stop codon E24X Frame shift at amino acid 249; stop codon at 256	No
3 Metastasis	226C>T in exon 2	Somatic	Stop codon R76X	No
4 Local recurrence	39delC in exon 1	Somatic¶	Frame shift at amino acid 13; stop codon at 20	Yes¶
5 Primary tumor Metastasis	23TGCG>GTG in exon 1 23TGCG>GTG in exon 1	Somatic Somatic	Frame shift at amino acid 8; stop codon at 20 Frame shift at amino acid 8; stop codon at 20	No No
6 Metastasis	664C>T in exon 7	Germ line	Stop codon R222X	Results uninformative
7 Primary tumor Local recurrence	373insA in exon 5 373insA in exon 5	Germ line Germ line	Frame shift at amino acid 125; stop codon at 130 Frame shift at amino acid 125; stop codon at 130	No No
8 Local recurrence	679insAG in exon 7 162C>G in exon 2	Germ line Somatic	Frame shift at amino acid 227; stop codon at 257** Stop codon Y54X	No
9 Primary tumor	16delA in exon 1 1230delC in exon 14	ND ND	Frame shift at amino acid 6; stop codon at 20 Frame shift at amino acid 410; stop codon at 427	ND
10 Local recurrence	60del10 in exon 1 700C>T in exon 7	ND ND	Frame shift at amino acid 20; stop codon at 22 Stop codon R234X	ND
11 Local recurrence	Wild type	—	—	ND
12 Metastasis 1 Metastasis 2 Metastasis 3 Metastasis 4	Wild type Wild type Wild type Wild type	— — — —	— — — —	No No No No
13 Primary tumor Local recurrence	Wild type Wild type	— —	— —	No No
14 Metastasis	Wild type	—	—	No
15 Metastasis	Wild type	—	—	ND

* ND denotes not determinable owing to the unavailability of germ-line DNA.

† Nucleotides are numbered from the initiation codon.

‡ For each frame shift, the amino acid position of the insertion or deletion is shown (numbered from the initiation codon), as is the position of the first premature stop codon in the altered reading frame.

§ A tumor scored positive for loss of heterozygosity (LOH) if either HRPT2 intragenic marker showed a loss of heterozygosity.

¶ The HRPT2 mutation plus loss of heterozygosity were documented to affect both HRPT2 alleles.

|| There was constitutional homozygosity.

**This is the predicted effect if there is no change in normal splicing between exons 7 and 8. The stop codon is located within exon 8.

DETECTION OF HRPT2 MUTATIONS

The 21 samples of parathyroid-carcinoma DNA were analyzed for mutations in the HRPT2 gene by direct sequencing of both strands. The 17 exons of the gene, which encode a protein of 531 amino acids, were amplified as 15 different fragments with the use of primers derived from flanking intronic or 3'- or 5'-untranslated-region sequences (listed in Supplementary Appendix 1, available with the full text of this article at <http://www.nejm.org>). After amplification, primers were removed by digestion with 10 U of exonuclease I (Amersham Pharmacia Biotech) and 1 U of shrimp alkaline phosphatase (Amersham).

Sequencing reactions were performed with the use of the BigDye Terminator cycle sequencing kit (Applied Biosystems) as described previously.²⁷ Data were analyzed with the use of Sequencing Analysis and AutoAssembler software (Applied Biosystems), and all mutations were confirmed by repeated forward and reverse sequencing of the involved exon or intron from an independent polymerase chain reaction (PCR). When mutations were detected in tumor DNA, the same exons or introns in corresponding germ-line DNA samples, when available, were sequenced in a similar manner. Amplified exons for which sequencing in both directions showed a clear chromatogram and an apparently normal sequence on one side of a specific nucleotide position abruptly followed by an unclear sequence on the other were interpreted as suggesting a frame-shift mutation. To determine definitively whether an insertion or a deletion was present in these cases, or to confirm the loss of a specific allele in one instance, amplified exons were resequenced after cloning to separate the alleles. PCR products were cloned into the PCR4-TOPO vector with use of the TOPO TA Cloning Kit (Invitrogen), transformed into *Escherichia coli*, and plated onto LB agar to which 50 µg of ampicillin per milliliter had been added. Then, 8 to 10 distinct colonies were picked and resuspended in PCR mix for amplification and sequencing.

Sequencing of all HRPT2 exons (1, 2, 3, 4, 5, 7, and 14) for which germ-line mutations were identified in this study (or in kindreds with the HPT-JT syndrome¹⁹) was performed as described previously¹⁹ in 150 unrelated healthy control subjects.

LOSS-OF-HETEROZYGOSITY ANALYSIS

We analyzed 17 matched pairs of germ-line and tumor DNA samples from 11 patients for loss of het-

erozygosity at the HRPT2 locus by genotyping four microsatellite markers. D1S542 and D1S413 flank HRPT2 on its centromeric and telomeric sides, respectively (University of California Santa Cruz Human Genome Project Working Draft, available at <http://genome.ucsc.edu>). We also searched the human-genome-sequence data base for previously unreported dinucleotide repeat sequences within HRPT2 that might serve as the basis for new intragenic HRPT2 polymorphisms, identified two such regions within intron 10 and intron 14, and designed primers from unique flanking sequences for PCR amplification: 5'TGATTCTCATGCATTTCCTG3' (intron 10 forward primer), 5'TAACTACCTGAAACCCATCAC3' (intron 10 reverse primer), 5'AATTAGTGTCACAGTATCTTA3' (intron 14 forward primer), and 5'CTCAAAGTATCTATTAGGTA3' (intron 14 reverse primer). These new intragenic markers were highly polymorphic, showing substantial frequencies of heterozygosity in our patients: 60 percent for intron 10 and 50 percent for intron 14. Electrophoresis of fluorescently labeled products in an ABI Prism 377 Sequencer was followed by pattern analysis with the use of Genescan and Genotyper software (Applied Biosystems).²⁷ An allelic imbalance was identified by an analysis of the heights of allele peaks in tumor and control samples²⁷ and was considered to indicate a loss of heterozygosity when the contribution of the minority allele was repeatedly negligible.

RESULTS

We identified HRPT2 mutations in parathyroid carcinomas from 10 of 15 patients with apparently sporadic disease. All mutations were predicted to inactivate the encoded protein, parafibromin, which is also affected in the HPT-JT syndrome.¹⁹ A total of 15 different HRPT2 mutations, spanning six exons, were identified in 12 tumors from 10 of the 15 patients (Table 1). Five mutations resulted directly in a premature stop codon, and 10 gave rise to an altered reading frame, typically with an early stop codon also following shortly in the altered frame (Table 1). Testing of germ-line DNA showed that eight HRPT2 mutations (from six patients) were somatic, and two distinct somatic mutations were found in tumors from two of these patients. Two mutations were also found in each tumor from two additional patients, but it was not possible to determine the somatic or germ-line status of these mutations.

Unexpectedly, HRPT2 mutations found in the parathyroid carcinomas of three patients were identified as germ-line mutations (Table 1 and Fig. 1), even though none of these patients had a known family history of the HPT-JT syndrome or presented with clinical evidence thereof. None of these germ-line mutations were present in 150 control subjects, nor were other mutations found in the sequenced exons. Two of these germ-line mutations (664C>T and 373insA) were not reported in a previous study of kindreds with the HPT-JT syndrome,¹⁹ whereas one (679insAG) was.¹⁹ Patient 8 had a germ-line mutation in one allele of HRPT2 and a tumor-specific somatic HRPT2 mutation in the other allele (Table 1).

The availability of germ-line DNA from 11 of the 15 patients allowed examination of their tumors for loss of heterozygosity within or near HRPT2. Loss of heterozygosity was identified in one carcinoma, from Patient 4 (Table 1 and Fig. 1B). Parathyroid carcinomas from 6 of the 10 patients whose tumors revealed mutations had two HRPT2 lesions (Table 1). In tumor tissue from Patient 4, one allele contained a somatic frame-shift mutation and the other was deleted (Table 1 and Fig. 1B); each of the other five tumors had two distinct intragenic HRPT2 mutations (Table 1 and Fig. 1).

In the four instances in which more than one tumor sample was available from a single patient, representing primary tumor plus a local recurrence or a metastasis or multiple metastases, all samples had the same HRPT2 gene status (Table 1). These patients included two (Patients 5 and 7) for whom the identical somatic mutation was present in both primary tumor and a metastasis or a local recurrence (Table 1).

DISCUSSION

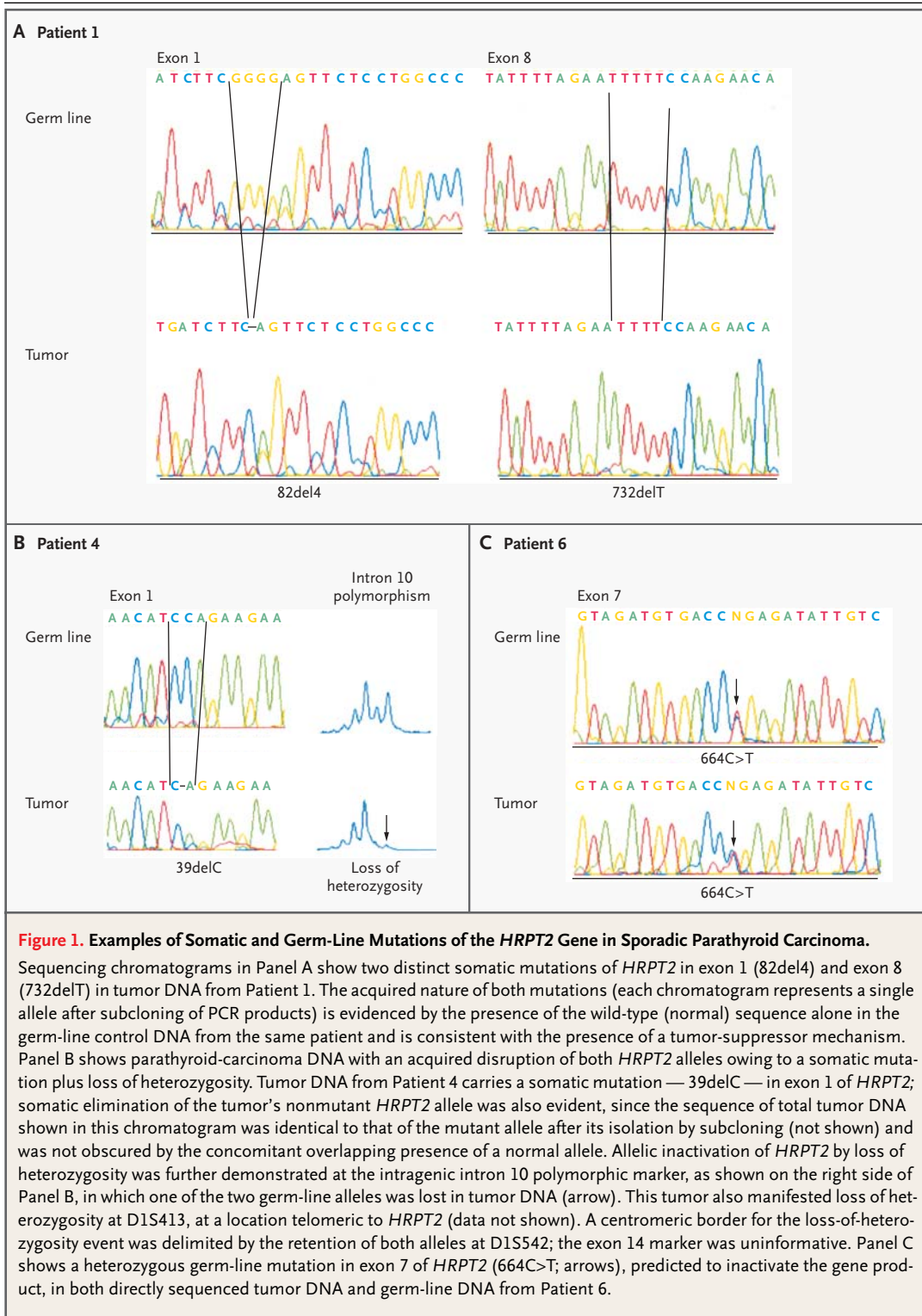
There has been considerable progress in elucidating the pathogenesis of sporadic parathyroid adenomas,^{19,28,29} but the molecular roots of parathyroid carcinoma are obscure. The identification of mutations in HRPT2 in patients with the HPT-JT syndrome, in which parathyroid carcinoma is over-represented despite the more common presence of benign parathyroid tumors, led us to evaluate whether HRPT2 is involved in sporadic parathyroid carcinoma. We found mutations of the HRPT2 gene in sporadic parathyroid carcinomas from 10 of 15 patients. The demonstration of tumor-specific, ac-

quired HRPT2 mutations in multiple parathyroid carcinomas marks these tumors as clonal expansions, each derived from an original cell that had undergone HRPT2 mutation and had gained a selective advantage. Therefore, HRPT2 mutation is very likely an important contributor to the pathogenesis of parathyroid carcinoma. Consistent with this conclusion are the findings of unexpected germ-line mutations in HRPT2 in certain patients with apparently sporadic parathyroid carcinoma. The concordant HRPT2 status among tumors in patients who had more than one sample available for analysis is consistent with the concept that HRPT2 mutation may influence the phenotype of parathyroid carcinoma, including its metastatic potential, at an early stage of tumorigenesis. The likelihood that the observed mutations inactivated the HRPT2 gene product and the finding that multiple parathyroid carcinomas each contained such distinct inactivating HRPT2 lesions indicate that a tumor-suppressor gene mechanism¹⁶ is involved in HRPT2's contribution to tumorigenesis.

Atypical parathyroid adenomas and parathyromatosis³⁰ are lesions that share some phenotypic features with parathyroid carcinoma but fail to fulfill rigorous criteria for cancer; study of their HRPT2 status should help clarify the extent to which they resemble parathyroid carcinoma on a molecular level. The mechanisms of action of parafibromin — the protein encoded by HRPT2 — in cell physiology and tumor suppression are unknown. Nonetheless, our findings indicate that parafibromin is a potential target for new therapeutic agents that could benefit patients with parathyroid carcinoma.

Patients with apparently sporadic parathyroid carcinoma who carry germ-line mutations in HRPT2 may, on further investigation of their clinical features and relatives, turn out to have the HPT-JT syndrome^{31,32} or phenotypic variants of the syndrome, perhaps with altered penetrance of the mutation. Two of the germ-line mutations we identified (664C>T and 373insA) were not reported in a previous study of kindreds with the HPT-JT syndrome,¹⁹ whereas one (679insAG) was,¹⁹ raising the possibility of a mutational “hot spot” or a familial relationship unknown to the patient.

The identification of a germ-line HRPT2 mutation in a patient with apparently sporadic parathyroid carcinoma requires clinicians to reconsider the approach to this patient and raises new management issues with respect to his or her relatives.



When hyperparathyroidism recurs or worsens in such a patient, a new and distinct primary parathyroid tumor, benign or malignant, should be carefully sought in addition to a recurrence or progression of the original carcinoma, because asynchronous primary parathyroid neoplasms can develop in patients with the HPT-JT syndrome. Surveillance for renal and jaw neoplasia may also be indicated.

Susceptibility to the development of parathyroid carcinoma or other manifestations of the HPT-JT syndrome may exist in the relatives of a patient with apparently sporadic parathyroid carcinoma who has an HRPT2 germ-line mutation if they also carry the mutation. Monitoring of serum calcium levels is warranted in such family members, with the goal of early diagnosis and treatment of an incipient or premetastatic parathyroid cancer. If primary hyperparathyroidism develops in a relative who is at risk, surgery aimed at identifying and examining all parathyroid glands could be advocated, even if a more limited approach might otherwise have been chosen.

We suggest that HRPT2 germ-line DNA testing should be seriously considered for patients presenting with apparently sporadic parathyroid carcinoma.

The identification of a coding mutation would be definitive, although a negative result would not rule out the existence of an undetected, noncoding mutation, and indeed, the latter are expected, since germ-line HRPT2 coding mutations were not found in affected members of almost half of families with classic HPT-JT syndrome.¹⁹ A separate question is whether and when genetic testing should be offered to at-risk relatives of a patient who has parathyroid carcinoma with an HRPT2 germ-line mutation. Genotyping of such family members for this recognized mutation would enable the focused implementation of clinical and biochemical monitoring of carriers of the mutation and offer reassurance to family members who do not have the mutation. Monitoring serum calcium levels in all persons at risk provides an alternative to definitive DNA diagnosis.

Supported by the Murray-Heilig Fund in Molecular Medicine, the Swedish Cancer Foundation, the Nilsson-Ehle Foundation, the Robert Lundberg Foundation, the Torsten and Ragnar Söderberg Foundations, the Gustav V Jubilee Foundation, and the Emil and Vera Cornell Foundation.

We are indebted to Dr. Anders Höög for expert histopathological evaluations and to Ms. Pamela Vachon for expert administrative assistance.

REFERENCES

1. Wang CA, Gaz RD. Natural history of parathyroid carcinoma: diagnosis, treatment, and results. *Am J Surg* 1985;149:522-7.
2. Shane E. Clinical review 122: parathyroid carcinoma. *J Clin Endocrinol Metab* 2001;86:485-93.
3. Bondeson L, Sandelin K, Grimelius L. Histopathological variables and DNA cytometry in parathyroid carcinoma. *Am J Surg Pathol* 1993;17:820-9.
4. Roth SI. Pathology of the parathyroids in hyperparathyroidism. *Arch Pathol* 1962;73:495-510.
5. Schantz A, Castleman B. Parathyroid carcinoma: a study of 70 cases. *Cancer* 1973;31:600-5.
6. Vetto JT, Brennan ME, Woodruff J, Burt M. Parathyroid carcinoma: diagnosis and clinical history. *Surgery* 1993;114:882-92.
7. Sandelin K, Thompson NW, Bondeson L. Metastatic parathyroid carcinoma: dilemmas in management. *Surgery* 1991;110:978-86.
8. Sandelin K, Auer G, Bondeson L, Grimelius L, Farnebo LO. Prognostic factors in parathyroid cancer: a review of 95 cases. *World J Surg* 1992;16:724-31.
9. Anderson BJ, Samaan NA, Vassilopoulos-Sellin R, Ordonez NG, Hickey RC. Parathyroid carcinoma: features and difficulties in diagnosis and management. *Surgery* 1983;94:906-15.
10. Cryns VL, Thor A, Xu HJ, et al. Loss of the retinoblastoma tumor-suppressor gene in parathyroid carcinoma. *N Engl J Med* 1994;330:757-61.
11. Pearce SH, Trump D, Wooding C, Sheppard MN, Clayton RN, Thakker RV. Loss of heterozygosity studies at the retinoblastoma and breast cancer susceptibility (BRCA2) loci in pituitary, parathyroid, pancreatic and carcinoid tumors. *Clin Endocrinol (Oxf)* 1996;45:195-200.
12. Dotzenrath C, Teh BT, Farnebo F, et al. Allelic loss of the retinoblastoma tumor suppressor gene: a marker for aggressive parathyroid tumors? *J Clin Endocrinol Metab* 1996;81:3194-6.
13. Kytölä S, Farnebo F, Obara T, et al. Patterns of chromosomal imbalances in parathyroid carcinomas. *Am J Pathol* 2000;157:579-86.
14. Imanishi Y, Palanisamy N, Tahara H, et al. Molecular pathogenetic analysis of parathyroid carcinoma. *J Bone Miner Res* 1999;14:Suppl 1:S421.
15. Agarwal SK, Schrock E, Kester MB, et al. Comparative genomic hybridization analysis of human parathyroid tumors. *Cancer Genet Cytogenet* 1998;106:30-6.
16. Haber D, Harlow E. Tumor-suppressor genes: evolving definitions in the genomic age. *Nat Genet* 1997;16:320-2.
17. Shattuck TM, Kim TS, Costa J, et al. Mutational analyses of RB and BRCA2 as candidate tumor suppressor genes in parathyroid carcinoma. *Clin Endocrinol (Oxf)* 2003;59:180-9.
18. Costa J, Shattuck TM, Imanishi Y, et al. Mutational analyses of connexin 26, connexin 30 and connexin 46 as candidate tumor suppressor genes in parathyroid carcinoma. *J Endocr Genet* 2003;3:57-62.
19. Carpten JD, Robbins CM, Villablanca A, et al. HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. *Nat Genet* 2002;32:676-80.
20. Jackson CE, Norum RA, Boyd SB, et al. Hereditary hyperparathyroidism and multiple ossifying jaw fibromas: a clinically and genetically distinct syndrome. *Surgery* 1990;108:1006-12.
21. Wassif WS, Farnebo F, Teh BT, et al. Genetic studies of a family with hereditary hyperparathyroidism-jaw tumour syndrome. *Clin Endocrinol (Oxf)* 1999;50:191-6.
22. Kennett S, Pollick H. Jaw lesions in familial hyperparathyroidism. *Oral Surg Oral Med Oral Pathol* 1971;31:502-10.
23. Eversole LR, Leider AS, Nelson K. Ossifying fibroma: a clinicopathologic study of sixty-four cases. *Oral Surg Oral Med Oral Pathol* 1985;60:505-11.
24. Szabo J, Heath B, Hill VM, et al. Hereditary hyperparathyroidism-jaw tumor syndrome: the endocrine tumor gene HRPT2

- maps to chromosome 1q21-q31. *Am J Hum Genet* 1995;56:944-50.
25. Teh BT, Farnebo F, Kristoffersson U, et al. Autosomal dominant primary hyperparathyroidism and jaw tumor syndrome associated with renal hamartomas and cystic kidney disease: linkage to 1q21-q32 and loss of the wild-type allele in renal hamartomas. *J Clin Endocrinol Metab* 1996;81:4204-11.
26. Haven CJ, Wong FK, van Dam EW, et al. A genotypic and histopathological study of a large Dutch kindred with hyperparathyroidism-jaw tumor syndrome. *J Clin Endocrinol Metab* 2000;85:1449-54.
27. Shattuck TM, Costa J, Bernstein M, Jensen RT, Chung DC, Arnold A. Mutational analysis of Smad3, a candidate tumor suppressor implicated in TGF- β and menin pathways, in parathyroid adenomas and enteropancreatic endocrine tumors. *J Clin Endocrinol Metab* 2002;87:3911-4.
28. Arnold A, Shattuck TM, Mallya SM, et al. Molecular pathogenesis of primary hyperparathyroidism. *J Bone Miner Res* 2002;17:Suppl 2:N30-N36.
29. Marx SJ. Hyperparathyroid and hypoparathyroid disorders. *N Engl J Med* 2000;343:1863-75. [Errata, *N Engl J Med* 2001;344:240, 696.]
30. Fitko R, Roth SI, Hines JR, Roxe DM, Cahill E. Parathyromatosis in hyperparathyroidism. *Hum Pathol* 1990;21:234-7.
31. Marx SJ, Simonds WF, Agarwal SK, et al. Hyperparathyroidism in hereditary syndromes: special expressions and special managements. *J Bone Miner Res* 2002;17:Suppl 2:N37-N43.
32. Simonds WF, James-Newton LA, Agarwal SK, et al. Familial isolated hyperparathyroidism: clinical and genetic characteristics of 36 kindreds. *Medicine (Baltimore)* 2002;81:1-26.

Copyright © 2003 Massachusetts Medical Society.

APPLY FOR JOBS ELECTRONICALLY AT THE NEW NEJM CAREER CENTER

Physicians registered at the new NEJM Career Center can now apply for jobs electronically using their own cover letters and CVs. You can now keep track of your job-application history with a personal account that is created when you register with the Career Center and apply for jobs seen online at our Web site. Visit www.nejmjobs.org for more information.