Am. J. Hum. Genet. 63:1743–1748, 1998

A Gene Predisposing to Familial Thyroid Tumors with Cell Oxyphilia Maps to Chromosome 19p13.2

Federico Canzian,[1] Patrizia Amati,[2] H. Rubén Harach,[5] Jean-Louis Kraimps,[3] Fabienne Lesueur,[1] Jacques Barbier,[3] Pierre Levillain,[4] Giovanni Romeo,[1] and Dominique Bonneau[2]

[1]Unit of Genetic Cancer Susceptibility, International Agency for Research on Cancer, Lyon; Service de [2]Génétique Médicale, [3]Chirurgie Endocrinienne, and [4]Anatomie Pathologique, Poitiers, France; and [5]Department of Histopathology, Saint Bartholomew's Hospital, London

Summary

Familial nonmedullary thyroid cancer (FNMTC) is a clinical entity characterized by a phenotype more aggressive than that of its sporadic counterpart. Families with recurrence of nonmedullary thyroid cancer (NMTC) have been repeatedly reported in the literature, and epidemiological data show a very high relative risk for first-degree relatives of probands with thyroid cancer. The transmission of susceptibility to FNMTC is compatible with autosomal dominant inheritance with reduced penetrance, or with complex inheritance. Cases of benign thyroid disease are often found in FNMTC kindreds. We report both the identification of a new entity of FNMTC and the mapping of the responsible gene, named "*TCO*" (thyroid tumors with cell oxyphilia), in a French pedigree with multiple cases of multinodular goiter and NMTC. *TCO* was mapped to chromosome 19p13.2 by linkage analysis with a whole-genome panel of microsatellite markers. Interestingly, both the benign and malignant thyroid tumors in this family exhibit some extent of cell oxyphilia, which, until now, had not been described in the FNMTC. These findings suggest that the relatives of patients affected with sporadic NMTC with cell oxyphilia should be carefully investigated.

Introduction

Thyroid neoplasms can be solitary or multiple, presenting clinically as uninodular or multinodular goiter (MNG), respectively. Thyroid carcinomas of follicular-cell origin have been repeatedly recorded as occurring in families (Lote et al. 1980; Cooper et al. 1981; Couch et al. 1986; Stoffer et al. 1986; Ozaki et al. 1988; Gorson 1992; Grossman et al. 1995; Burgess et al. 1997; Kraimps et al. 1997), and the proportion of these familial nonmedullary thyroid carcinomas (FNMTC [MIM 188550]) is estimated to be ~3%–7% of all thyroid tumors. These kindreds typically show two to four affected relatives, with as many as eight cases of FNMTC in the same family (Lote et al. 1980; Burgess et al. 1997). Recent studies (Grossman et al. 1995) have indicated that FNMTC is, in contrast to sporadic cases, usually multifocal, recurs more frequently, and shows an early age at onset. This is well exemplified by familial adenomatous polyposis–associated thyroid carcinoma, which, in addition, has been found to be a distinct morphological entity, rather than the papillary carcinoma that it previously had been believed to be (Harach et al. 1994). The mode of inheritance of some FNMTC is not clear. Most published pedigrees are compatible with inheritance of one autosomal dominant gene with reduced penetrance, but polygenic inheritance cannot be excluded. Epidemiological data show a very high relative risk for first-degree relatives of probands with thyroid cancer (Goldgar et al. 1994), but large families with many cases of thyroid cancer are rare, a situation that suggests, again, a possible polygenic inheritance. These hypotheses are supported also by the analysis of 125 pedigrees, each with at least two members affected with NMTC, collected through the international consortium for the study of genetic susceptibility to NMTC (see the Geocities Website). The structures of the families of this collection suggest a complex situation of genetic heterogeneity, polygenic inheritance, and gene-environment interaction. Studying complex traits is very difficult. However, rare monogenic forms of diseases that are otherwise inherited as complex traits do exist. In these cases the identification of the genes involved can give insights into the more widespread polygenic form of the trait.

One peculiar form of thyroid tumors is characterized by the presence of cell oxyphilia. Oxyphil cells are found in a minority of thyroid tumors, either benign or malignant. They are characterized by the presence of a large

Received August 3, 1998; accepted for publication October 6, 1998; electronically published November 6, 1998.

Address for correspondence and reprints: Dr. Giovanni Romeo, Unit of Genetic Cancer Susceptibility, International Agency for Research on Cancer, F-69372 Lyon Cedex 08, France. E-mail: romeo@iarc.fr

0002-9297/98/6306-0021$02.00

volume of granular eosinophilic cytoplasm, and they are very rich in mitochondria. Tumors with cell oxyphilia are frequently associated with autoimmune thyroiditis. There is substantial controversy about the classification and behavior of tumors with cell oxyphilia. Their occurrence in families has been reported elsewhere (Katoh et al. 1998), and here we report the mapping, to chromosome 19p13, of a gene responsible for familial thyroid tumors with cell oxyphilia.

Patients, Material, and Methods

Patients

Family PL, whose pedigree is shown in figure 1, had been previously reported in a series of seven families affected with familial papillary thyroid carcinoma (PTC) (Kraimps et al. 1997). In brief, in this three-generation family from a nonendemic-goiter area of western France, six individuals were affected with MNG, and three were affected with PTC. Six patients were followed up by two of us (J.B. and J.-L.K.) in the endocrine-surgery unit in the hospital in Poitiers. Of these six individuals, five underwent thyroid surgery, and the sixth (III-5) was considered as affected because he was shown to have MNG, by both thyroid palpation and echography. Complete clinical and pathological information was obtained from the remaining three affected subjects, who were followed up elsewhere. The age range at which each affected individual was operated on was 10–63 years (mean 24 years). PTCs were diagnosed in individuals II-5, III-3, and III-7 at the ages of 41, 27, and 11 years, respectively. Thyroid-tumor recurrence occurred in four patients during follow-up. None of the patients had history of any other type of cancer-prone syndrome or radiation exposure. There is no history of autoimmune disease in the family. Individuals III-2, III-4, and III-6 were considered "unaffected" because the results of clinical examination and thyroid-image analysis were normal. All clinical and ultrascintigraphic examinations were performed before the molecular studies were started.

Genotyping

Blood samples were collected from the nine affected persons and from eight unaffected relatives (spouses and siblings of patients), after their informed consent was obtained, according to the French law. Genomic DNAs were extracted according to a standard phenol-chloroform protocol. The 17 samples were genotyped with microsatellite markers from the Cooperative Human Linkage Center, the Généthon collections, or other sources (Collin et al. 1996). Heterozygosity was >70% for all markers, and the average spacing between the markers used in the genome scan was 12 cM. Each PCR reaction included 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 50 ng DNA, 0.2 U Red Hot DNA polymerase (Advanced Biotechnologies), and 5 pmol of each primer (one of them fluorescently labeled), for a total volume of 8 μl. Alternatively, unlabeled primers were used, and the PCR reactions were supplemented with 0.5–2 μM fluorescently labeled dCTP (Perkin-Elmer). The reactions were run in a 9600 GeneAmp PCR System (Perkin-Elmer), with the following thermal profile: 96°C for 5 min; 30 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s; and final extension at 72°C for 5 min. The fluorescent products for each template were appropriately pooled, and an aliquot was loaded onto a 4.8% polyacrylamide 8-M urea gel and was run in an automated sequencer (model 377; Applied Biosystems). The data were automatically collected and analyzed by GeneScan and Genotyper software (Applied Biosystems).

Linkage Analysis

Linkage power for this family was studied by a simulation by means of the SLINK and MSIM programs of the LINKAGE package, version 5.1 (Ott 1989). Patients with MNG, PTC, or both were considered as equally affected. Two-point linkage analysis was performed with the MLINK program of LINKAGE (Lathrop and Lalouel 1984). Parametric and nonparametric multipoint analysis was performed with Genehunter (Kruglyak et al. 1996). For the parametric analysis, the trait was assumed to be autosomal dominant, with a disease-allele frequency of .001 and penetrance of .85. Allele frequencies for the microsatellite markers either were obtained from Genome Database and the Cooperative Human Linkage Center or were considered as equal (i.e., 1/no. of alleles).

Morphological Studies

Bouin-and-formalin–fixed, paraffin-embedded and hematoxylin-eosin–stained histological sections from cases I-1, II-5, II-7, III-3, and III-7 were available for histology. Representative slides from all cases were also studied for the presence of thyroglobulin and calcitonin, by an immunoperoxidase method.

Results

Linkage Mapping

Segregation of the disease in the family shows the presence of a single disease gene with an autosomal dominant mode of inheritance and very high penetrance. Simulation of linkage with SLINK and MSIM in family PL yielded a maximum LOD score (Z_{max}) of 2.97 at a recombination fraction (θ) of 0 and 100% penetrance. A random genome screening was undertaken. Markers intragenic or mapping near major candidate genes (whose alterations have been frequently reported in he-

reditary or sporadic thyroid tumors) yielded negative LOD scores. After genotyping a total of 226 microsatellites covering ~75% of the genome, we found a LOD score of 2.41 at $\theta = 0$, for marker D19S586. Additional markers were typed in the region (to a density as high as 10 markers/21.5 cM) and were found to be in linkage, with LOD scores peaking at markers D19S916 ($Z_{max} = 3.01$ at $\theta = 0$) and D19S413 ($Z_{max} = 2.95$ at $\theta = 0$). Table 1 summarizes the findings. The LOD scores did not change significantly if calculated with different values of penetrance (data not shown). The information content calculated by Genehunter (Kruglyak et al. 1996) was 100% for markers D19S916 and D19S413, thus explaining why a LOD score equal to the theoretical maximum has been reached. Although the trait is very likely to be transmitted in an autosomal dominant fashion with high penetrance, its mode of inheritance is not known a priori; therefore, we also performed a nonparametric multipoint analysis of the data, with Genehunter. The result was an nonparametric-linkage score of 9.1 ($P = .0048$) throughout the region of linkage, thus corroborating the result of the parametric analysis (data not shown). Haplotypes for the region of linkage were reconstructed in the family (fig. 1), and critical

Table 1

Analysis of Linkage of MNG and Thyroid Carcinoma Susceptibility to Chromosome 19p13.2

	LOD Score at θ =								
Marker	.00	.01	.05	.10	.20	.30	.40	Z_{max}	θ_{max}
D19S1034	−7.61	−1.09	.15	.55	.72	.61	.36	.72	.18
D19S884	−1.61	.34	.89	1.01	.93	.70	.38	1.02	.12
D19S391	1.74	1.72	1.61	1.48	1.18	.84	.45	1.74	.00
D19S916	3.01	2.96	2.77	2.51	1.95	1.32	.65	3.01	.00
D19S413	2.95	2.90	2.70	2.45	1.90	1.28	.63	2.95	.00
D19S586	2.41	2.37	2.21	2.00	1.54	1.03	.49	2.41	.00
D19S583	.60	.59	.54	.47	.32	.17	.05	.60	.00
D19S535	2.40	2.36	2.20	2.00	1.50	1.00	.48	2.40	.00
D19S221	−1.31	.63	1.17	1.27	1.13	.84	.46	1.27	.10
D19S432	−1.30	.91	1.43	1.50	1.32	.98	.53	1.50	.08

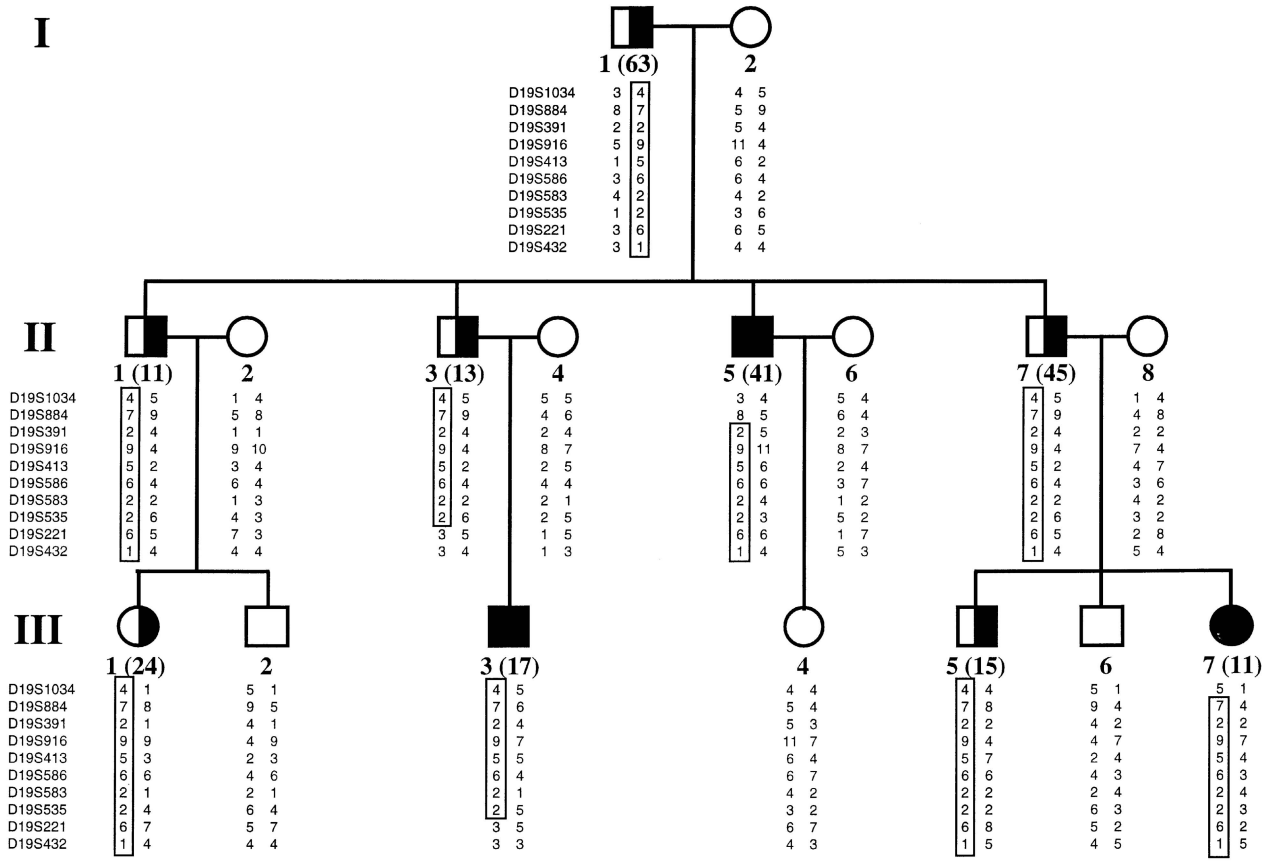

Figure 1 Pedigree of family PL, with haplotype analysis in the region of linkage to susceptibility to thyroid cancer and MNG, on chromosome 19. Circles represent females, and squares represent males; unblackened symbols denote unaffected individuals, blackened symbols denote cases of thyroid cancer, and half-blackened symbols denote cases of MNG. In parentheses are the ages at diagnosis/surgery of the family members affected with NMTC or MNG. Critical recombinations in individuals II-3 and II-5 define a 2-9-5-6-2-2 haplotype that is coinherited with the disease and that is not shared by the unaffected family members.

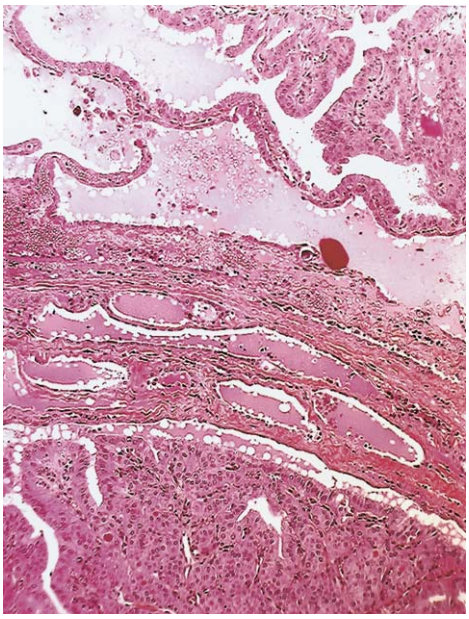

Figure 2 Two well-circumscribed tumors separated by normal thyroid tissue and predominantly composed of papillary structures lined by oxyphil cells (× 45; hematoxylin and eosin).

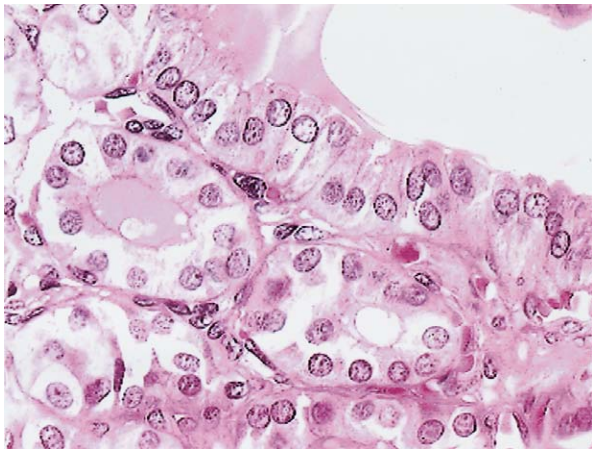

Figure 3 Adenoma showing follicular structures lined by cells with oxyphil cytoplasm and regular, sometimes apical nuclei with conspicuous nucleoli. Nuclear grooving and cytoplasmic inclusions are not seen (× 220; hematoxylin and eosin).

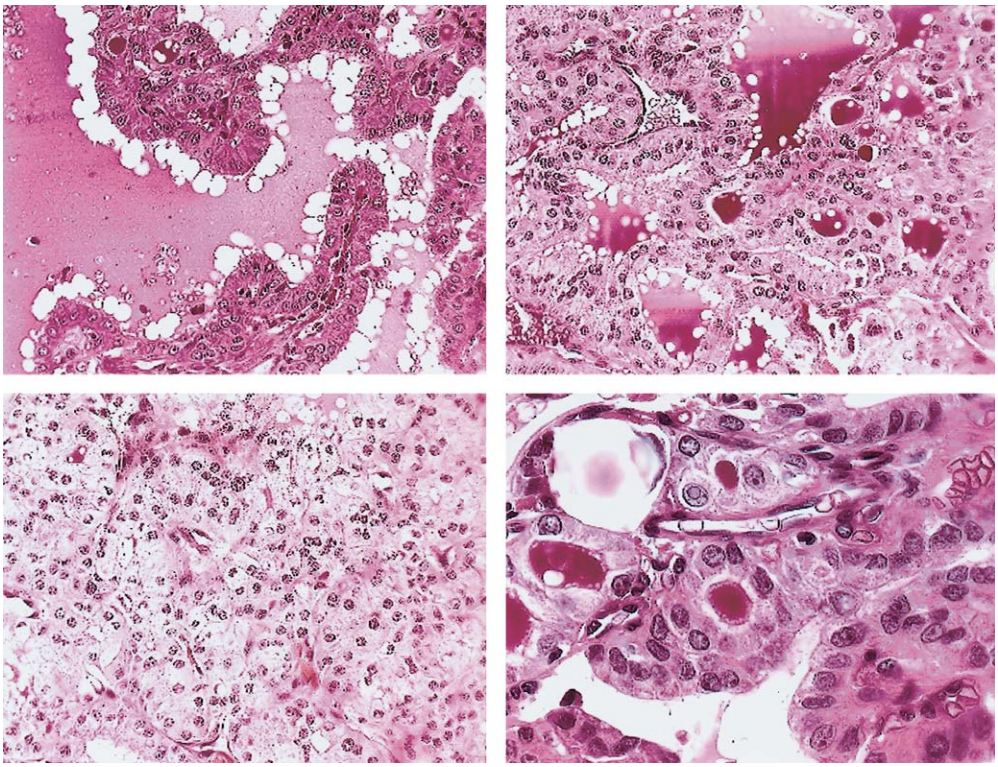

Figure 4 Papillary carcinoma. Oxyphil cells forming papillary (*upper left*), follicular (*upper right*), and solid (*lower left*) structures (× 180; hematoxylin and eosin). At the lower right the oxyphil cells show irregular, often-grooved nuclei, an occasional nuclear-cytoplasmic inclusion, and conspicuous nucleoli. Note the fragmented psammoma body at the lower right (× 290; hematoxylin and eosin).

recombinations in individuals II-5 and II-3 identified a region of linkage, encompassing markers DS19S391–2.5 cM–D19S916–1.1 cM–D19S413–0.55 cM–D19S586–0 cM–D19S583–0.8 cM–D19S535. The interval between the closest unlinked markers on either side (D19S884 and D19S221) is 9.8 cM (on the basis of a map of the Marshfield Medical Research Foundation). In fact, since patient I-1 is homozygous at marker locus D19S391 (fig. 1), it is equally probable that the critical interval lies in the 7.4 cM between D19S391 and D19S221. The markers defining the region of linkage have been physically mapped to a region of chromosome 19p13.2, spanning ~4 Mb (Ashworth et al. 1995; also see the "Maps of Human Chromosome 19" Website).

Histopathological Findings

Histology results available from five cases showed multiple, usually well-demarcated tumors of varying sizes, composed of follicular structures, papillae, solid/trabecular areas, or an admixture. The neoplastic cells from the majority of the lesions showed variable cytoplasmic eosinophilia (figs. 2–4). Tumors from cases I-1 and II-7 showed no evidence of malignancy and were regarded as multiple adenomas with variable cell oxyphilia (fig. 3). Cases II-5 and III-3 showed, in addition, malignant tumors that were regarded as oxyphil papillary carcinoma (fig. 4). Histology results available from case III-7 showed an invasive single oxyphil-cell tumor showing neither classic nuclear features of papillary carcinoma nor psammoma bodies, which therefore was classified as oxyphil-cell carcinoma. All tumors showed thyroglobulin immunoreactivity and were negative for calcitonin. Nonnodular background thyroid tissue was unremarkable in all cases.

Discussion

Here we have presented evidence for a new entity of genetic disease, characterized by thyroid carcinomas and adenomas, multiple or isolated, with cell oxyphilia and a compound architecture of follicular, papillary, and solid structures, not resembling any of the typical subclasses of thyroid tumors. Adenomas and carcinomas share the same properties and represent only different stages of progression to malignancy. We mapped, by linkage analysis in a French family, a gene on chromosome 19p13.2, named "*TCO*" (thyroid tumors with cell oxyphilia), that accounts for susceptibility to this phenotype.

A proportion of sporadic thyroid tumors of follicular-cell origin shows some of the features present in family PL—that is, cell oxyphilia with variable architecture. Also, occasional familial aggregation has been suggested for this type of tumor (Katoh et al. 1998). The latter may represent the entity that we are herein describing, in which adenomas and carcinomas share some morphological properties.

The susceptibility to thyroid tumors, which we have described here for the first time and have characterized from the morphological and genetic point of view, is distinct from previously reported hereditary predisposition to thyroid disease. Three conditions are known to be associated with hereditary predisposition to nonmedullary thyroid tumors; one is familial adenomatous polyposis coli (Harach et al. 1994), the second is Cowden syndrome (Liaw et al. 1997), and the third is a form of MNG and PTC (Bignell et al. 1997; MIM 138800). The tumors found in family PL are morphologically distinct from those observed in these three conditions. During the genome scan, we excluded linkage to the involved genes (*APC, PTEN,* and *MNG1,* respectively). Linkage to another important candidate gene, *TSHR,* whose somatic mutations are found in sporadic thyroid adenomas (Parma et al. 1993) and whose germ-line mutations predispose to familial hyperthyroidism (Duprez et al. 1994), has been excluded by use of an intragenic marker. We excluded, as well, linkage to *RET*, *TRK,* and *MET,* three oncogenes that are known to be frequently altered in sporadic NMTC (Bongarzone et al. 1989; Di Renzo et al. 1992; Pasini et al. 1996). Studying somatic alterations of these genes in the tumors of family PL will be of great interest, since it will provide clues to the mechanism of action of the *TCO*-gene product.

Several genes have been mapped in the critical area of linkage (Ashworth et al. 1995), although none appears as a striking candidate for the indentification of *TCO*. Among the genes mapping to 19p13.2, *ICAM1* is expressed in PTCs and is silent in normal thyroid tissue (Nakashima et al. 1994). Several zinc-finger–protein genes also map to the region of interest. Moreover, the oncogene *JUNB* is localized just at the centromeric border of the region of linkage (Ashworth et al. 1995).

Once *TCO* has been cloned and its identity elucidated, it will be of interest to verify its involvement in the etiology of thyroid tumors with cell oxyphilia—that is, to search both for *TCO* germ-line mutation in hereditary and apparently sporadic cases and for somatic mutations in sporadic ones. Our findings should prompt clinicians to investigate the family history of patients with nonmedullary thyroid carcinoma, adenoma, or MNG showing morphological features similar to those described here.

Acknowledgments

This work was funded by Electricité de France contract RB97-06, Association pour la Récherche sur le Cancer contract 4078-96, European Union contract PL-96 2107, and la Ligue Contre le Cancer (Comité Départemental de la Vienne). The

expert technical collaboration of Tatiana Tocco is acknowledged. F.L. is the recipient of a Special Training Award from International Agency for Research on Cancer.

Electronic-Database Information

Cooperative Human Linkage Center, http://www.chlc.org
Généthon, http://www.genethon.fr
Genome Database, http://gdbwww.gdb.org
Geocities, http://www.geocities.com/ResearchTriangle/4485/fnmtc.html
Maps of Human Chromosome 19, http://www-bio.llnl.gov/genome/html/chrom_map.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FNMTC [MIM 188550] and MNG and PTC [MIM 138800])

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