Clinical and genetic analysis of an inherited case of thyroid adenoma/cancer

Hideki Asakawa, Tetsuro Kobayashi1, Yoshifumi Komoike1, Yoshiaki Nakano1, Yasuhiro Tamaki1, Yuji Matsuzawa and Morito Monden1

The Second Department of Internal Medicine and 1Department of Surgery II, Osaka University Medical School, Osaka, Japan

(Correspondence should be addressed to T Kobayashi, Department of Surgery II, Osaka University Medical School, 2–2, Yamadaoka, Suita, Osaka, 565 Japan)

Abstract

We studied a family in which thyroid neoplasms appeared to occur through genetic inheritance. Six blood relations, including the two probands, had thyroid carcinoma, and six others had benign thyroid tumors. When both parents had a thyroid neoplasm, their children frequently had thyroid neoplasms; this was confirmed through two generations of this family. To clarify the mechanism of inheritance, we performed chromosomal analysis, Southern blot analysis of three variable number of tandem repeats markers and HLA typing on two probands, and examined their RET proto-oncogenes, and p53 and RB tumor suppressor genes. We could not find any positive data on genetic analysis, although our data were limited. In conclusion, we studied a family in which thyroid neoplasms have occurred partly through genetic inheritance, although environmental factors may have influenced the occurrence of thyroid diseases. A search for a predisposing gene, using the microsatellite technique, is required to clarify the genetic factors involved.

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Introduction

Medullary thyroid carcinoma (MTC) and papillary thyroid carcinoma (PTC) display familial inheritance as part of the MEN type 2 syndrome and Gardner’s syndrome respectively (1, 2); however, most thyroid neoplasms are sporadic. Hereditary occurrence has not been proven, although several familial occurrences of thyroid carcinoma other than in the setting of MEN type 2 and Gardner’s syndrome have been reported (3–8). Among our patients, we encountered two sisters with papillary thyroid carcinoma, several of whose blood relations had benign and malignant thyroid neoplasms.

Specific chromosomal alterations have been shown to be associated with the malignant phenotype in some human neoplasms (9), and several chromosome abnormalities have been reported in PTC and MTC (10). However, these abnormalities are at the somatic chromosomal level. Various familial cancers also have been reported, and the gene abnormalities responsible have been clarified (11). In this study, we performed chromosomal and Southern blot analyses of three variable number of tandem repeats (VNTR) markers from the two probands, and screened their RET proto-oncogenes and p53 and RB tumor suppressor genes for germ line abnormalities (11–16). We also examined the HLA types of the probands, as some studies have found an association between HLA and differentiated thyroid carcinoma (17, 18).

Patients and methods

Patients

All family members examined had neither been victims of an atomic bomb blast, nor lived in iodine-deficient areas. As the first generation members all died before World War II, we could not obtain their clinical records; information was collected from their families. The clinical records of all benign and malignant thyroid diseases of the second to fifth generations were collected through the following hospitals: Yoshida Hospital (Nara, Japan), Tenri-Yorozu-Soudansho Hospital (Nara), Nara Prefectural Hospital (Nara), Noguchi Hospital (Oita, Japan), Yao Municipal Hospital (Osaka, Japan), Takeda-Sougou Hospital (Kyoto, Japan), National Kyoto South Ryouyousho Hospital (Kyoto), and Kagoshima Prefectural Hokusatsu Hospital (Kagoshima, Japan). Family members not affected were interviewed about their cervical status; their health screening data were obtained from their school or company. We obtained blood samples from the two probands, with their informed consent, and performed HLA typing and chromosome and gene analyses.
**Chromosomal analysis and HLA typing**

Heparinized peripheral blood samples were fractionated using the standard Ficoll–hypaque technique. The lymphocytes were cultured with phytohemagglutinin (PHA) for 72 h. Twenty metaphases from each sample were analysed using G-band staining methods (19). HLA typing was performed using a microcytotoxicity assay.

**Southern blot analysis of VNTR**

Genomic DNA was prepared by the proteinase K–phenol–chloroform extraction method, digested with HaeIII, electrophoresed on 0.7% agarose gels, denatured and transferred to nylon filters. The transferred DNA was fixed by u.v. irradiation. The filters were hybridized with 32P-labeled VNTR marker probes, washed, dried and exposed to radiographic film. The hybridization probes were YNH-24 (D2S44), TBQ-7 (D10S28) and CMM101 (D14S13) (Promega, WI, USA).

**Southern blot analysis of RB**

Genomic DNA was prepared by the proteinase K–phenol–chloroform extraction method, digested with HindIII, electrophoresed on 0.8% agarose gels, denatured and transferred to nylon filters. The transferred DNA was fixed by u.v. irradiation. The filters were hybridized with 32P-labeled DNA probes under stringent conditions, washed, dried and exposed to radiographic film. The hybridization probes were two fragments representing the full coding sequence of the RB gene. The 3′ region of the gene was probed with a 3.8 kb fragment of an RB cDNA clone, and the 5′ region was probed with a DNA sequence generated by PCR. This latter probe was prepared by amplifying the 5′ region of RB cDNA using the primers corresponding to exons 1 and 10 (20).

**PCR–single strand conformational polymorphism (SSCP) analysis of p53**

For the analysis of genomic DNA by the SSCP technique, the extracted DNAs were subjected to PCR to amplify exons 5, 6, 7 and 8 of the p53 gene, using primers labeled with [γ-32P]ATP (Amersham, IL, USA). After denaturation for 5 min at 80°C, the samples were electrophoresed on a 5% acrylamide gel containing 5% glycerol at 40 W for 2 h. The gel was dried and exposed to radiographic film with intensifying screens at −80°C overnight.

**RET DNA sequence of exons 10 and 11, and PCR–restriction fragment length polymorphism (RFLP) analysis of exons 13 and 16**

Exons 10 and 11 of the RET gene were amplified separately using genomic DNAs as templates with the oligonucleotide primers. The amplified products were completely digested with EcoRI, and subcloned into the EcoRI site of pBluescript KS(−). A mixture of at least 50 subclones was used as the template for DNA sequencing with primers, using a T7 sequencing kit (Pharmacia, Uppsala, Sweden). To detect the mutations of codon 768 in exon 13 and codon 918 in exon 16, exons 13 and 16 were amplified by PCR. The amplified product of exon 13 was digested with AluI, and electrophoresed on a 3% acrylamide gel; that of exon 16 was digested with PstI, and electrophoresed on an 8% acrylamide gel. The gel was dried and exposed to radiographic film with intensifying screens at −80°C overnight.

**Results**

**Patients**

The family pedigree is shown in Fig. 1. Proband 1 (subject 28) underwent operations for thyroid nodules three times. Histological examination at the third operation revealed PTC. In 1996, at age 60 years, this proband underwent gastrectomy for gastric adenocarcinoma. Proband 2 (subject 32) underwent surgery for thyroid nodules twice. Histological examination at her second operation also showed PTC. There was no family history of lipoma, osteoma or intestinal polyposis suggestive of Gardner’s syndrome; however, patients with thyroid related diseases accumulated in this family (Table 1), and six members had other types of cancer (Table 2).

A sister and two brothers of the probands had adenomatous goiters, two brothers had no thyroid abnormalities, and two brothers died of an accident or enteritis at ages 22 and 4 years respectively. The father of the probands was diagnosed as having inoperable thyroid carcinoma with pulmonary metastases and
underwent external beam radiation to the neck. His clinical status worsened rapidly, and he died of respiratory failure at age 87 years, 6 months after his diagnosis. The mother of the probands underwent an operation for a thyroid mass at age 60 years. The grandmother of the probands died of thyroid carcinoma, as did her sister. Subject 27 underwent an operation for PTC at age 55 years; her daughter underwent an operation for thyroid adenoma and her son twice underwent operations for thyroid nodules. The granddaughter (subject 45) of the eldest sister (subject 25) of the probands had thyroidectomy and dissection of the cervical lymph nodes for PTC with lymph node metastases at age 16 years.

**Chromosomal analysis**

No abnormalities in the chromosomes of the two probands were found (data not shown).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subjects with thyroid related disease.</th>
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<td>Subject</td>
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</tr>
<tr>
<td>2</td>
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<tr>
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<td>5</td>
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<tr>
<td>45</td>
<td>16</td>
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<tr>
<td>50</td>
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</tr>
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**HLA typing**

HLA typing in the A, B, C, and DR loci was performed on the two probands. Their phenotypes were completely identical (A locus: A11, A24; B locus: B54, B67; C locus: CW1, CW7; DR locus: DR2, DR4).

**Southern blot analysis of VNTR**

We performed Southern blot analysis using three different VNTR marker probes. The DNA patterns of two probands were same (data not shown).

**Abnormalities of RET, RB and p53**

We examined the RB gene by Southern blot analysis to detect rearrangements. We did not find any rearrangement of this gene in the two probands (data not shown). PCR–SSCP of the p53 gene showed no mutations in the exons examined (data not shown). The DNA sequence of exons 10 and 11 of the RET gene did not harbor any mutations, and PCR–RFLP analysis of exons 13 and 16 showed no mutation of codons 768 and 918 respectively (data not shown).

**Discussion**

We report here a family in which thyroid neoplasms accumulated, probably through genetic inheritance. A previous study has reported that other thyroid abnormalities are found among the close relatives of patients.
Inherited case of thyroid neoplasms

with PTC (3). In our case, a sister and two brothers of the probands had adenomatous goiters, and underwent operations at young ages. In addition, the mother of the probands had a thyroid tumor, and their father died of thyroid carcinoma. The wife of the older brother of the probands also had PTC, and two of their three children also had thyroid neoplasms at a young age. In families in which both parents had a thyroid neoplasm, the children frequently had thyroid neoplasms. This was confirmed through two generations of this family, although the absence of any thyroid disease of some family members (generation IV, subjects 35, 36 and 37) may indicate a default of penetrance or a complex genetic influence. Two patients with autoimmune thyroiditis were also identified.

In this family, thyroid adenomas, adenomatous goiters and PTC were observed. The multi-step process of carcinogenesis in colorectal cancer is well known, but the process of carcinogenesis in thyroid neoplasias is unclear, although several hypotheses have been proposed (21, 22). Transformation from thyroid adenomas or adenomatous goiters to carcinoma has not been proven. Our study makes it clear that genetic factors are related to familial thyroid tumorigenesis. However, the reasons why some members have malignant and some have benign thyroid tumors remain obscure.

Lote et al. (5) have shown that the patient’s age at the time of diagnosis is younger, and lymph node metastases more frequent, in familial than in non-familial cases of PTC from the same region. Our two probands underwent their first operations at young ages and had relapses, and the granddaughter of their eldest sister had an operation for PTC with metastases of cervical lymph nodes at a young age. These facts are compatible with the findings of the study by Lote’s group, although our probands’ father died of thyroid carcinoma at age 87 years. However, the age of family members at the time of diagnosis was mostly young, irrespective of their having benign or malignant thyroid tumors. This indicates that thyroid epithelial cell tumorigenesis occurred at an early age in genetically affected family members.

Several studies have demonstrated an association between HLA and differentiated thyroid carcinoma. Sridama et al. (18) have reported an association between the HLA-DR7 gene and non-radiation-associated thyroid carcinoma in the United States. Panza et al. (17) have shown an association between the HLA-DR1 gene and differentiated thyroid carcinoma in Italy. In Japan, Ozaki et al. (8) studied the association between HLA and familial thyroid carcinoma, and reported that the B7 and DR1 phenotypes were predominant in patients with familial thyroid carcinoma, compared with non-familial patients and normal Japanese, and that the B7-CW7-DR1 haplotype was observed in five of 13 patients tested. Our data did not agree with those from previous studies, although we were able to obtain blood samples only from the two probands. We also performed Southern blot analysis using three different VNTR marker probes, and found that the DNA patterns of two probands were the same. Further Southern blot analysis using various VNTR marker probes is required to identify the predisposing genes.

The RB locus is responsible for the hereditary susceptibility to retinoblastoma and sarcoma, and to a lesser degree that to brain tumors, melanoma and bladder cancers (11, 13–15). We could not find germ-line RB rearrangements in our two probands, although most germline mutations at the RB locus are obviously too small to be identified by Southern blot analysis, and require different detection techniques (14, 15). Germ-line mutations in the p53 suppressor gene are found in the Li-Fraumeni syndrome, brain tumors in young adults and the familial brain tumor syndrome (23–25). We could not find germline mutations of p53 exons 5, 6, 7 or 8 in our probands. These exons contain most of the reported mutation sites. Germline mutations in the RET proto-oncogene result in MEN 2A and MEN 2B syndromes and familial medullary thyroid carcinoma (1, 12, 16); we could not find germline mutations of RET in the two probands.

In conclusion, we encountered a family in which thyroid neoplasms accumulated partly through genetic inheritance, although environmental factors may have influenced the occurrence of thyroid diseases. We found no abnormalities on chromosomal analysis or germline gene mutation analysis in two affected members. A search for the predisposing gene, using a microsatellite technique, is required to clarify the genetic factors involved.

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References


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