

## Cytogenetic and molecular genetic studies of follicular and papillary thyroid cancers.

M A Herrmann, I D Hay, D H Bartelt Jr, S R Ritland, R J Dahl, C S Grant, R B Jenkins

*J Clin Invest.* 1991;88(5):1596-1604. <https://doi.org/10.1172/JCI115472>.

### Research Article

Cytogenetic studies have shown frequent clonal abnormalities in papillary carcinoma (PTC) and follicular carcinoma (FTC). Loss of heterozygosity (LOH) may suggest the presence of tumor suppressor genes and has not been reported in these neoplasms. These studies were undertaken to determine if consistent chromosomal abnormalities are associated with thyroid cancer, to determine likely regions for molecular genetic investigations, and to determine if there is allelic loss in thyroid tumors. Cytogenetic analysis of 26 PTC and 5 FTC showed clonal abnormalities in 9 and included -Y, +5, or inv(10)(q11.2q21.2) in PTC, and -Y or near haploidy in FTC. Using DNA probes specific for chromosomes 1, 3, 10, 16, and 17, we carried out restriction fragment length polymorphism analysis on 6 FTC, 3 follicular adenomas (FA), and 12 PTC. LOH of all informative loci on chromosome 3p was observed in all 6 FTC, but not in FA or PTC. No LOH was observed for loci mapped to chromosome 10 in PTC. Our results suggest: cytogenetic abnormalities of chromosome 10q are associated with PTC; cytogenetic and molecular abnormalities of chromosome 3 are associated with FTC; and a tumor suppressor gene may be present on the short arm of chromosome 3 important for the development or progression of FTC.

**Find the latest version:**

<https://jci.me/115472/pdf>



# Cytogenetic and Molecular Genetic Studies of Follicular and Papillary Thyroid Cancers

Marie A. Herrmann,\* Ian D. Hay,<sup>‡</sup> Duane H. Bartelt, Jr.,\* Steven R. Ritland,\* Richard J. Dahl,\* Clive S. Grant,<sup>§</sup> and Robert B. Jenkins\*

\*Department of Laboratory Medicine and Pathology, Section of Laboratory Genetics; <sup>‡</sup>Division of Endocrinology/Metabolism and Internal Medicine; and <sup>§</sup>Section of Gastroenterologic and General Surgery, Mayo Clinic and Foundation, Rochester, Minnesota 55905

## Abstract

Cytogenetic studies have shown frequent clonal abnormalities in papillary carcinoma (PTC) and follicular carcinoma (FTC). Loss of heterozygosity (LOH) may suggest the presence of tumor suppressor genes and has not been reported in these neoplasms. These studies were undertaken to determine if consistent chromosomal abnormalities are associated with thyroid cancer, to determine likely regions for molecular genetic investigations, and to determine if there is allelic loss in thyroid tumors. Cytogenetic analysis of 26 PTC and 5 FTC showed clonal abnormalities in 9 and included  $-Y$ ,  $+5$ , or  $inv(10)(q11.2q21.2)$  in PTC, and  $-Y$  or near haploidy in FTC. Using DNA probes specific for chromosomes 1, 3, 10, 16, and 17, we carried out restriction fragment length polymorphism analysis on 6 FTC, 3 follicular adenomas (FA), and 12 PTC. LOH of all informative loci on chromosome 3p was observed in all 6 FTC, but not in FA or PTC. No LOH was observed for loci mapped to chromosome 10 in PTC. Our results suggest: cytogenetic abnormalities of chromosome 10q are associated with PTC; cytogenetic and molecular abnormalities of chromosome 3 are associated with FTC; and a tumor suppressor gene may be present on the short arm of chromosome 3 important for the development or progression of FTC. (*J. Clin. Invest.* 1991; 88:1596–1604.) Key words: chromosome analysis • tumorigenesis • carcinoma • endocrine tumors • neoplasia

## Introduction

Papillary (PTC)<sup>1</sup> and follicular thyroid carcinoma (FTC), the most frequently occurring primary thyroid cancers, both arise from thyroid epithelium, but each has a characteristic histopathology and clinical significance (1). Thus far, relatively few

genetic analyses of these tumors have been reported (2–15). A preliminary study of 12 thyroid carcinomas from our group (6) demonstrated abnormalities of chromosomes 3 and 10 in FTC and PTC, respectively. Others have observed anomalies of chromosome 7, 10, or 11 in PTC (3–5, 7, 9). An oncogene associated with PTC, formed by rearrangement of the *ret* oncogene (mapped to 10q11.2) and H4, another chromosome 10 gene of unknown function (mapped to 10q21.2), has been described on chromosome 10 at q11–q12 (16–18). Although loss of heterozygosity (LOH) of specific chromosome loci may suggest the presence of tumor suppressor genes important for the development or progression of particular tumors (19), we are not aware that such studies have been performed on nonmedullary thyroid neoplasms. The present cytogenetic and LOH studies were performed to determine if consistent cytogenetic abnormalities are associated with various grades and morphologic types of differentiated thyroid cancer, to determine likely chromosomal regions for molecular genetic investigations, and to determine if there is evidence of allelic loss in thyroid neoplasia.

## Methods

**Cytogenetic analysis.** Chromosome studies were performed on fresh surgical specimens from 31 patients who were seen at the Mayo Clinic between August 1989 and May 1990. Details of our methods for solid tumor culture and cytogenetic analysis have been described previously (20). Briefly, small pieces of tissue from each specimen were minced and treated with an enzyme solution, washed, and suspended in 50:50 MEM alpha:Chang's media with 20% fetal bovine serum. Cells were cultured on 16 chamber slides, then harvested in situ on days 2–26, when there was sufficient mitotic activity for chromosome analysis. In most cases 30 metaphases from each tumor were analyzed using quinacrine mustard for fluorescent Q-bands. Most specimens were also stained with Leishman's stain after trypsinization for G-bands (GTL-banding), and Distamycin A/DAPI as needed. The karyotype of each cell was described according to the International System for Human Cytogenetic Nomenclature (21).

We strictly defined clonality. An abnormal clone consisted of two or more metaphases with the same additional chromosome or structural abnormality, or three or more metaphases lacking the same chromosome. In addition, the abnormalities must have occurred in two or more independent cultures. Using this definition we separated our cytogenetic results into four categories: a normal specimen contained no apparent chromosome abnormality; a nonclonal specimen contained no consistent abnormality; a simple clonal specimen contained metaphases with two or less consistent chromosome abnormalities; and a complex clonal specimen contained metaphases with three or more consistent chromosome abnormalities.

**Loss of heterozygosity studies.** Restriction fragment length polymorphism (RFLP) analysis was performed on matched samples of peripheral blood leukocytes and thyroid tumors from 21 patients. Genomic DNA was extracted from 12 PTC, 6 FTC, and 3 follicular adenoma (FA). The tumor samples were reviewed histologically (M. A.

This work was presented in part at the 10th International Thyroid Congress, The Hague, The Netherlands, February 1991, and at the Fourth International Workshop on Chromosomes in Solid Tumors, Tucson, AZ, February 1991.

Address correspondence to Robert B. Jenkins, M.D., Department of Laboratory Medicine and Pathology, Section of Laboratory Genetics, Mayo Clinic and Foundation, 200 First Street S. W., Rochester, MN 55905.

Received for publication 16 May 1991 and in revised form 9 July 1991.

1. *Abbreviations used in this paper:* FA, follicular adenoma; FTC, follicular thyroid carcinoma; LOH, loss of heterozygosity; PTC, papillary thyroid carcinoma; RCC, renal cell carcinoma; SCC, small cell lung carcinoma.

The Journal of Clinical Investigation, Inc.  
Volume 88, November 1991, 1596–1604

Herrmann) by frozen section and each contained > 50% neoplastic cells. Tissue samples were frozen-sectioned, and homogenized in a 1.0-ml glass conical homogenizer (Bellco Glass, Inc., Vineland, NJ) with ice-cold isotonic high pH buffer (140 mM NaCl, 10 mM Tris-Cl, 1.5 mM MgCl<sub>2</sub>, pH 8.0) containing 0.5% NP-40 (Sigma Chemical Co., St. Louis, MO) and fresh 0.01% diethylpyrocarbonate (Sigma). The homogenate was centrifuged and the pelleted nuclei were digested with protease K, 2× lysis buffer (IBI) and CDTA (*trans*-1,2-diaminocyclohexane-*N,N,N',N'*,tetra acetic acid) at 55°C overnight. The DNA in the resulting solution was extracted with phenol and chloroform, then precipitated with ethanol and NaCl. The precipitated DNA was washed twice with 70% ethanol, air dried, dissolved in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0) with RNase (50 µg/ml), and then quantitated spectrophotometrically. Genomic DNA was extracted from peripheral blood leukocytes using a high salt and chloroform method (22).

Eight aliquots of genomic DNA (2.5 µg) from tumor tissue and peripheral blood leukocytes were digested with each of eight restriction enzymes (Pst I, Taq I, Bam HI, Hind III, Rsa I, Bgl II, Pvu II, and Msp I) and separated by electrophoresis (45 V for 16 h) through 0.8% agarose gels. The DNA fragments were alkali denatured then neutralized in the gels and transferred to nylon membranes (Nytran; Schleicher & Schuell, Keene, NH) with 10× SSC (sodium chloride sodium citrate) (3 M NaCl, 0.3 M NaCitrate, pH 7.0) by the Southern technique (23). The nylon membranes were prehybridized in a solution containing 5× SSC, 50% formamide, 5× Denhardt's, 0.05 M Na Phosphate (pH 6.5), 0.5% SDS, 0.2 mg/ml RNA (type III; Sigma), and 10 mg/ml salmon sperm DNA (type VII; Sigma) for 6 h at 42°C. We used seven polymorphic DNA probes specific for chromosome 3 and nine probes specific for chromosome 10. Single polymorphic DNA probes for loci on chromosomes 1, 16, and 17 were used as controls since cytogenetic abnormalities of these chromosomes are infrequent in nonmedullary thyroid tumors (2–15). Table I lists the polymorphic DNA probes, loci, map position, and restriction enzymes detecting RFLP.

The DNA probes (25 nG) were radiolabeled with deoxycytidine-5'-

alpha-<sup>32</sup>P-triphosphate using a multiprime DNA labeling system (Multiprime Labeling kit; Amersham Corp., Arlington Heights, IL) (43). Radiolabeled probes (typically 10 × 9 cpm/µg) were applied to the prehybridized membranes in a hybridization solution containing 50% formamide, 5× SSC, 1× Denhardt's, 0.02 M NaPhosphate (pH 6.5), 0.5% SDS, 10% dextran sulfate, 0.2 mg/ml RNA, and 10 mg/ml salmon sperm DNA. After hybridization at 42°C for 16 h the membranes were washed in 2× SSC/0.1% SDS at 42°C for 40 min, then once each at 60°C for 40 min in 2× SSC/0.1% SDS, 1× SSC/0.1% SDS, 0.2× SSC/0.1% SDS, and 0.1× SSC/0.1% SDS. The membranes were exposed to x-ray film (Kodak XOMAT AR) with intensifying screens at -70°C for autoradiography. Membranes were stripped before rehybridization with 0.4N NaOH for 30 s followed by 10-min neutralization (0.2 M Tris, 2× SSC, pH 7.5).

## Results

**Cytogenetic analysis.** 26 PTC and 5 FTC from 18 female and 13 male patients were successfully cultured and studied. The patients ranged in age from 17 to 86 yr. Table II summarizes cytogenetic and LOH results from this study and includes previously reported cytogenetic results of patients 1, 13, 14, and 15 (2, 6). (Patient 1 had a complex clonal karyotype [6], including losses of chromosomes 8, 14, 15, 17, gain of 11, and structural anomalies of chromosomes 1, 3, 6, 10, 11, 14, and 17. Patient 14 had a simple clonal karyotype [2]: 46,XX,t[8;14]-[q13;q24.1].)

Complex karyotypes were observed in one grade 2 PTC, one grade 3 PTC, and in two grade 2 FTC. Simple clonal karyotypes were seen in four grade 1 tumors (one FTC, three PTC) and in one grade 2 PTC. Normal or nonclonal karyotypes were observed in 20 of 26 PTC and 2 of 5 FTC.

Table III summarizes the cytogenetic findings in the three FTC and six PTC with clonal abnormalities. One grade 2 PTC (patient 50) had trisomy 5 and two low grade PTC (patients 33 and 35) had loss of the Y chromosome as the sole clonal abnormality. Another low grade PTC (patient 42) had an inv(10)(q11.2q21.2) in 3 of 29 cells (Fig. 1). One grade 2 PTC (patient 39) had three abnormal cell lines with gains of chromosomes 5, 7, 20, and/or X. A grade 3 PTC (patient 20) had a complex karyotype with three abnormal clones and a modal chromosome number of 38–47 with loss of chromosomes 9, 11, 14, 19, 22, and Y and structural abnormalities of 1p, 9q, 10p, 17q, and 19q. A karyotype representing some of these complex abnormalities is shown in Fig. 2.

One low grade FTC (patient 43) had loss of the Y chromosome as the sole clonal abnormality. Two grade 2 FTC (patients 46 and 47), one locally recurrent and one primary, were hypodiploid and had similar complex karyotypes. The modal chromosome numbers of these two cases were near haploid and both had relative gains of chromosomes 5, 7, 12, 19, and 20. Neither of these tumors had clonal structural abnormalities. Representative karyotypes of these tumors are shown in Figs. 3 and 4. Each cytogenetically abnormal FTC was an oxyphil cell variant (1).

**Loss of heterozygosity studies.** Our LOH study group consisted of 12 PTC, 6 FTC, and 3 FA (Table II). Representative autoradiography results of eight follicular tumors after restriction enzyme digestion with Hind III and hybridization with radiolabeled probe pH3H2 are illustrated in Fig. 5. LOH of all informative loci on chromosome 3p was observed in all six FTC but not in the three FA (Fig. 6). Loss of heterozygous alleles on chromosomes 16p and 17p was observed in one FTC (patient 46). LOH of chromosome 10 loci was observed in

Table I. Loci and Map Positions of DNA Probes Used in This Study\*

Probe	Locus	Map location	Enzyme	Reference
pEB8	AMY	1p21	PstI	24
p627	RAFI	3p25	TaqI	25
pHeA4	THRB	3p24.1-p22	BamHI	26
pH3H2	D3F15S2	3p21	HindIII	27
pHF12-32	D3S2	3p21	MspI	28
pYNZ86.1	D3S30	3p	MspI	29
p30-1-60	D3S86	3p	RsaI	30
pHS-3	D3S1	3q12	HindIII	31
pTBQ7	D10S28	10pter-p13	RsaI	32
pTHH54	D10S14	10q21.1-q23	RsaI	33
p1.2	TST1	10q11.2	TaqI	34
H.4IRBP	RBP3	10q11.2	BglII	35
pTB10.163	D10S22	10q21.1	MspI	36
p9-12A	D10S5	10q21.1	TaqI	37
pTB10.171	D10S19	10q21.1-q22	PvuII	38
p1-101	D10S4	10q22-q23	TaqI	39
5-1	D10S1	10q22-q23	TaqI	40
3HVR	D16S85	16p13.3	RsaI	41
pYNZ22.1	D17S5	17p13.3	BamHI	42

\* Probe p1.2 was obtained from Dr. Massimo Santoro, Oncologia Sperimentale del C.N.R., II Facolta di Medicina e Chirurgia, Naples, Italy. Remaining probes were obtained from American Type Culture Collection, Rockville, MD.

Table II. Demographics, Karyotype Characteristics, and LOH Results on 38 Study Patients

Patient no.	Age (yr)	Sex	Tumor location	Grade	Karyotype characteristics	Chromosomes showing LOH
<b>Papillary carcinoma</b>						
20	19	M	Anterior neck	3	Complex clonal	ND
21	45	F	Rt. thyroid	1	Nonclonal	ND
22	33	F	Lt. thyroid	1	Nonclonal	ND
23	45	M	Lt. thyroid	2	Nonclonal	ND
24	36	F	Lt. thyroid	1	Normal	ND
25	62	M	Lt. thyroid	1	Nonclonal	No LOH
26	41	M	Lt. thyroid	1	Nonclonal	No LOH
27	47	F	Thyroid isthmus	1	Nonclonal	No LOH
28	31	M	Lymph nodes	1	Nonclonal	ND
29	41	M	Rt. thyroid	1	Nonclonal	ND
30	76	F	Laryngeal tissue	2	Nonclonal	No LOH
31	36	F	Lt. thyroid	1	Normal	No LOH
32	34	M	Rt. thyroid	1	Nonclonal	ND
33	70	M	Lymph node	1	Simple clonal	No LOH
34	31	F	Lt. thyroid	1	Nonclonal	ND
35	83	M	Lymph nodes	1	Simple clonal	No LOH
36	50	F	Lt. thyroid	1	Nonclonal	ND
37	29	F	Rt. thyroid	1	Nonclonal	ND
38	68	F	Rt. thyroid	1	Nonclonal	ND
39	73	F	Rt. thyroid	2	Complex clonal	ND
40	60	F	Lung	1	Nonclonal	ND
41	42	F	Lt. thyroid	1	Nonclonal	No LOH
42	21	F	Lymph nodes	1	Simple clonal	No LOH
49	65	F	Lt. thyroid	1	Nonclonal	ND
50	86	F	Lt. thyroid	2	Simple clonal	ND
51	76	F	Thyroid	1	Nonclonal	ND
52	65	M	Lt. thyroid	1	ND	No LOH
53	16	F	Rt. thyroid	1	ND	No LOH
54	46	F	Rt. thyroid	1	ND	No LOH
<b>Follicular carcinoma</b>						
1	40	M	Larynx	3	Complex clonal	3p, 3q, 10p
43	54	M	Lymph node	1	Simple clonal	3p
44	17	M	Rt. thyroid	1	Nonclonal	3p
45	75	M	Lt. neck	2	Nonclonal	3p, 10p, 10q
46	79	M	Thyroid bed	2	Complex clonal	3p, 10p, 10q, 16p, 17p
47	71	F	Lt. thyroid	2	Complex clonal	3p, 3q
<b>Follicular adenoma</b>						
13	40	F	Lt. thyroid	—	Nonclonal	No LOH
14	72	F	Lt. thyroid	—	Simple clonal	16p
15	55	F	Lt. thyroid	—	Normal	10q

ND, not done.

three FTC (patients 1, 45, and 46). One FA (patient 15) demonstrated LOH for one allele on chromosome 10; another (patient 14) showed loss on chromosome 16p (Table IV). No LOH was observed for loci mapped to chromosomes 1, 3, 10, 16, or 17 in any of 12 PTC. The frequency of LOH for each 3p probe was greater than that for the single control probes on chromosomes 1, 16, and 17, as well as for each chromosome 10 probe.

## Discussion

In the United States it is estimated that there will be ~ 12,400 new cases of thyroid cancer in 1991, and thyroid cancer deaths

will account for ~ 0.2–0.4% of all cancer deaths (44). Although papillary and follicular thyroid cancers have a relatively good prognosis they have an unpredictable recurrence risk. Flow cytometric determination of nuclear DNA content in thyroid malignancies has identified subsets of patients at “high risk” of cancer-related death (45), but little is known about the molecular basis of the pathobiology of these tumors. Williams (46) has suggested that normal follicular thyroid epithelium undergoes tumorigenesis in the direction of either PTC or FA (and subsequent FTC). It has further been suggested that the progression to FTC from FA occurs as a result of a multistage process of oncogene activation, often involving *ras* (47–50).

Table III. Summary of Cytogenetic Findings in Six PTC and Three FTC with Clonal Abnormalities

Patient no.	Clone*	Cells	Karyotype
<b>Papillary carcinoma</b>			
20	N	2	46,XY
	a	11	45,X,-Y
	b	14	38-44,XY,-9,-11,-14,-19,-22,dup(1)(p22→p36),del(1)(p13),der(9)t(9;?)(q13;?),del(17)(q23),der(18)t(18;?)(q23;?),+0-8mar
	c	5	45-46,XY,-3,-6,-11,-16,-18,-21,-22,del(8)(q22),der(10)t(10;?)(p13;?),del(12)(p11.2),der(17)t(17;?)(q25;?),+der(19)t(19;?)(q13.3;?),+5-6mar
33	N	26	46,XY
	a	4	45,X,-Y
35	N	20	46,XY
	a	10	45,X,-Y
39	a	18	47,XX,+5
	b	3	47,XX,+20
	c	9	49,XX,+X,+5,+7
42	N	26	46,XX
	a	3	46,XX,inv(10)(q11.2q21.2)
50	N	24	46,XX
	a	6	47,XX,+5
<b>Follicular carcinoma</b>			
43	N	21	46,XY
	a	8	45,X,-Y
46	N	5	46,XY
	a	17	29,XY,-1,-2,-3,-4,-6,-8,-9,-10,-11,-13,-14,-15,-16,-17,-18,-21,-22
47	N	14	46,XX
	a	12	36,X,-X,-1,-2,-3,-8,-9,-11,-14,-18,-22

\* Clone refers to different cell populations within the same specimen; e.g., N, no apparent chromosome abnormality; a-c, chromosomally abnormal cell populations.

Cytogenetic studies of at least 152 nonmedullary thyroid neoplasms have been reported (2-15). Approximately 50% of the 61 nonmedullary thyroid carcinomas had an abnormal karyotype, while in our present study of 31 thyroid cancers 9 (28%) had clonal abnormalities. Loss of heterozygosity studies have not been previously reported for nonmedullary thyroid tumors.

As in our initial study (6) we observed that tumors of higher histologic grade were more often cytogenetically abnormal. 4 of 7 grade 2 tumors had clonal abnormalities, while only 4 of 23 grade 1 tumors were abnormal. Moreover, the grade 3 PTC had a complex abnormal karyotype. Most of the cytogenetically abnormal tumors were higher grade primary tumors (grade 2), or from local recurrences (grade 3 and grade 2). Four cytogenetically abnormal grade 1 tumors were from regional lymph node metastases. Tumor grade has previously been reported at Mayo to be prognostically important in both PTC and FTC (51). Cytogenetic reports of thyroid cancers from other groups, however, have not considered the tumor grade.

We also determined that there were specific chromosomal

abnormalities associated with the morphologic subtypes of PTC and FTC. Previously we had reported that FTC showed complex clonal karyotypes, with structural abnormalities of 3p (6). We did not observe structural 3p anomalies in the five FTC we analyzed in our current series; however, two tumors were hypodiploid with consistent loss of chromosome 3. Including this report, cytogenetic studies of 17 FTC have been reported (5, 6, 12). Structural aberrations of the p-arm of chromosome 3, occurring with other structural and numerical anomalies, have been observed in four of the eight structurally abnormal tumors (5, 6). Teyssier et al. (5) reported clonal abnormalities in five of eight FTC studied; two tumors contained complex anomalies, including one with loss of chromosome 3 and del(3)(p22). These cytogenetic studies suggest that alterations of the short arm of chromosome 3 are consistent genetic events in many FTC, and may be important in their pathogenesis. Our LOH data showed loss of each informative locus on chromosome 3p in each FTC and not in FA or PTC. While it is likely that the LOH on chromosome 3 in the tumors from patients 46 and 47 reflects the cytogenetically detected monosomy 3, this is clearly not the case in the other four FTC with disomy 3. These findings support the hypothesis that alterations of chromosome 3 are important in FTC, and further suggests the presence of a tumor suppressor gene on chromosome 3p which may play a role in the pathogenesis of FTC. Additional LOH studies of FTC using more probes specific for shorter segments on chromosome 3p may help to elucidate which region may be specific for these malignant thyroid tumors.

Cytogenetic abnormalities of the short arm of chromosome 3 have also been frequently observed in ovarian adenocarcinoma, small cell lung carcinoma (SCC), renal cell carcinoma (RCC), bladder cancer, gastric cancer, mesothelioma, and pleomorphic adenoma of the salivary gland (52). In contrast to LOH studies of medullary thyroid carcinoma that have not shown loss on chromosome 3p (53), such studies performed on other tumor types such as RCC and SCC have shown loss of alleles on chromosome 3p (54, 55). The critical region in RCC appears to be 3p13-p14.2, while the region of 3p14-p23 appears to be important in SCC (54, 55). This region of 3p(p21-p14) has recently been reported to contain a *ras*-related gene, ARH12 (56).

From a review of the literature and the results presented here, candidate chromosome abnormalities that may be implicated in the pathogenesis of PTC include trisomy 5, loss of Y, deletion 11q23, structural or numerical abnormalities of chromosome 7, or structural abnormalities of chromosome 10. Two of our cases had trisomy 5, one as the sole clonal abnormality. Trisomy 5 is infrequent in other solid tumors (57), has not been reported in PTC, but has been observed in FA in addition to other chromosome gains (8, 12). Loss of the Y chromosome has been observed in the bone marrow of normal elderly males (58), nonneoplastic brain tissue (59), and in many kinds of tumors (57), and is therefore not specific for thyroid or other neoplasms. Olah et al. (7) observed abnormalities at 11q23 in three cases of PTC and suggested that deletion of 11q may be specific for PTC. However, this abnormality has not been reported by others in PTC. Structural or numerical abnormalities of chromosome 7 have now been observed in five cases of PTC from four groups (4, 5, 11; current report), and in four cases they represented the sole clonal abnormality. Alterations of chromosome 7 have been frequently observed in

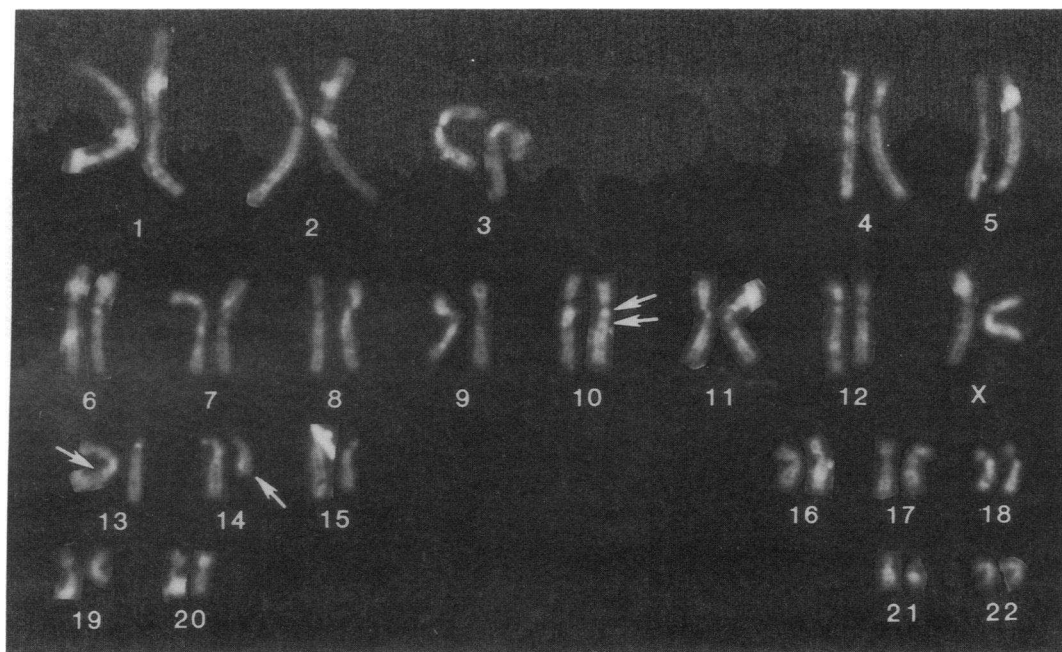


Figure 1. Q-banded karyotype, grade 1 PTC (patient 42). 46,XX,inv(10)(q11.2q21.2),t(13;14)(q32;q22). The t(13;14) was nonclonal, occurring in only one cell.

other tumor types such as melanoma (60, 61), bladder carcinoma (62, 63), and mesothelioma (64) in which they have been correlated with poor clinical outcome. Teyssier et al. (5) suggest that chromosome 7 abnormalities in thyroid carcinoma may have similar significance. The PTC in this report with trisomy 7 had other chromosome gains and was from a grade 2 carcinoma,

which implies a more advanced tumor with a less favorable prognosis (45).

Abnormalities of chromosome 10 in the region of q11-q26 have now been described in 11 PTC (3-6, 11; Pierotti, M. A., et al., submitted for publication; and current report) and in 9 of these cases structural 10q abnormalities were the sole clonal

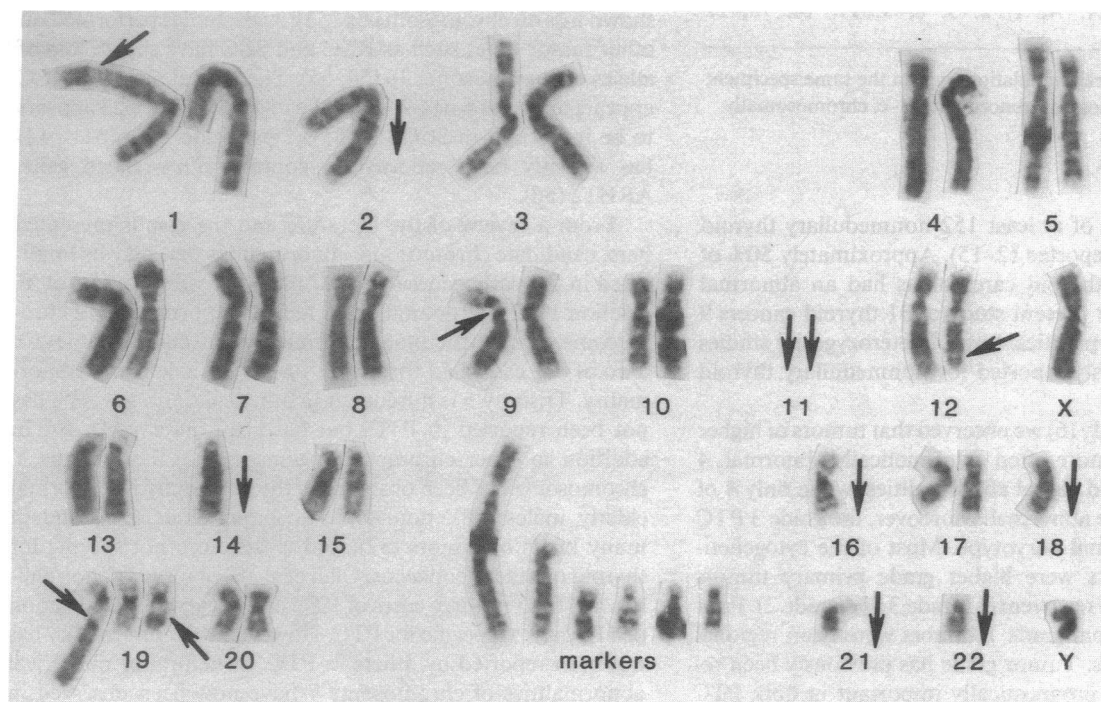


Figure 2. GTL-banded karyotype, grade 3 PTC (patient 20). 45,XY,-2,-11,-11,-14,-16,-18,-21,der(1)t(1;?)p36;?,tdic(9;22)(p13;p12),der(12)t(12;?)q24;?,der(19)t(19;?)q13.3;?,+der(19)t(19;?)q13.3;?,+6mar. The t(9;22) and der(12) were nonclonal.



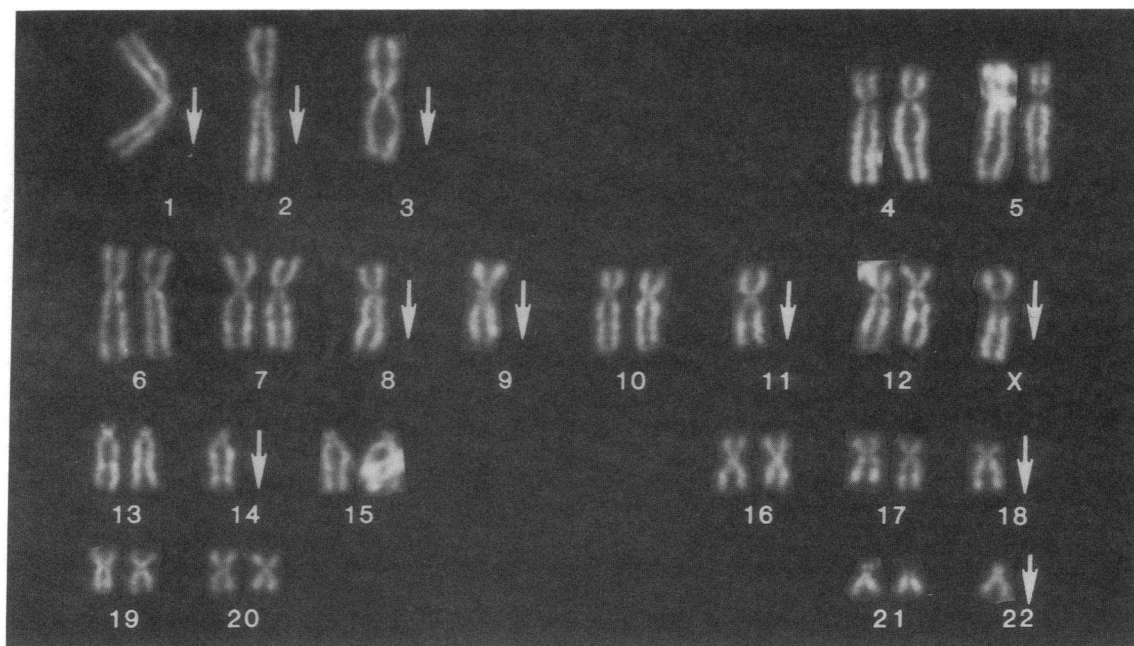


Figure 3. Q-banded karyotype, grade 2 FTC (patient 46). 36,X,-X,-1,-2,-3,-8,-9,-11,-14,-18,-22.

abnormality. Moreover, at least six of these abnormalities were *inv(10)(q11.2q21.2)* (3, 6; Pierotti et al., submitted for publication; and current report). Four of the papillary tumors with *inv(10)(q11.2q21.2)* have been shown to contain the PTC oncogene (Pierotti et al., submitted for publication), which is formed by rearrangement of the *ret* oncogene (mapped to

10q11.2) and another chromosome 10 gene of unknown function, H4 (mapped to 10q21.2) (18). This suggests that *inv(10)(q11.2q21.2)* is specific for PTC and may represent a primary event in the pathogenesis of a significant number of PTC. Our finding of no loss of alleles on chromosome 10 in PTC further supports this hypothesis.

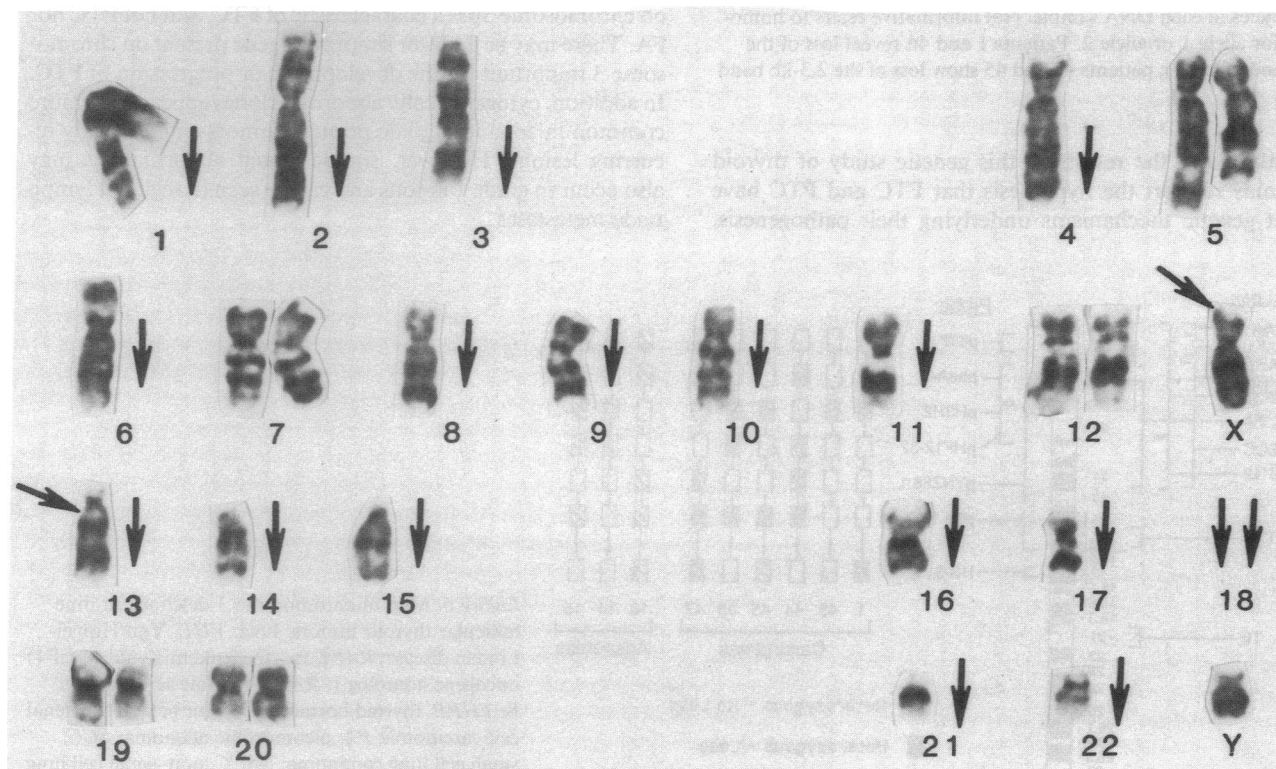
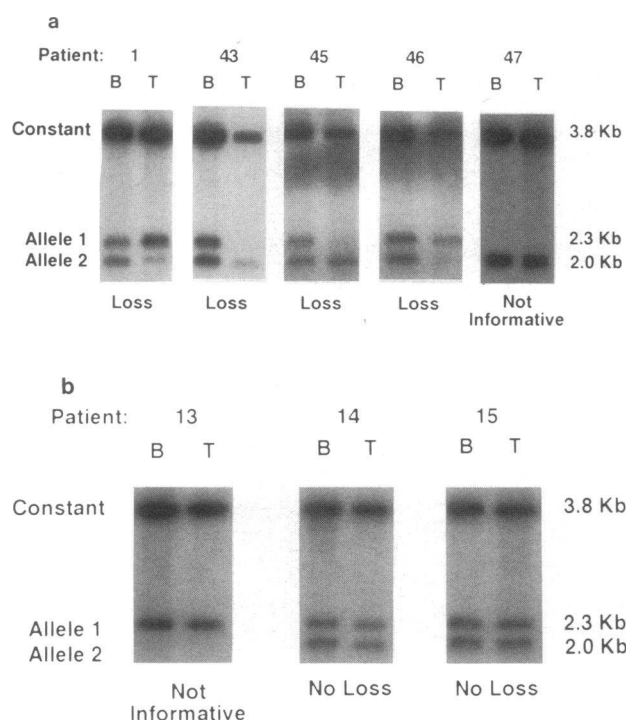


Figure 4. GTL-banded karyotype, grade 2 FTC (patient 47). 28,Y,del(X)(p22),-1,-2,-3,-4,-6,-8,-9,-10,-11,-13,-14,-15,-16,-17,-18,-18,-21,-22,der(13)t(13;?)(q12;?). The del(X) and der(13) were nonclonal.



**Figure 5.** (a) and (b). Representative autoradiography results of normal peripheral blood leukocyte (B) DNA and tumor (T) DNA from five patients with FTC (a) and three patients with FA (b). Hind III restriction enzyme digestion and hybridization with P-32 labeled probe pH3H2 (locus, D3F15S2; map location, 3p21) results in one constant 3.8-kb band and two alleles, allele 1 at 2.3 kb and allele 2 at 2.0 kb. Loss refers to absence of one of the alleles in the tumor DNA compared with the normal DNA. No loss indicates presence of both alleles in each DNA sample. Not informative refers to homozygosity for allele 1 or allele 2. Patients 1 and 46 reveal loss of the 2.0-kb band (allele 1); patients 43 and 45 show loss of the 2.3-kb band (allele 2).

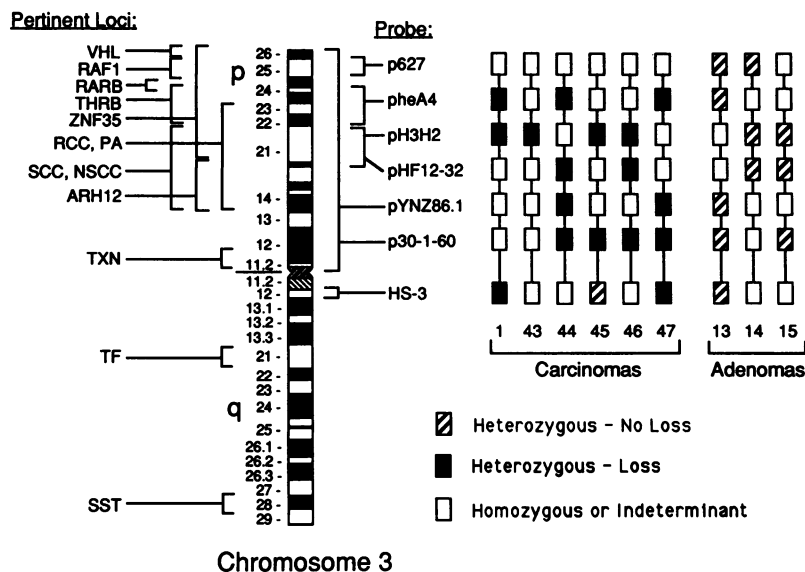
In summary, the results of this genetic study of thyroid carcinomas support the hypothesis that FTC and PTC have different genetic mechanisms underlying their pathogenesis.

**Table IV.** Summary of LOH Results in 6 FTC, 3 FA, and 12 PTC

Tumor type	Chromosome arm						
	1p	3p	3q	10p	10q	16p	17p
<b>Follicular carcinoma</b>							
Number of patients	6	6	6	6	6	6	6
Number informative*	2	6	3	6	6	4	4
Number with loss	0	6	2	3	2	1	1
<b>Follicular adenoma</b>							
Number of patients	3	3	3	3	3	3	3
Number informative	0	3	1	3	3	1	2
Number with loss	0	0	0	0	1	1	0
<b>Papillary carcinoma</b>							
Number of patients	11	12	12	12	12	12	12
Number informative	2	12	3	10	12	8	7
Number with loss	0	0	0	0	0	0	0

\* Informative refers to number of patients who were heterozygous for at least one locus on a chromosome arm. LOH was observed for each FTC patient using probes for 3p (Fig. 6 illustrates the details of losses on chromosome 3 for FTC and FA patients). LOH was observed on chromosome 10: probe TBQ7—FTC patients 1, 45, and 46; probe pTB10.163—FTC patient 46; probe p9-12A—FTC patients 45 and 46; probe 1-101—FTC patient 45 and probe pTHH54—FA patient 15. LOH observed on chromosome 16: FTC patient 46 and FA patient 14. LOH observed on chromosome 17: FTC patient 46.

Structural cytogenetic abnormalities of chromosome 10 appear to be associated with a primary event in papillary tumorigenesis, while cytogenetic and molecular genetic anomalies of chromosome 3 are associated with FTC. Loss of alleles at loci on chromosome 3p is a characteristic of FTC, and not PTC nor FA. There may be a tumor suppressor gene present on chromosome 3 important for the development or progression of FTC. In addition, cytogenetically abnormal clones appear to be more common in advanced grade primary tumors and in locally recurring lesions. However, simple clonal abnormalities may also occur in grade 1 lesions and can be seen in regional lymph node metastases.



**Figure 6.** Map of chromosome 3 deletions in nine follicular thyroid tumors. Loci: *VHL*, Von Hippel-Lindau disease; *RAF1*, murine leukemia viral (v-raf-1) oncogene homolog 1; *RARB*, retinoic acid receptor B; *THRB*, thyroid hormone receptor beta; *RCC*, renal cell carcinoma; *PA*, pleomorphic adenoma; *SCC*, small cell lung carcinoma; *NSCC*, non-small cell lung carcinoma; *TF*, transferrin; *SST*, somatostatin; *ARH12*, *ras* related gene; *TXN*, human thioredoxin; *ZNF35*, zinc finger protein gene.



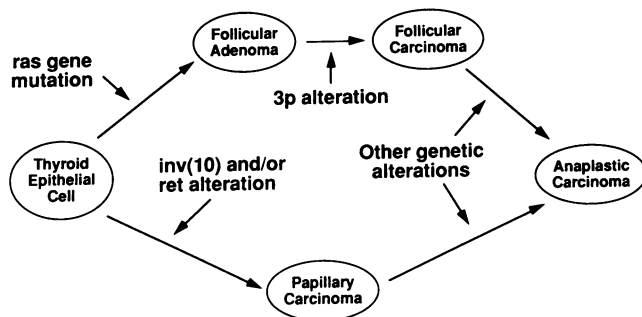


Figure 7. A model for the pathogenesis of nonmedullary thyroid neoplasia.

We favor the model of thyroid tumor formation and progression proposed by Lemoine et al. (48), and would further propose that alterations on chromosome 3p may facilitate the progression of FA to FTC, while *inv(10)*, with rearrangement of the *ret* oncogene, is specifically involved in the pathogenesis of PTC (Fig. 7). Other, as yet unknown, genetic events may contribute to the rare transformation to an undifferentiated (anaplastic) carcinoma (1, 51).

While the overall prognosis of PTC and FTC is good, thus making clinical correlation of these cytogenetic and molecular genetic findings premature, perhaps the risk for metastases or recurrence may eventually be predicted from the presence or absence of genetic abnormalities. For the present, however, we believe that the results of these studies have provided a substrate for further more sophisticated genetic investigation of these relatively common thyroid tumors.

## Acknowledgments

The authors wish to thank Sharon Cooke for assistance in the preparation of karyotypes for this manuscript, and Karen Erwin for assistance in preparation of the manuscript.

## References

- Hedinger, C., E. D. Williams, and L. H. Sobin. 1989. The WHO histologic classification of thyroid tumors: a commentary on the second edition. *Cancer (Phila.)* 63:908-911.
- Herrmann, M. A., I. D. Hay, D. H. Bartelt, Jr., J. L. Spurbeck, R. J. Dahl, C. S. Grant, and R. B. Jenkins. 1991. Cytogenetics of six follicular adenomas including a case report of an oxyphil variant with a t(8;14)(q13;q24.1). *Cancer Genet. Cytogenet.* In press.
- Herrmann, M. E., G. Talpas, A. Mohamed, A. Saxe, S. Ratner, and S. R. Wolman. 1991. Genetic markers in thyroid tumors. *Surgery (St. Louis)*. In press.
- Schreck, R. R., T. Ross, and J. A. Fagin. 1991. Cytogenetic analysis of follicular and papillary thyroid tumors. *Cancer Genet. Cytogenet.* In press.
- Teyssier, J. R., F. Liautaud-Roger, D. Ferre, M. Patey, and J. Dufer. 1990. Chromosomal changes in thyroid tumors. Relation with DNA content, karyotypic features, and clinical data. *Cancer Genet. Cytogenet.* 50:249-263.
- Jenkins, R. B., I. D. Hay, J. F. Herath, C. G. Schultz, J. L. Spurbeck, C. S. Grant, J. R. Goellner, and G. W. Dewald. 1990. Frequent occurrence of cytogenetic abnormalities in sporadic nonmedullary thyroid carcinoma. *Cancer (Phila.)* 66:1213-1220.
- Olah, E., E. Balogh, F. Bojan, F. Juhasz, V. Stenszky, and N. R. Farid. 1990. Cytogenetic analyses of three papillary carcinomas and a follicular adenoma of the thyroid. *Cancer Genet. Cytogenet.* 44:119-129.
- Van den Berg, E., J. W. Oosterhuis, B. de Jong, J. Buist, A. M. Vos, A. Dam, and B. Vermeij. 1990. Cytogenetics of thyroid follicular adenomas. *Cancer Genet. Cytogenet.* 44:217-222.
- Antonini, P., A. M. Venuat, G. Linares, B. Caillou, R. Berger, and C. Parmentier. 1989. A translocation (7;10)(q35;q21) in a differentiated papillary carcinoma of the thyroid. *Cancer Genet. Cytogenet.* 41:139-144.
- Herrmann, M. E., J. Bullerdiek, S. Bartnitzke, B. Lebert, H. Lobeck, and W. Zuschneid. 1989. Cytogenetic studies on 15 thyroid adenomas. *Cancer Genet. Cytogenet.* 41:65. (Abstr.)
- Antonini, P., A. M. Venuat, R. Berger, G. Caillou, J. L. Rossignol, and C. Parmentier. 1989. Cytogenetic abnormalities in thyroid tumors. *Cancer Genet. Cytogenet.* 38:190. (Abstr.)
- Bondeson, L., A. Bengtsson, A. G. Bondeson, R. Dahlenfors, L. Grime-lius, B. Wedell, and J. Mark. 1989. Chromosome studies in thyroid neoplasia. *Cancer (Phila.)* 64:680-685.
- Bartnitzke, S., M. E. Herrmann, H. Lobeck, W. Zuschneid, P. Neuhaus, and J. Bullerdiek. 1989. Cytogenetic findings on eight follicular thyroid adenomas including one with a t(10;19). *Cancer Genet. Cytogenet.* 39:65-68.
- Mark, J., C. Ekedahl, R. Dahlenfors, and B. Wester-Mark. 1987. Cytogenetic observations in five human anaplastic thyroid carcinomas. *Hereditas* 107:163-174.
- Teyssier, J. R. 1987. Nonrandom chromosomal changes in human solid tumors: application of an improved culture method. *JNCI (J. Natl. Cancer Inst.)* 79:1189-1198.
- Fusco, A., M. Grieco, M. Santoro, M. T. Berlingieri, S. Pilotti, M. A. Pierotti, G. Della Porta, and G. Vecchio. 1987. A new oncogene in human thyroid papillary carcinomas and their lymphnodal metastases. *Nature (Lond.)* 328:170-172.
- Donghi, R., G. Sozzi, M. A. Pierotti, I. Biunno, M. Miozzo, A. Fusco, M. Grieco, M. Santoro, G. Vecchio, N. K. Spurr, and G. Della Porta. 1989. The oncogene associated with human papillary thyroid carcinoma (PTC) is assigned to chromosome 10 q11-q12 in the same region as multiple endocrine neoplasia type 2A (MEN2A). *Oncogene* 4:521-523.
- Grieco, M., M. Santoro, M. T. Berlingieri, R. M. Melillo, R. Donghi, I. Bongarzone, M. A. Pierotti, G. Della Porta, A. Fusco, and G. Vecchio. 1990. PTC is a novel rearranged form of the *ret* proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell* 60:557-563.
- Fearon, E. R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759-767.
- Spurbeck, J. L., R. O. Carlson, J. E. Allen, and G. W. Dewald. 1988. Culturing and robotic harvesting of bone marrow, lymph nodes, peripheral blood, fibroblasts, and solid tumors with *in situ* techniques. *Cancer Genet. Cytogenet.* 32:59-66.
- Standing Committee on Human Cytogenetic Nomenclature. 1985. An international system for human cytogenetic nomenclature. *Birth Defects Orig. Artic. Ser.* 21:1-117.
- Müllénbach, R., P. J. L. Lagoda, and C. Welter. 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genet.* 5:391.
- Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503-517.
- Gumucio, D. C., M. H. Meisler, and J. R. Kidd. 1985. Detection of two RFLPs near the human salivary amylase gene on the short arm of human chromosome 1. *Am. J. Hum. Genet.* 37:A155. (Abstr.)
- Seizinger, B., G. A. Rouleau, L. J. Ozelius, A. H. Lane, G. E. Farmer, J. M. Lamiell, J. Haines, J. W. M. Yuen, D. Collins, D. Majoor-Krakauer, et al. 1988. Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. *Nature (Lond.)* 332:268-269.
- Weinberger, C., C. C. Thompson, E. S. Ong, R. Lebo, D. J. Gruol, and R. M. Evans. 1986. The *c-erb-A* gene encodes a thyroid hormone receptor. *Nature (Lond.)* 324:641-646.
- Kok, K., J. Osinga, B. Carritt, M. B. Davis, A. H. van der Hout, A. Y. van der Veen, R. M. Landsvater, L. F. M. H. de Leij, H. H. Berendsen, P. E. Postmus, et al. 1987. Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. *Nature (Lond.)* 330:578-581.
- Naylor, S. L., A. Y. Sakaguchi, B. U. Zabel, D. Barker, R. White, and T. B. Shows. 1984. Assignment of polymorphic DNA segments to human chromosomes. *Cytogenet. Cell Genet.* 37:553.
- Nakamura, Y., M. Culver, S. Gillilan, P. O'Connell, M. Leppert, G. M. Lathrop, J. M. Lalouel, and R. White. 1987. Isolation and mapping of a polymorphic DNA sequence pYNZ86.1 on chromosome 3 (D3S30). *Nucleic Acids Res.* 15:10079.
- Litt, M., R. Litt, L. Ballard, and M. Leppert. 1989. An anonymous single-copy clone, p30-1-60, identifies a frequent RFLP on chromosome 3p. *Nucleic Acids Res.* 17:2883.
- Kok, K., S. L., A. Y. Sakaguchi, D. Barker, R. White, and T. B. Shows. 1984. DNA polymorphic loci mapped to human chromosomes 3, 5, 9, 11, 17, 18, and 22. *Proc. Natl. Acad. Sci. USA* 81:2447-2451.
- Bragg, T., Y. Nakamura, C. Jones, and R. White. 1988. Isolation and mapping of a polymorphic DNA sequence (cTBQ7) on chromosome 10 (D10S28). *Nucleic Acids Res.* 16:11395.
- Holm, T., Y. Nakamura, L. Ballard, P. O'Connell, M. Leppert, G. M. Lathrop, J. M. Lalouel, and R. White. 1988. Isolation and mapping of a polymorphic DNA sequence pTHH54 on chromosome 10 (D10S14). *Nucleic Acids Res.* 16:372.
- Radice, P., R. Donghi, G. Sozzi, M. A. Pierotti, M. Miozzo, A. Longoni,

- A. Fusco, M. Grieco, M. Santoro, G. Vecchio, N. K. Spurr, and G. Della Porta. 1989. DNA sequences linked to the human papillary thyroid carcinoma (PTC) oncogene map to chromosome 10q11-q12 and identify Taq I and Hinc II RFLPs. *Cytogenet. Cell Genet.* 51:934.
35. Liou, G. I., Y. Li, C. Wang, S. L. Fong, S. Bhattacharya, and C. D. B. Bridges. 1987. Bgl II RFLP recognized by a human IRBP cDNA localized to chromosome 10. *Nucleic Acids Res.* 15:3196.
36. Bragg, T., Y. Nakamura, E. Fujimoto, P. O'Connell, M. Leppert, G. M. Lathrop, J. M. Lalouel, and R. White. 1988. Isolation and mapping of a polymorphic DNA sequence (pTB10.163) on chromosome 10 (D10S22). *Nucleic Acids Res.* 16:4185.
37. McDermid, H. E., P. J. Goodfellow, A. M. V. Duncan, K. R. Brasch, N. E. Simpson, C. D. Souza, J. J. A. Holden, and R. White. 1987. A polymorphic locus, D10S5, at 10q21.1. *Nucleic Acids Res.* 15:5498.
38. Nakamura, Y., T. Bragg, R. Myers, P. O'Connell, M. Leppert, G. M. Lathrop, J. M. Lalouel, and R. White. 1988. Isolation and mapping of a polymorphic DNA sequence (pTB10.171) on chromosome 10 (D10S19). *Nucleic Acids Res.* 16:4187.
39. Litt, M., O. T. Mueller, T. B. Shows, and R. Litt. 1987. A single copy subclone, p1-101, from cosmid 3-3B, defines three RFLPs on 10pter-q23. *Nucleic Acids Res.* 15:2783.
40. Farrer, L. A., C. M. Castiglione, J. R. Kidd, S. Myers, N. Carson, N. E. Simpson, and K. K. Kidd. 1988. A linkage group of five DNA markers on human chromosome 10. *Genomics.* 3:72-77.
41. Reeders, S. T., M. H. Breuning, K. E. Davies, R. D. Nicholls, A. P. Jarman, D. R. Higgs, P. L. Pearson, and D. J. Weatherall. 1985. A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. *Nature (Lond.)* 317:542-544.
42. Nakamura, Y., L. Ballard, M. Leppert, P. O'Connell, G. M. Lathrop, J. M. Lalouel, and R. White. 1988. Isolation and mapping of a polymorphic DNA sequence (pYNZ22) on chromosome 17p (D17S30). *Nucleic Acids Res.* 16:5707.
43. Feinberg, A. P., and B. L. Vogelstein. 1984. Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266-267.
44. Boring, C. C., T. S. Squires, and T. Tong. 1991. Cancer Statistics, 1991. *CA-Cancer J. Clin.* 41:19-39.
45. Hay, I. D. 1989. Prognostic factors in thyroid carcinoma. *Thyroid Today.* 12:1-9.
46. Williams, E. D. 1979. The aetiology of thyroid tumors. *Clin. Endocrinol. Metab.* 8:193-207.
47. Lemoine, N. R., E. S. Mayall, F. S. Wyllie, C. J. Farr, D. Hughes, R. A. Padua, V. Thurston, E. D. Williams, and D. Wynford-Thomas. 1988. Activated *ras* oncogenes in human thyroid cancers. *Cancer Res.* 48:4459-4463.
48. Lemoine, N. R., E. S. Mayall, F. S. Wyllie, E. D. Williams, M. Goyns, B. Stringer, and D. Wynford-Thomas. 1989. High frequency of *ras* oncogene activation in all stages of human thyroid tumorigenesis. *Oncogene.* 4:159-164.
49. Wyllie, F. S., N. R. Lemoine, E. D. Williams, and D. Wynford-Thomas. 1989. Structure and expression of nuclear oncogenes in multi-stage thyroid tumorigenesis. *Br. J. Cancer.* 60:561-565.
50. Wright, P. A., N. R. Lemoine, E. S. Mayall, F. S. Wyllie, D. Hughes, E. D. Williams, and D. Wynford-Thomas. 1989. Papillary and follicular thyroid carcinomas show a different pattern of *ras* oncogene mutation. *Br. J. Cancer.* 60:576-577.
51. Brennan, M. D., E. J. Bergstralh, J. A. van Heerden, and W. M. McConahey. 1991. Follicular thyroid cancer treated at the Mayo Clinic 1946 through 1970: initial manifestations, pathologic findings, therapy, and outcome. *Mayo Clin. Proc.* 66:11-22.
52. Teyssier, J. R. 1989. The chromosomal analysis of human solid tumors. A triple challenge. *Cancer Genet. Cytogenet.* 37:103-125.
53. Khosla, S., V. M. Patel, I. D. Hay, D. J. Schaid, C. S. Grant, J. A. Van Heerden, and S. N. Thibodeau. 1991. Loss of heterozygosity suggests multiple genetic alterations in pheochromocytoma, and medullary thyroid carcinomas. *J. Clin. Invest.* 87:1691-1699.
54. Kovacs, G., R. Erlandsson, F. Boldog, S. Ingvarsson, R. Müller-Brechlin, G. Klein, and J. Sümegi. 1988. Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma. *Proc. Natl. Acad. Sci. USA.* 85:1571-1575.
55. Mooibroek, H., J. Osinga, P. E. Postmus, B. Carritt, and C. H. C. M. Buys. 1987. Loss of heterozygosity for a chromosome 3 sequence presumably at 3p21 in small cell lung cancer. *Cancer Genet. Cytogenet.* 27:361-365.
56. Naylor, S. L., and B. Carritt. 1990. Report of the committee on the genetic constitution of chromosome 3. Human gene mapping 10.5. *Cytogenet. Cell Genet.* 55:92-96.
57. Mitelman, F., Y. Kaneko, and J. M. Trent. 1990. Report of the committee on chromosome changes in neoplasia. Human gene mapping 10.5. *Cytogenet. Cell Genet.* 55:358-386.
58. Pierre, R. V., and H. C. Hoagland. 1971. 45,X cell lines in adult men: loss of Y chromosome, a normal aging phenomenon? *Mayo Clin. Proc.* 46:52-55.
59. Heim, S., N. Mandahl, Y. Jin, S. Stromblad, E. Lindstrom, L. G. Salford, and F. Mitelman. 1989. Trisomy 7 and sex chromosome loss in human brain tissue. *Cytogenet. Cell Genet.* 52:136-138.
60. Becher, R., Z. Gibas, C. Karakousis, and A. A. Sandberg. 1983. Nonrandom chromosome changes in malignant melanoma. *Cancer Res.* 43:5010-5016.
61. Pedersen, M. I., J. W. Bennett, and N. Wang. 1986. Nonrandom chromosome structural aberrations and oncogene loci in human malignant melanoma. *Cancer Genet. Cytogenet.* 20:11-27.
62. Smeets, W., R. Pauwels, L. Laarakkers, F. Debruyne, and J. Geraedts. 1987. Chromosomal analysis of bladder cancer. II. Nonrandom alterations. *Cancer Genet. Cytogenet.* 29:29-41.
63. Vanni, R., R. M. Scarpa, M. Nieddu, and E. Usai. 1988. Cytogenetic investigation on 30 bladder carcinomas. *Cancer Genet. Cytogenet.* 30:35-42.
64. Popescu, N. C., A. P. Chahinian, and J. A. Dipaolo. 1988. Nonrandom chromosome alterations in human malignant mesothelioma. *Cancer Res.* 48:142-147.