Hum Genet (1997) 99 : 342–349 © Springer-Verlag 1997

ORIGINAL INVESTIGATION

Filip Farnebo · Bin Tean Teh · Cornelia Dotzenrath
Wassif S. Wassif · Ann Svensson · Irene White
Regina Betz · Peter Goretzki · Kerstin Sandelin
Lars-Ove Farnebo · Catharina Larsson

Differential loss of heterozygosity in familial, sporadic, and uremic hyperparathyroidism

Received: 28 October 1996 / Revised: 16 November 1996

Abstract Various genetic loci harboring oncogenes, tumor suppressor genes, and genes for calcium receptors have been implicated in the development of parathyroid tumors. We have carried out loss of heterozygosity (LOH) studies in chromosomes 1p, 1q, 3q, 6q, 11q, 13q, 15q, and X in a total of 89 benign parathyroid tumors. Of these, 28 were sporadic parathyroid adenomas from patients with no family history of the disease, 41 were secondary parathyroid tumors, 5 were from patients with a history of previous irradiation to the neck, 12 were from patients with a family history of hyperparathyroidism, and 3 were parathyroid tumors related to multiple endocrine neoplasia type 1 (MEN1). In addition, we determined the chromosomal localization of a second putative calcium-sensing receptor, CaS, for inclusion in the LOH studies. Based on analysis of somatic cell hybrids and fluorescent in situ hybridization to metaphase chromsomes, the gene for CaS was mapped to chromosomal region 2q21-q22. The following results were obtained from the LOH studies: (1) out of the 24 tumors that showed LOH, only 4 had more than one chromosomal region involved, (2) in the tumours from uremic patients, LOH of chromosome 3q was detected in a subset of the tumors, (3) LOH of the MEN1 region at 11q13 was the most common abnormality found in both MEN1-related and sporadic parathyroid tumours but was not a feature of the other forms of parathyroid tumors, (4) LOH in 1p and 6q was not as frequent as previously reported, and (5) tumor suppressor genes in 1q and X might have played a role, particularly on the X chromosome, in the case of familial parathyroid adenomas. We therefore conclude that the tumorigenesis of familial, sporadic, and uremic hyperparathyroidism involves different genetic triggers in a non-progressive pattern.

F. Farnebo (✉) · B. T. Teh · I. White · R. Betz · C. Larsson
Department of Molecular Medicine, Endocrine Tumor Unit, Karolinska Hospital L6 Building, S-171 76 Stockholm, Sweden
Fax: + 46-8-327734; e-mail: ffa@gen.ks.se

C. Dotzenrath · P. Goretzki
Department of Surgery, Heinrich-Heine-Universität, Düsseldorf, Germany

W. S. Wassif
Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, London, UK

A. Svensson · K. Sandelin · L.-O. Farnebo
Department of Surgery, Karolinska Hospital, S-171 76 Stockholm, Sweden

Introduction

Various genetic abnormalities have been reported as taking part in parathyroid tumorigenesis. Most studies concern loci known to harbor genes involved in the formation of neoplasia, oncogenes and tumor suppressor genes. A few studies have investigated mechanisms regulating parathyroid hormone (PTH) secretion.

Loss of heterozygosity (LOH) in 11q13, the region that harbors the putative tumor suppressor gene of multiple endocrine neoplasia type 1 (MEN1), has been demonstrated not only in MEN1-related parathyroid tumors, but also in up to 30% of sporadic parathyroid tumors (Larsson et al. 1988; Arnold et al. 1988; Byström et al. 1990; Friedman et al. 1992). Tumor development in uremic hyperparathyroidism has also been suggested to involve deletions of the MEN1 locus (Falchetti et al. 1993), but subsequent studies indicate that this alteration only occurs in a small subset of these cases (Arnold et al. 1995; Farnebo et al. 1996).

The cell-cycle regulator PRAD1/cyclin D1 has been established to play a role in a subset of tumors (Rosenberg et al. 1991), probably by overexpression of the wild-type PRAD1 sequence. Another hyperparathyroidism-related gene has been localized to chromosome 1q21-q32, the locus for the hyperparathyroidism-jaw tumor syndrome (HPT-JT, HPRT2; Szabo et al. 1995). Although there is no evidence so far of LOH in HPT-JT-associated parathyroid tumors, LOH has been found in associated "renal hamartomas" (Teh et al. 1996). Since the loss always involves the wild-type allele derived from the unaffected parent, this suggests the inactivation of a tumor supressor gene in

the region (Teh et al. 1996). Deletions at the retinoblastoma gene locus (RB1) on chromosome 13q occur frequently in parathyroid carcinoma and atypical recurrent disease (Cryns et al. 1994; Dotzenrath et al. 1996). An allelotyping study of primary sporadic parathyroid tumors has also implied that hitherto unidentified tumor supressor genes on chromosome 1p, 6q, 15q, and X are involved (Tahara et al. 1996). The enlarged parathyroid glands found in patients with secondary HPT have been shown to be mainly monoclonal in nature (Arnold et al. 1995), but a recurrent chromosomal abnormality has not yet been identified in this subgroup.

The secretion of PTH is controlled by extracellular calcium via receptors on the surface of the parathyroid cell. The gene coding for the calcium receptor (CaR), a seven transmembrane receptor with a well-characterized function, is located on chromosome 3q (Brown et al. 1993, 1995). Inactivating heterozygous mutations cause familial hypocalcuric hypercalcemia (FHH), whereas homozygous mutations cause neonatal severe hyperparathyroidism, in which parathyroid hyperplasia also occurs (Pollak et al. 1993; Heath et al. 1996). It has been speculated that mutations in CaR could also induce parathyroid tumor formation.

Another protein with possible calcium-sensing properties has been described. This protein (CaS) is a member of the low density lipoprotein receptor super family (Lundgren et al. 1994). Immunohistochemistry has demonstrated reduced expression of this protein in parathyroid adenomas and in the enlarged glands of secondary HPT, compared with normal parathyroid glands (Juhlin et al. 1989). Monoclonal antibodies against CaS block the effect of increased extracellular calcium on the secretion of PTH in vitro (Juhlin et al. 1987; Nygren et al. 1988).

We have analyzed tissue samples from five different types of benign parathyroid lesions in 70 patients, i.e., a total of 89 tumors, for LOH in chromosomal regions implicated in the development of sporadic parathyroid tumors. The human chromosomal position of the gene for CaS has also been determined by analysis of somatic cell hybrids and fluorescence in situ hybridization (FISH).

Materials and methods

Patients and tumor samples

This study included matched pairs of constitutional and tumor samples from 70 patients with various types of benign parathyroid lesions. A total of 89 tumors were studied, including 28 sporadic tumors, 41 tumors from patients with secondary hyperparathyroidism, 5 tumors from patients who had previously undergone radiation treatment to the neck, 12 tumors from patients with hereditary predisposition to hyperparathyroidism, and 3 tumors from patients with MEN1.

Immediately after surgical removal, parathyroid tissue was frozen in liquid nitrogen and stored at –70°C. High molecular weight DNA was extracted from fresh frozen tumor tissues and peripheral blood leukocytes by using standard methods. In some cases, tumor DNA was isolated from paraffin-embedded parathyroid tissue (Shibata 1994).

LOH studies

The following microsatellite markers were selected: D1S468, D1S228, D1S507, and D1S513 on 1p; D1S428, D1S412, and D1S413 at the HPT-JT locus on 1q; D2S141, D2S156, D2S389, and D2S311at the CaS locus on 2q21-q22; D3S1215, D3S1303, D3S1267, D3S1269, D3S1316, and D3S1309 flanking the FHH locus on 3q; D6S297 on chromosome 6q; PYGM (muscle glycogen phosphorylase) and D11S97 in the MEN1 region at 11q13; D13S267, D13S287, D13S164, RB1.20 (retinoblastoma), D13S153, and D13S71 at 13q; GABRB3 (gamma aminobutyric acid A receptor beta 3) and D15S120 on chromosome 15; and MAOB (monoamine oxidase B), DXS453, and DXS3 on the X chromosome (Fig. 1). Polymerase chain reactions (PCR) were performed in a total volume of 10 µl containing 50–100 ng constitutional or tumor DNA, 50 mM KCl, 10 mM TRIS-HCl (pH 8.3), 1.5 mM $MgCl_2$, 125 µM of each dNTP, 2 pmol of each oligodeoxynucleotide primer (one of which was end-labeled with [^{32}P]), and 0.2 U DNA polymerase (Dynazyme, Finnzyme Oy). Samples were amplified by using a hot-start of 5 min at 94°C, 25 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s (alternative cycle 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s) followed by a further elongation at 72°C for 7 min. Polyacrylamide gel electrophoresis was carried out on the PCR products, followed by either autoradiography or digital imaging (Bio-imaging analyzer Bas 1000, Fuji). LOH was defined either as a total absence or as a reduction of $\geq$50% of the signal intensity of one of the constitutional alleles in the tumor compared with the constitutional DNA.

Somatic cell hybrids

High molecular weight DNA was isolated from peripheral human leukocytes, Chinese hamster cells, mouse liver, and somatic hybrid cell lines (hamster/human and mouse/human) retaining individual human chromosomes (NIGMS Coriell Cell Repositories). To establish on which human chromosome the CaS gene was located, the somatic cell hybrid panels were screened by PCR. Primers were designed to match a CaS-specific sequence of the cDNA clone published by Lundgren et al. (1994). Approximately 1 µg DNA from each cell hybrid was combined with 10 pmol of each primer (5′-GAGTCTGCCTGGATGAATGGAG and 5′-GCTTCCTTTGCAATGACTCTCC), and combined with 2.5 U *Taq* polymerase in 25 µl PCR buffer (10 mM TRIS-HCl pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatine, 0.2 mM dNTPs). Amplification was carried out by using a hot-start at 94°C, 35 cycles of denaturing at 94°C for 60 s, annealing at 65°C for 60 s, and elongation at 72°C for 60 s. The PCR products were analyzed by electrophoresis in a 2% agarose gel.

Isolation of a genomic clone for CaS

A human placenta cosmid library in pWE15 was transferred to nitrocellulose filters and screened with the ^{32}P-labeled PCR fragment of CaS derived as described above. After hybridization, the filters were washed under stringent conditions, and the positive clones were re-screened until pure, by using standard methods. DNA from the purified cosmids was isolated by means of Quiagen columns and verified by Southern blot analysis after *Eco*RI cleavage.

Fluorescence in situ hybridization

Slides with human metaphase chromosomes were prepared from 5-bromodeoxyuridine-synchronized lymphocyte cultures from normal controls (Benn and Perle 1988). The slides were post-fixed, RNAse-treated, and denatured as previously described (Blennow et al. 1992; Pinkel et al. 1986). The cosmid clone for CaS was used as a probe after labeling with biotin-16-dUTP by means of a nick-translation kit (BRL). The probe (50 ng) was pre-annealed with Cot-1 DNA (4 µg) for 30–60 min at 37°C after denaturing in 68°C for 10 min. Hybridization was performed in 50% formamide at

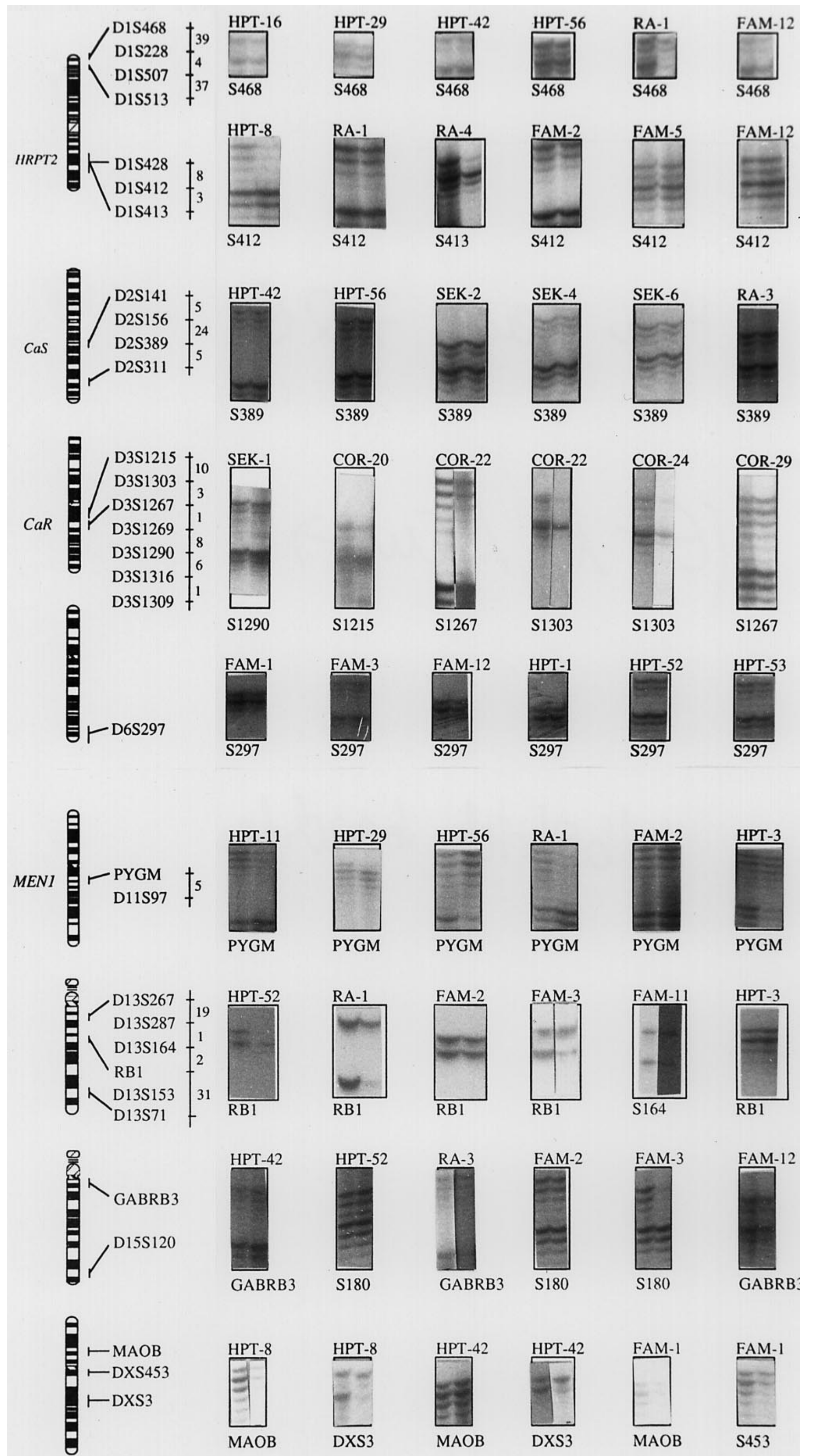

Fig. 1 Autoradiograms showing retention or loss of heterozygosity at loci on each of the investigated chromosomes. The physical location of and genetic distances (in cM) between the markers are indicated on the idiograms (*left*)

Table 1 Summary of LOH detected in the 89 benign parathyroid tumors studied (*SHPT* secondary hyperparathyroidism, *RA* radiation associated)

Locus		Classification of tumor											
		Sporadic		SHPT		RA		Familial		MEN1		Total	
		Inf	LOH	Inf	LOH	Inf	LOH	Inf	LOH	Inf	LOH	Inf	LOH
Total no. cases		28		41		5		12		3		89	
1p	S468	14	–	31	–	1	1	4	–	2	–	52	1
	S228	16	–	29	–	1	–	8	–	1	–	55	–
	S507	20	–	28	–	2	1	5	–	1	–	56	1
	S513	19	–	30	–	1	–	2	–	1	–	53	–
	Total	22	–	38	–	2	1	12	–	2	–	76	1
1q	S428	11	2	9	–	1	–	4	–	1	–	26	2
	S412	5	1	7	–	1	–	10	–	1	–	24	1
	S413	14	1	33	–	4	1	6	–	1	–	58	2
	Total	20	2	33	–	5	1	12	–	3	–	73	3
2q	S141	13	–	15	–	3	–	4	–	1	–	36	–
	S156	12	–	15	–	3	–	11	–	1	–	42	–
	S389	15	–	15	–	4	–	5	–	1	–	40	–
	S311	13	–	18	–	1	–	5	–	1	–	38	–
	Total	20	–	18	–	4	–	11	–	1	–	54	–
3q	S1215			14	1							14	1
	S1303			13	2							13	2
	S1267	20	–	30	2	3	–	10	–	2	–	65	2
	S1269			15	1							15	1
	S1290	20	–	33	–	5	–	5	–	1	–	64	–
	S1316	16	–	26	–	4	–	4	–	1	–	51	–
	S1309	15	–	20	–	3	–	5	–	1	–	44	–
	Total	23	–	39	4	5	–	11	–	2	–	80	4
6q	S297	8	–	8	–	1	–	7	1	–	–	24	1
11q	PYGM	25	6	31	1	4	1	9	–	3	1	72	9
	S97									3	2	3	2
	Total	25	6	31	1	4	1	9	–	3	2	72	10
13q	S267	15	–	17	–	–	–	5	1	1	–	38	1
	S287	11	–	11	–	1	–	4	–	1	–	28	–
	S164	11	–	7	–	–	–	5	–	1	–	24	–
	RB1	24	1	32	–	5	1	6	1	3	–	70	3
	S153							4	1			4	1
	S71							4	–			4	–
	Total	26	1	37	–	5	1	11	3	3	–	82	5
15q	GABRB3	17	–	13	–	1	1	7	1	1	–	39	2
	S120	7	–	12	–	–	–	8	1	1	–	28	1
	Total	18	–	17	–	1	1	12	1	1	–	49	2
X	MAOB	15	1	4	–	3	–	4	1	–		26	2
	S453	8	–	3	–	2	–	5	2	1	–	19	2
	S3	11	2	2	–	1	–	3	1	1	–	18	3
	Total	18	2	16	–	3	–	11	2	1	–	49	4

42°C overnight in a moist chamber. The slides were then washed three times for 5 min in 50% formamide, 2 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at 42°C and then three times for 5 min in 0.1 × SSC at 60°C. After being washed, the slides were left in BT-buffer (0.1 M $NaHCO_3$ pH 8.0, 0.05% Tween 20) for 10 min. Probe detection and signal amplification were performed by applying two alternating layers of fluorescein-avidin DCS (Vector Lab) and biotinylated anti-avidin antibodies (Vector Lab). After dehydration, the slides were mounted in glycerol containing 2.3% 1,4-diazabicyclo-(2,2,2) octane as antifade, and 4,6-diamino-2-phenyl-indole (DAPI) at 0.5 µg/ml as counterstain. The signal was visualized by using a Zeiss Axioskop fluorescence microscope equipped with a cooled charge-coupled device camera (Photometrics Sensys) controlled by a Power Macintosh Quadra 950 computer. Gray-scale images were captured, pseudocolored, and merged using SmartCapture software (Digital Scientific, Cambridge). Two independent experiments were performed, a minimum of 10 metaphases being scored in each experiment.

Table 2 Details of the 24 parathyroid tumors showing LOH (*LOH* loss of heterozygosity, – non-informative, i.e., constitutional homozygosity, + retained heterozygosity, *blank* not done, *SHPT* secondary hyperparathyroidism, *RA* radiation associated)

Tumor type	Tumor no.	Sex (M/F)	Chromosome								
			1p	1q	2q	3q	6q	11q	13q	15q	X
Sporadic	HPT-7	F	+	+	+	+	–	LOH	–	–	–
	HPT-8	F	+	LOH	+	+	–	+	+	+	LOH
	HPT-11	F	+	+	+	+	–	LOH	+	+	+
	HPT-16	F	+	LOH	+	+	–	+	+	+	+
	HPT-29	F	+	+	+	+	+	LOH	+	+	+
	HPT-42	F	+	+	+	+	+	+	+	+	LOH
	HPT-47	F	+	+	+	+	–	LOH	+	+	+
	HPT-48	F	+	+	+	+	–	LOH	+	+	+
	HPT-52	F	+	+	+	+	+	+	LOH	+	+
	HPT-56	F	+	+	+	+	+	LOH	+	+	+
SHPT	SEK-1	M	+	+	+	+	–	LOH	+	+	–
	COR-20	F	+	+		LOH		+	+		
	COR-22	F	+	+		LOH		+	+		
	COR-24	M	+	–		LOH		+	–		
	COR-32	M	+	+		LOH		+	+		
RA	RA-1	F	LOH	+	+	+	–	LOH	LOH	–	+
	RA-3	F	–	+	+	+	–	–	+	LOH	+
	RA-4	F	–	LOH	–	+	–	+	+	–	–
Familial	FAM-1	F	+	+	+	+	+	+	+	+	LOH
	FAM-3	F	+	+	+	+	+	–	LOH	LOH	LOH
	FAM-10	M	+	+	+	–	+	+	LOH	+	+
	FAM-12	F	+	+	+	+	LOH	+	LOH	+	+
MEN1	HPT-3	F	+	+	+	+	–	LOH	+	+	+
	HPT-9	F	–	+	–	–	–	LOH	+	–	–
Total no. LOH			1	3	0	4	1	10	5	2	4

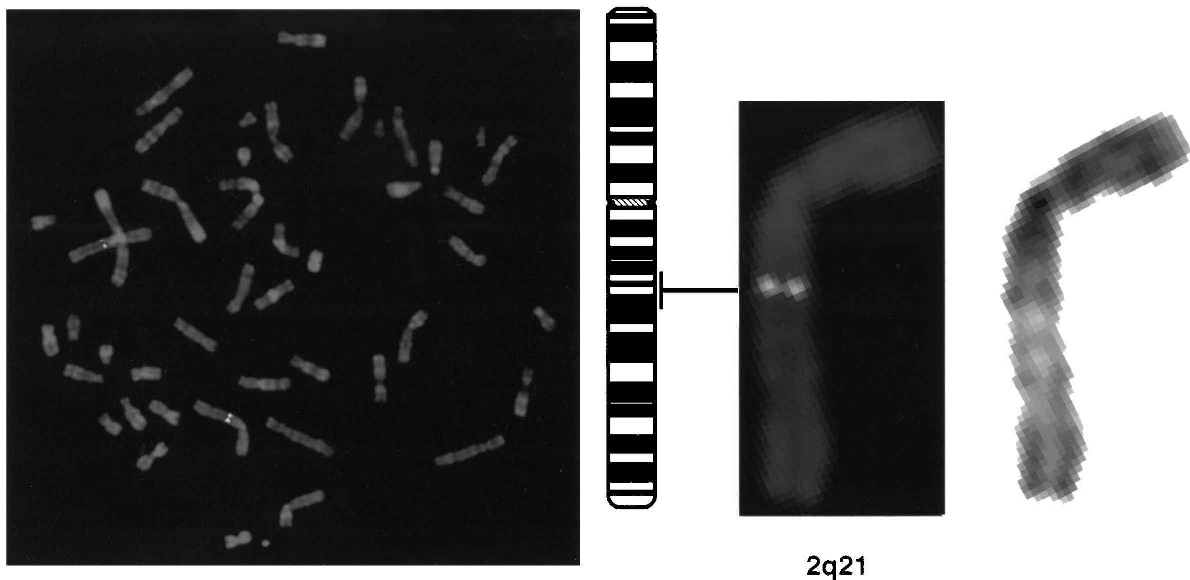

Fig. 2 Assignment of the CaS gene to human chromosomal region 2q21-q22. FISH mapping using a CaS cosmid is shown to the *left* of the idiogram; an enlargement of one chromosome 2 and the banding pattern is given to the *right* of the idiogram

Results

The results from the LOH analysis are summarized in Tables 1 and 2, and Fig. 1. The overall rate of LOH was low. Only 24 of the 89 tumors studied showed LOH, which was restricted to one chromosomal region in all but four cases. The tumors were divided into five groups based on

clinical characteristics. The sporadic adenomas showed loss of the MEN1 locus in 11q13 in 24% of the informative cases (6/25). Losses were also found on 1q in two additional tumors, on 13q in one tumor, and on the X chromosome in two tumors (Table 2). Only one tumor showed loss at two chromosomal regions, viz., 1q and X. Four patients with secondary hyperparathyroidism exhibited loss on 3q (10%). In another patient, one out of four tumors showed loss at 11q13. Three out of five tumors from patients previously given irradiation to the neck possessed random losses at several regions (Table 2). Four familial tumors showed losses that, in three cases, involved 13q and, in two cases, the X chromosome. However, none of the familial tumors exhibited LOH involving the MEN1 locus. With regard to the MEN1 tumors, even though the number of tumors was small, loss of markers at 11q13 (2/3) was the only observed abnormality.

To establish on which human chromosome CaS was situated, PCR was performed on somatic cell hybrids that retained individual human chromosomes in mouse or hamster cells. A single product was obtained from total human DNA and from the cell line containing human chromosome 2 only (not shown). Subsequently, the PCR product was used to isolate a genomic clone for CaS from a total human cosmid library. The cosmid clone was then used for FISH to determine more precisely the position of CaS. Hybridization to chromosome 2 was observed, and the signal was detected as symmetrical spots on the two homologous chromosomes. CaS was regionally assigned to 2q21-22 by means of DAPI staining (Fig. 2).

Discussion

Previous studies of the tumorigenesis of parathyroid glands have provided no evidence of clear-cut tumor progression steps as exemplified in the case of colon cancer (Fearon and Vogelstein 1990). Parathyroid glands are not as readily accessible as the colon and thus, prior to the known presence of biochemical abnormality, they are not subject to biopsy, even in individuals who have a predisposition to parathyroid tumors. Furthermore, parathyroid glands are endocrine organs under hormonal and metabolic regulation and are subject to the influence of gender and age.

Whether benign parathyroid tumors develop into malignant forms is still the subject of debate, since parathyroid cancer is a rarity. Deletions at the RB1 locus on chromosome 13q have been reported to be specific for parathyroid carcinoma (Cryns et al. 1994). However, it has also been found in atypical recurrent parathyroid disease (Dotzenrath et al. 1996), and in four benign parathyroid tumors in the present study (Table 2).

Genetically, HPT has been associated with a number of familial diseases, such as MEN1, MEN2, neurofibromatosis type 1, and HPT-JT. Accordingly, the genes of these syndromes can be expected to play a role not only in the familial, but also in some of the sporadic cases. Interestingly, parathyroid cancer has been associated with one of the syndromes, viz., HPT-JT syndrome (Jackson et al. 1990).

In view of these factors, a complex mechanism(s) for parathyroid tumorigenesis can be expected. In the majority of cases, different genetic triggers probably contribute separately, rather than in a progressive pattern. This is supported by the present study, since out of the 24 tumors that show LOH, only four have LOH in more than one chromosomal region. Among the investigated loci, MEN1 is most frequently involved.

The MEN1 gene has previously been shown to be frequently involved in both MEN1-related and sporadic parathyroid tumors. Although only three MEN1-related tumors have been included in this series, they indicate that the MEN1 gene, as expected, plays the most important role. The sporadic tumors showing loss at the MEN1 locus are significantly larger ($P < 0.01$; chi-square) than those showing retention (> 900 mg or < 560 mg, respectively) in agreement with previous findings (Friedman et al. 1992). It is interesting to note that four tumors exhibit LOH in chromosomes 1q, 13q, and X, rather than in the MEN1 region.

The calcium-sensing receptor(s) plays a crucial role in the regulation of PTH secretion. The increased set-point seen in primary and secondary HPT may be the result of a defective (mutated) receptor or a reduced number of normal receptors (Garrett et al. 1995; Kifor et al. 1996). The possible involvement of CaR in the development of parathyroid neoplasia has also been discussed (El-Deiry and Levine 1995). Thompson et al. (1995) have shown LOH by using markers flanking the FHH/CaR region in approximately 10% of sporadic parathyroid adenomas. We have not detected any LOH in 28 informative cases; this argues against a major role of this gene in the development of sporadic adenomas. In a previous study, Hosokawa et al. (1995) have reported the absence of mutations in CaR in both sporadic adenomas and tumors from patients with secondary HPT. It should be noted, however, that intronic and promoter mutations might escape detection with the type of assay that those authors have used, i.e., the RNAse protection assay. On the other hand, the present study demonstrates LOH in 10% of tumors from patients with secondary HPT, suggesting that the inactivation of CaR may be specific for a subset of secondary HPT tumors. Since these tumors could still contain one copy of the gene, it might be worthwhile to carry out mutation analysis of the gene.

Extensive work, physiological and immunohistochemical, has been performed to characterize a second putative calcium-sensing receptor, CaS (Nygren et al. 1988; Juhlin et al. 1987, 1989, 1990). These studies have shown that the expression of CaS is reduced both in sporadic parathyroid adenomas and in secondary HPT glands. Part of the CaS protein has recently been sequenced, and the gene cloned (Lundgren et al 1994; Korenberg et al. 1994). Using FISH analysis, we have now shown that the gene maps to chromosome 2q21-q22. Our studies of this region have not revealed LOH in tumors from any group, thus suggesting that inactivation of the gene by somatic deletion is not a mechanism for tumorigenesis in the parathyroid. However, other inactivating mechanisms, such as point mutations, might play a role.

As far as we are aware, this is the first study to investigate parathyroid tumors of patients previously irradiated to the neck. Because of radiation-associated damage, more genetic abnormalities can be expected in these tumors. We have found LOH in 3/5 informative cases (60%), the losses involving five different random chromosomal regions (Table 2).

In addition to MEN1, there have been many reports of familial hyperparathyroidism. Some of these families have subsequently been found to have ossifying jaw fibromas, suggesting that they have HPT-JT, a separate entity mapping to chromosome 1q. Previous studies of HPT-JT-associated parathyroid tumors have not revealed LOH in this region (Szabo et al. 1995). Our studies show that only one radiation-associated parathyroid tumor has LOH in this region, but this tumor also has LOH in the MEN1 region and on 13q. These results, together with previous studies, indicate that inactivation by gross deletion of the HPT-JT gene is not frequent in the tumorigenesis of any type of parathyroid tumors.

LOH in chromosome 1p36 has frequently been found in various kinds of malignancies, such as neuroblastoma, pheochromocytoma, melanoma, and breast cancer. Cryns et al. (1995) have reported LOH in up to 40% of parathyroid adenomas studied. Surprisingly, we have not found LOH in any of the 28 informative sporadic cases. Contamination of normal tissue can be excluded, since LOH has been detected in other loci. However, the existence of a small interstitial deletion beyond our detection method or point mutation of a candidate gene in this region cannot be ruled out.

One interesting finding is the LOH involving the X chromosome in four tumors. Two of them were sporadic cases, whereas the other two were familial with no evidence of MEN1 or jaw tumors to date. This is consistent with a previous study that has also shown LOH of the X chromosome in 2 out of 4 tumors in a family with familial hyperparathyroidism (Arnold et al. 1995), suggesting the existence of an X-chromosome tumor-suppressor gene. We have recently shown reduced penetrance of hyperparathyroidism in female patients in HPT-JT syndrome (Teh et al. 1996); we hypothesize that an X-chromosome modifier gene might be involved and that, in these familial cases, the female requires two mutations, whereas the male only requires one. It will be interesting to analyze these tumors when both genes are cloned.

Acknowledgements This work was supported by the Swedish Cancer Foundation, the Medical Research Council, the Fredrik and Ingrid Thuring Foundation, the Gustav V Jubilee Fund, the Magn. Bergvall Foundation, the Torsten and Ragnar Söderberg Memory Foundation, the Sven och Ebba-Christina Hagbergs Foundation, the Martin Rind Foundation, and the Cancer Society of Stockholm.

References

Arnold A, Staunton CE, Kim HG, Randall AB, Gaz D, Kronenberg HM (1988) Monoclonality and abnormal parathyroid hormone genes in parathyroid adenomas. N Engl J Med 318:658–662

Arnold A, Brown MF, Urena P, Gaz RD, Sarfati E, Drueke TB (1995) Monoclonality of parathyroid tumors in chronic renal failure and in primary parathyroid hyperplasia. J Clin Invest 95:2047–2053

Benn PA, Perle MA (1988) Chromosome staining and banding techniques. In: Rooney DE, Czepulkowski BH (eds) Human cytogenetics: a practical approach, vol 1. IRL Press, Oxford Washington, pp 91–118

Blennow E, Telenius H, Larsson C, Vos D de, Bajalica S, Ponder BA, Nordenskjöld M (1992) Complete characterization of a large marker chromosome by reverse and forward chromosome painting. Hum Genet 90:371–374

Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger A, Lytton J, Hebert SC (1993) Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. Nature 366:575–580

Browne EM, Pollak M, Hebert SC (1995) Molecular mechanisms underlying the sensing of extracellular Ca^{2+} by parathyroid and kidney cells. Eur J Endocrinol 132:523–531

Byström C, Larsson C, Blomberg C, Sandelin K, Falkmer U, Skogseid B, Öberg K, Werner S, Nordenskjöld M (1990) Localization of the MEN1 gene within a small region of 11q13 by deletion mapping in tumors. Proc Natl Acad Sci USA 87:1968–1972

Cryns VL, Thor A, Xu H-J, Hu S-X, Wierman ME, Vickery AL, Benedict WF, Arnold A (1994) Loss of the retinoblastoma tumor-suppressor gene in parathyroid carcinoma. N Engl J Med 330:757–761

Cryns VL, Yi SM, Tahara H, Gaz RD, Arnold A (1995) Frequent loss of chromosome arm 1p DNA in parathyroid adenomas. Genes Chromosom Cancer 13:9–17

Dotzenrath C, Teh BT, Farnebo F, Cupisti K, Svensson A, Toll A, Goretzki P, Larsson C (1996) Allelic loss of the retinoblastoma tumour suppressor gene: a marker for aggressive parathyroid tumours? J Clin Endocrinol Metab 81:3194–3196

El-Deiry S, Levine MA (1995) Molecular overtones of primary hyperparathyroidism. J Clin Endocrinol Metab 80:3105–3106

Falchetti A, Bale AE, Amorosi A, Bordi C, Cicchi P, Bandini S, Marx SJ, Brandi ML (1993) Progression of uremic hyperparathyroidism involves allelic loss on chromosome 11. J Clin Endocrinol Metab 76:139–144

Farnebo F, Farnebo L-O, Nordenström J, Larsson C (1997) Allelic loss on chromosome 11 is rare in hypercalcemic secondary hyperparathyroidism. Eur J Surg (in press)

Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767

Friedman E, De Marco L, Gejman P, Norton JA, Bale AE, Aurbach GD, Spiegel AM, Marx SJ (1992) Allelic loss from chromosome 11 in parathyroid tumors. Cancer Res 52:6804–6809

Garrett JE, Capuano IV, Hammerland LG, Hung BCP, Brown EM, Hebert SC, Nemeth EF, Fuller F (1995) Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. J Biol Chem 270:12919–12925

Heath H, Odelberg S, Jackson CE, Teh BT, Hayward N, Larsson C, Buist NRM, Krapcho KJ, Hung BC, Capuano IV, Garrett JE, Leppert MF (1996) Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. J Clin Endocrinol Metab 81:1312–1317

Hosokawa Y, Pollak MR, Brown EM, Arnold A (1995) Mutational analysis of the extracellular Ca^{2+}-sensing receptor gene in human parathyroid tumors. J Clin Endocrinol Metab 80:3107–3110

Juhlin C, Johansson H, Holmdahl R, Gylfe E, Larsson R, Rastad J, Åkerström G, Klareskog L (1987) Monoclonal anti-parathyroid antibodies interfering with a Ca^{2+}-sensor of human parathyroid cells. Biochem Biophys Res Commun 143:570–574

Juhlin C, Rastad J, Klareskog L, Grimelius L, Åkerström G (1989) Parathyroid histology and cytology with monoclonal antibodies recognizing a calcium sensor of parathyroid cells. Am J Pathol 135:321–328

Juhlin C, Lundgren S, Johansson H, Lorentzen J, Rask L, Larsson E, Rastad J, Åkerström G, Klareskog L (1990) 500-Kilodalton calcium sensor regulating cytoplasmic Ca^{2+} in cytotrophoblast cells of human placenta. J Biol Chem 265:8275–8279

Jackson CE, Norum RA, Boyd SB, Talpos GB, Wilson SD, Taggart T, Mallette LE (1990) Hereditary hyperparathyroidism and multiple ossifying jaw fibromas: a clinically and genetically distinct syndrome. Surgery 108:1006–1013

Kifor O, Moore FD, Wang P, Goldstein M, Vassilev P, Kifor I, Hebert SC, Brown EM (1996) Reduced immunostaining for the extracellular Ca sensing receptor in primary and secondary hyperparathyroidism. J Clin Endocrinol Metab 81:1598–1606

Korenberg JR, Argraves KM, Chen XN, Tran H, Strickland DK, Argraves WS (1994) Chromosomal localization of human genes for the LDL receptor family member glycoprotein 330 (LRP2) and its associated protein RAP (LRPAP1). Genomics 22:88–93

Larsson C, Skogseid B, Öberg K, Nakamura Y, Nordenskjöld M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332: 85–87

Lundgren S, Hjälm G, Hellman P, Ek B, Juhlin C, Rastad J, Klareskog L, Åkerström G, Rask L (1994) A protein involved in calcium sensing of the human parathyroid and placental cytotrophoblast cells belongs to the LDL-receptor protein superfamily. Exp Cell Res 212:344–350

Nygren P, Larsson R, Johansson H, Ljunghall S, Rastad J, Åkerström G (1988) 1,25(OH)2D inhibits hormone secretion and proliferation but not functional differentiation in bovine parathyroid cells. Calcif Tissue Int 42: 213–218

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83: 2934–2938

Pollak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidmann CE, Seidmann JG (1993) Mutations in the human $Ca(^{2+})$-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 75:1297–1303

Rosenberg CL, Kim HG, Shows TB, Kronenberg HM, Arnold A (1991) Rearrangement and overexpression of D11S287E, a candidate oncogene on chromosome 11q13 in benign parathyroid adenoma. Oncogene 6:449–453

Shibata D (1994) Extraction of DNA from paraffin-embedded tissue for analysis by polymerase chain reaction: new tricks from an old friend. Hum Pathol 25:561–563

Szabo J, Heath B, Hill VM, Jackson CE, Zarbo RJ, Mallette LE, Chew SL, Besser GM, Thakker RV, Huff V, Leppert MF, Heath H III (1995) Hereditary hyperparathyroidism-jaw tumor syndrome: the endocrine gene HRPT2 maps to chromosome 1q21-q31. Am J Hum Genet 56:944–950

Tahara H, Smith AP, Gaz RD, Cryns VL, Arnold A (1996) Genomic localization of novel tumor suppressor gene loci in human parathyroid adenomas. Cancer Res 56:599–605

Teh BT, Farnebo F, Kristoffersson U, Sundelin B, Cardinal J, Axelson R, Yap A, Epstein M, Heath H III, Cameron D, Larsson C (1996) Autosomal dominant primary hyperparathyroidism-jaw tumor syndrome associated with renal hamartoma and cysts: linkage to 1q21-q32 and loss of the wild-type allele in hamartomas. J Clin Endocrinol Metab 81 : 4204–4211

Thompson DB, Samowitz WS, Odelberg S, Davis RK, Szabo J, Heath H III (1995) Genetic abnormalities in sporadic parathyroid adenomas: loss of heterozygosity for chromosom 3q markers flanking the calcium receptor locus. J Clin Endocrinol Metab 80:3377–3379