

Short Communication

Losses of Chromosomes 1p and 3q Are Early Genetic Events in the Development of Sporadic Pheochromocytomas

Hilde Dannenberg,*[†] Ernst J.M. Speel,[‡]
Jianming Zhao,[‡] Parvin Saremaslani,[‡]
Erwin van der Harst,[†] Jürgen Roth,[‡]
Philipp U. Heitz,[‡] H. Jaap Bonjer,[†]
Winand N.M. Dinjens,* Wolter J. Mooi,*
Paul Komminoth,[‡] and Ronald R. de Krijger*

From the Departments of Pathology* and Surgery,[†] Erasmus University and University Hospital Rotterdam, Rotterdam, The Netherlands; and the Department of Pathology,[‡] University of Zürich, Zürich, Switzerland

Despite several loss of heterozygosity studies, a comprehensive genomic survey of pheochromocytomas is still lacking. To identify DNA copy number changes which might be important in tumor development and progression and which may have diagnostic utility, we evaluated genetic aberrations in 29 sporadic adrenal and extra-adrenal pheochromocytomas (19 clinically benign tumors and 10 malignant lesions). Comparative genomic hybridization was performed using directly fluorochrome-conjugated DNA extracted from frozen (16) and paraffin-embedded (13) tumor tissues. The most frequently observed changes were losses of chromosomes 1p11–p32 (86%), 3q (52%), 6q (34%), 3p, 17p (31% each), 11q (28%), and gains of chromosomes 9q (38%) and 17q (31%). No amplification was identified and no difference between adrenal and extra-adrenal tumors was detected. Progression to malignant tumors was strongly associated with deletions of chromosome 6q (60% versus 21% in clinically benign lesions, $P = 0.0368$) and 17p (50% versus 21%). Fluorescence *in situ* hybridization confirmed the comparative genomic hybridization data of chromosomes 1p, 3q, and 6q, and revealed aneuploidy in some tumors. Our results suggest that the development of pheochromocytomas is associated with specific genomic aberrations, such as losses of 1p, 3q, and 6q and gains of 9q and 17q. In particular, tumor suppressor genes on chromosomes 1p and 3q may be involved in early tumorigenesis, and deletions of

chromosomes 6q and 17p in progression to malignancy. (*Am J Pathol* 2000, 157:353–359)

Pheochromocytomas (PCCs) represent neuroendocrine tumors derived from pheochromocytes within and outside the adrenal medulla. PCCs usually produce catecholamines and, as a result of inappropriate hormone secretion, can cause life-threatening disorders, such as myocardial infarction and cerebrovascular hemorrhage.

To date no reliable clinical or histopathological markers are available to distinguish benign from malignant PCCs. Metastases, occurring in approximately 10% of the tumors, are the only convincing sign of malignancy. They may already be present at the time of diagnosis or occur only after lag phases as long as 25 to 30 years.¹ Such a situation calls for more accurate markers that can predict the clinical behavior of these tumors.

The genetic mechanisms underlying the tumorigenesis of PCCs are poorly understood. Because of the known association with the inherited tumor syndromes multiple endocrine neoplasia type 2 (MEN2), Von Hippel-Lindau disease (VHL) and neurofibromatosis type 1 (NF1),^{2–4} PCCs have been investigated for involvement of the responsible *RET*, *VHL*, and *NF1* genes, respectively.^{5–9} However, only a subset of sporadic lesions harbors somatic mutations in these genes, suggesting that they do not play a prominent role in PCC tumorigenesis.^{10–13} Furthermore, no mutation has been detected in the *c-mos* proto-oncogene or in the p16 tumor suppressor gene (TSG) in benign and malignant lesions.^{14,15} Conflicting results exist with regard to the frequency of *TP53* gene alterations in PCCs as well as for the association of *c-erbB-2* expression with malignancy.^{16–19} Other onco-

Supported by the Dutch Cancer Society (Koningin Wilhelmina Fonds), the Jan Dekker and Dr. Ludgardina Bouwman Foundation, and the De Drie Lichten Foundation.

Accepted for publication May 10, 2000.

Address reprint requests to Ronald R. de Krijger, M.D., Ph.D., Department of Pathology, University Hospital Rotterdam, Josephine Nefkens Institute, Room 230A, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. E-mail: dekrijger@path.azr.nl.

Table 1. Patient Characteristics and Genetic Findings in Sporadic Pheochromocytomas

Type/no.	Age/Sex	Size (cm)	Location	CGH results				IHC p53*	FISH results			
				Number of changes			Chromosomal changes of chromosomes 1, 3, 6, 9, and 17		1p22 [†]	1p36 [†]	3q26 [†]	6q22 [†]
				All	Gain	Loss						
Benign												
1	63/F	4.7	EA	4	1	3	1p11-31-, 3p-, 9q33-qter+					
2	29/F	18	L	6	3	3	1p11-31-, 3p-, 9q33-qter+, 17pq+					
3	49/M	13	BI	7	2	5	1p-, 3pq-, 17p-, 17q+					
4	34/M	6	L	4	0	4	3pq-, 9q33-qter+		dis/tetras	dis/tetras	monos/dis	dis/tetras
5	75/F	5	EA	6	2	5	1p11-32-, 17pq+		4-2	4-2	tetras	tetras
6	52/F	5	R	9	4	2	9q32-34+					
7	43/F	3	R	3	1	2	1p11-32-, 3q-, 9q32-qter+					
8	42/M	6	L	5	1	4	1p11-32-, 3q-, 6q-, 9q32-qter+		2-1/4-2	dis/tetras	2-1/4-2	2-1/4-2
9	53/M	nk	L	4	0	4	1p11-31-					
10	27/F	11	L	6	2	4	1p-, 3pq-, 9q32-qter+, 17q+					
11	72/F	nk	R	14	2	12	1p11-32-, 3pq-, 6q-, 9q33-qter+, 17q+					
12	43/M	6	R	3	0	3	1p11-31-, 3q21-qter-, 17p-					
13	42/M	4.5	R	3	0	3	1p11-35-, 3q-	-				
14	45/F	2.5	L	7	2	5	1p11-31-, 6q-, 17q22-qter+	-				
15	66/M	3.2	L	6	1	5	1p-, 3pq-, 17p-	-				
16	79/M	5	R	5	1	4	1p11-35-, 3pq-	-				
17	51/F	5	L	3	0	3	1p11-32-, 3q-, 6q-	-				
18	57/M	15	nk	1	0	1		-				
19	47/M	nk	EA	4	0	4	1p-, 3q-, 17pq-	-				
Malignant												
20	49/F	4	Meta., LN	5	2	3	1p11-31-, 3q-, 6q-, 9q33-qter+, 17q22-qter+	+				
21	36/M	nk	nk	1	0	1	1p11-21-					
22	43/F	nk	Meta., LN	5	0	5	1p-, 3q-, 6q-, 17pq-		2-1	2-1	2-1	2-1
23	36/M	21	EA	3	1	2	1p11-31-, 9q32-qter+					
24	70/F	16	L	6	0	6	1p31-pter-, 6q-, 17p-	-				
25	65/F	11	R	13	6	7	1p11-31-, 9pq11-33-, 17p-, 17q23-qter+	+				
26	56/F	8.5	L	21	13	8	1p-, 3p-, 3q+, 6q-, 9pq+, 17pq-	+				
27	23/F	8	nk	4	2	2	1p11-32-, 3q-, 17q+	-				
28	62/F	12	L	16	9	7	6q-	-				
29	49/M	7.5	R	8	0	8	1p-, 6q22-qter-, 17p-	-				

*Data previously published (Ref. 20).

[†]In combination with the chromosome-specific centromere probe.

Abbreviations: M, male; F, female; L, left adrenal gland; R, right adrenal gland; BI, bilateral adrenal localization; EA, extra-adrenal localization; Meta., metastasis; LN, lymph node; nk, not known; n, no detectable changes; monos, monosomy; dis, disomy; tetras, tetrasomy; 4-2, tetrasomy with only two copies (loss) of the specific region; 2-1, disomy with allelic loss of the specific region.

genes that have been found to be overexpressed in PCCs, include *c-myc*, *c-fos*, and *Bcl-2*.^{19,20}

Several loss of heterozygosity (LOH) studies provided evidence for involvement of TSGs on chromosomes 1p, 3p, 11p, 17p, and 22q.²¹⁻²⁵ However, the impact of these data are limited, since small series of sporadic PCCs were analyzed and often no correlation with clinical data was presented. Moreover, the vast majority of the genome remains unexamined. We performed comparative genomic hybridization (CGH) analysis on 29 apparently sporadic PCCs, including 19 benign and 10 malignant lesions, to identify chromosome alterations which may be important in tumor development and behavior and might have diagnostic utility. Additionally, fluorescence *in situ* hybridization (FISH) experiments were carried out to independently confirm some of the CGH data.

Materials and Methods

Patients and Tumor Samples

Altogether, tumors of 29 patients with an apparently sporadic adrenal ($n = 25$) or extra-adrenal ($n = 4$) PCC

were analyzed, including 19 clinically benign and 10 proven malignant lesions (with metastases). A pheochromocytoma was considered sporadic if there was no family history of MEN2, VHL, or its constituent tumors and there were no other signs of MEN 2, VHL, or NF1 recorded by the clinician. Furthermore, previous analyses did not reveal germline mutations in the *RET* and *VHL* gene in these tumors^{10,11} (Dannenberg et al, unpublished data). The average age of the patients was 50.3 years (range: 23-79) and the mean size of the tumors was 7.1 ± 4.6 cm for benign lesions and 11.0 ± 4.7 cm for malignant lesions. Nine of 19 benign and 7 of 10 malignant tumors were from female patients. Except for two lymph node metastases, all specimens were primary tumors. Clinical follow-up was obtained in all benign cases with an average follow-up period of 7.6 years. Relevant characteristics of all patients are listed in Table 1.

DNA Extraction

Genomic DNA from 16 frozen tumors was isolated using the D-5000 Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). DNA extraction from 13 for-

malin-fixed, paraffin-embedded samples was performed by standard detergent-proteinase K lysis, followed by phenol/chloroform extraction and ethanol precipitation, as described elsewhere.²⁶

CGH and Digital Image Analysis

CGH was carried out as previously described.²⁶ One μg of high molecular weight tumor DNA was labeled with Spectrum Green-dUTPs (Vysis, Downers Grove, IL) by nick translation (BioNick kit, Life Technologies, Basel, Switzerland). Archival tumor DNA with fragment sizes of <1 kb was labeled with a platinum/D-Green complex (D-Green-ULS) using the Kreatech Universal Linkage System (ULS) D-Green labeling kit (kindly provided by Kreatech Morwell Diagnostics GmbH, Zürich, Switzerland), according to the manufacturer's recommendations. Briefly, 800 ng of tumor DNA and 1.6 μl of D-Green-ULS, adjusted to a total volume of 20 μl , was incubated at 65°C for 20 minutes. To reduce background signals, the ULS-labeled samples were purified using Qiagen nucleotide removal columns (Qiagen GmbH, Hilden, Germany).

The hybridization mixture consisted of 200 ng of Spectrum (or D-) Green-labeled tumor DNA, 200 ng of Spectrum Red-labeled sex matched normal reference DNA (Vysis), and 10 to 20 μg of human Cot-1 DNA (Life Technologies) dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2X SSC, pH 7.0). Hybridization to normal metaphase spreads (Vysis) took place for 3 days at 37°C.

Digital images were collected from six to seven metaphases using a Photometrics cooled CCD camera (Microimager 1400; Xillix Technologies, Vancouver, Canada). The QUIPS software program (Vysis) was used to calculate average green-to-red ratio profiles of at least four observations per autosome and two observations per sex chromosome in each analysis. Gains and losses of DNA sequences were defined as chromosome regions where the mean green-to-red fluorescence ratio was above 1.20 and below 0.80, respectively. Over-representations were considered amplifications when the fluorescence ratio values in a subregion of a chromosome arm exceeded 1.5. Because of some false positive results at chromosomes 1p32-pter, 16p, 19, and 22 found in normal tissues, gains of these known G-C-rich regions were excluded from all analyses.

FISH Analysis

To validate CGH data independently, touch preparations of four frozen PCCs were subjected to fluorescence *in situ* hybridization (FISH) analysis using a chromosome 1-specific centromere probe (pUC1.77) in combination with an 1p22-31-specific YAC probe (929-E-1) (kindly provided by S. Franke, Center for Human Genetics, University of Leuven, Belgium and J.R. Testa, Fox Chase Cancer Center, Philadelphia, PA), or a repeat probe (p179), mapping to the 1p36 region.²⁷⁻²⁹ Chromosomes 3 and 6 were examined using a chromosome 3 (p α 3.5) and 6 (p308) centromere probe, in combination with a

3q26-specific (*pancpin*) and 6q22-specific (cCl6-44) probe, respectively.³⁰⁻³³ Cell processing, probe labeling, *in situ* hybridization, and detection of the hybridized signals were performed as recently described.³⁴ For each tumor, hybridization signals of 100 interphase nuclei were scored. Numerical chromosomal aberrations were considered an aneusomy when the percentage of nuclei showing an abnormal number of centromere signals exceeded 30%. The presence of only half of the locus specific signals compared to the number of centromere signals in more than 30% of the nuclei was interpreted as a deletion.

Statistical Analysis

To calculate the statistical significance of differences in genomic changes between benign and malignant tumors, we used contingency table analysis, which was also used to analyze the relationship between genomic alterations and sex. Regression analysis was carried out to compare the number of chromosomal aberrations with tumor size.

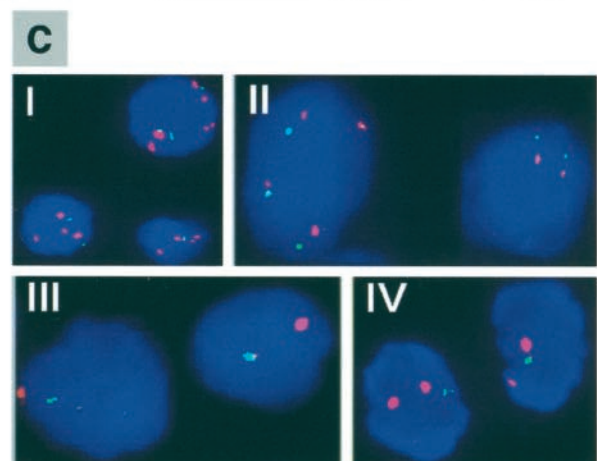
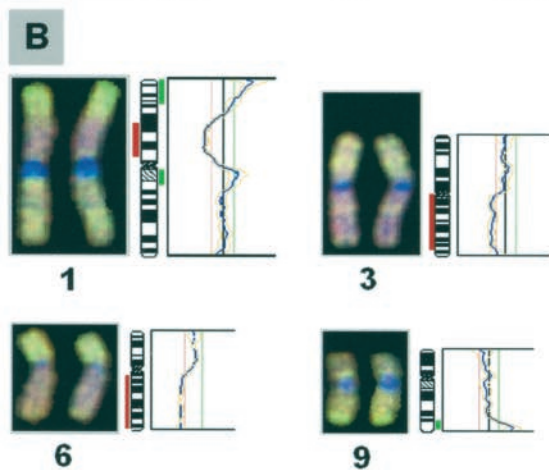
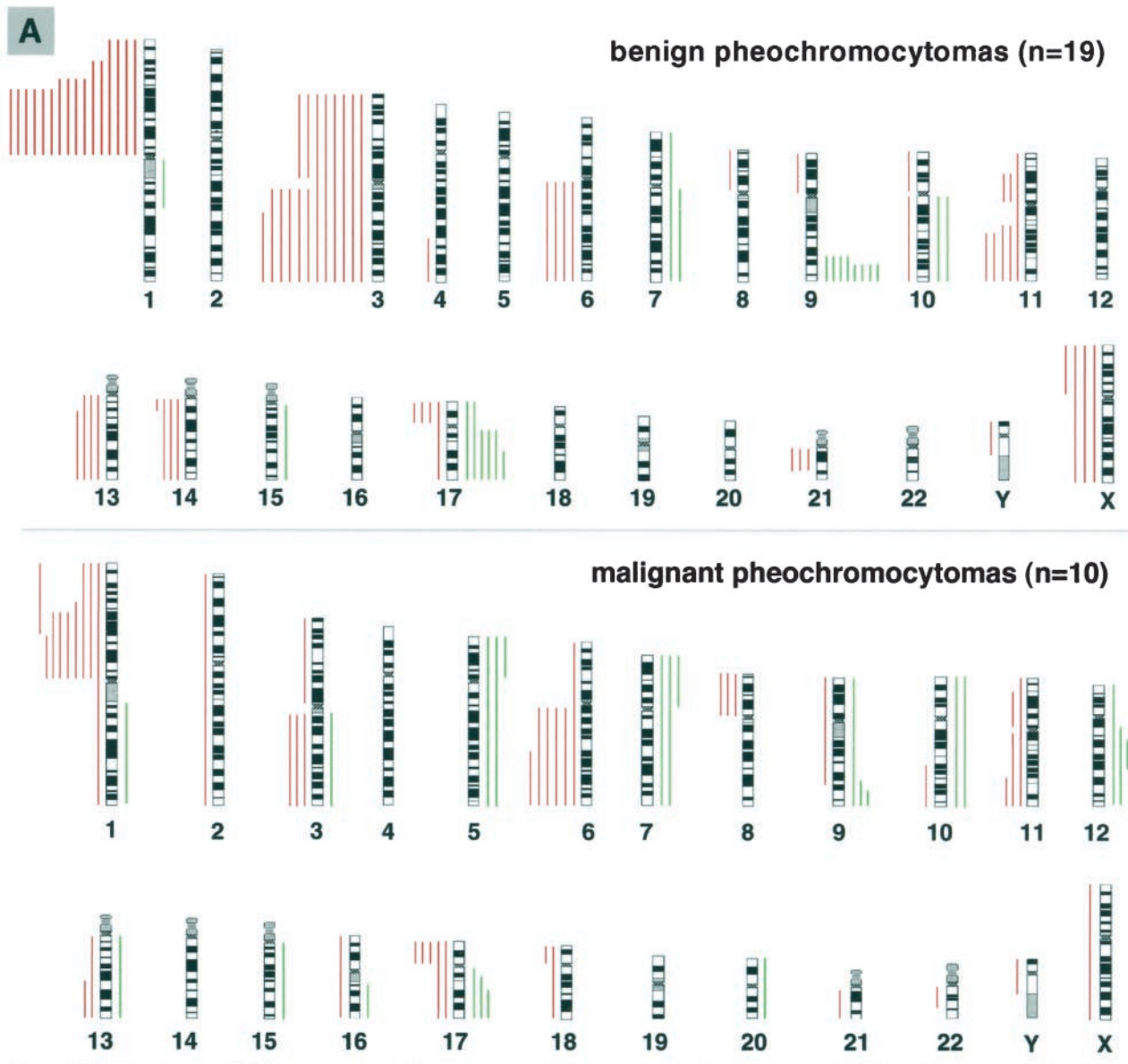
Results

Overview of CGH Findings

Figure 1A summarizes all DNA copy number changes identified in the 29 evaluated sporadic PCCs. Genetic alterations were observed in all PCCs, and the average number of chromosome arm aberrations per tumor was 6.3 ± 4.4 (range, 1-21). Chromosomal losses (mean, 4.3; range, 1-12) were more frequent than gains (mean, 1.9; range, 0-13) and no amplifications could be detected (Table 1). The average number of chromosomal alterations was marginally associated with malignancy (5.3 ± 2.7 versus 8.2 ± 6.1), principally due to a higher frequency of gains. The most common chromosome arm copy number changes included losses of chromosomes 1p11-p32 (86%), 3q (52%), 6q (34%), 3p, 17p (31% each), and 11q (28%), and gains of chromosome 9q (38%) and 17q (31%). Representative examples of CGH results are shown in Figure 1B.

Genetic Changes in Relation to Clinical Parameters

Ten of 29 PCCs fell into the category of malignant tumors, since regional or distant metastases occurred in these patients. In these lesions, losses of chromosome 6q were more often observed as compared to clinically benign lesions (60% versus 21%, $P = 0.0368$). To a lesser degree, losses of 17p were also related to metastatic disease (50% versus 21%), whereas deletions of chromosome 3p and 3q were preferably associated with benign tumors (42% versus 10% and 63% versus 30%, respectively). Although the malignant tumors were significantly larger in size (11.0 ± 4.7 cm versus 7.1 ± 4.6 cm, $P = 0.0470$) and demonstrated a slightly higher number of chromosome arm copy number changes, there was no



correlation between the number of genomic aberrations and the tumor size. No differences were seen between adrenal and extra-adrenal PCCs.

Additionally, a statistically significant correlation was found between the number of chromosomal alterations and female gender ($P = 0.0196$), predominantly due to a higher frequency of gains of different genomic regions.

Comparison of CGH and FISH

FISH analysis confirmed the CGH results of chromosomes 1p, 3q, and 6q in 4 tumors that showed chromosomal imbalances as detected by CGH (Table 1). Two of the 4 tumors presented with a partial loss of chromosome 1p (1p11–32). In one case, FISH analysis proved the loss of this region and the retention of 1p36. However, the other tumor appeared to have a complete loss of chromosome 1p, which could be expected, since the 1p32-pter region is known to generate some false positive results in CGH.

In addition, the three benign lesions revealed a tetrasomy in the major population of cells for all chromosomes analyzed. In contrast, the malignant tumor turned out to be diploid. In two of the three aneuploid lesions a subpopulation of diploid tumor cells (as demonstrated by two centromere signals and only one locus-specific signal per nucleus) could also be detected. Examples of FISH results are shown in Figure 1C.

Discussion

Our genome-wide survey of genetic alterations in sporadic benign and malignant PCCs identified specific aberrations associated with tumor initiation and progression, which might be useful in clinical diagnosis. Our results highlight several novel chromosome regions that may harbor genes critically involved in the pathogenesis of sporadic PCCs. A high frequency of losses of chromosomes 1p and 3q in the benign tumors suggests that they are important genetic events in early tumorigenesis. Deletions of chromosome 6q were strongly associated with metastatic disease and thus may help to define the malignant potential of PCCs.

The most commonly encountered chromosomal aberrations in our series of 29 sporadic PCCs involved 1p, 3q, 9q, 6q, 3p, 17p, 17q, and 11q. These findings support and extend the hypothesis of Koshla et al²² that multiple genes are involved in the pathogenesis of these tumors. Although LOH studies have shown loss of heterozygosity at 1p, 3p, 11q, 17p, and 22q in syndrome-related and sporadic PCCs,^{21–25} the here found losses of 3q and 6q and gains of 9q and 17q have not been reported previously. In contrast to a previous LOH analysis,²⁵ we ob-

served only one tumor with loss of 22q. Possible explanations for this discrepancy are the limited sensitivity of CGH for small losses (<10 Mb) and the fact that G-C-rich chromosome regions, like those in chromosome 22, are known to yield false positive CGH results.

We and others^{21–24} found a very high incidence of 1p loss in PCCs, suggesting that it might be an important tumorigenic event. Deletions of chromosome 1p are common in several other human neoplasms, including adrenocortical carcinomas and neuroectodermally derived tumors, such as neuroblastomas.^{35,36} The smallest common region of overlap as detected by CGH in our study involves the 1p22–32 region. Because of known false positive CGH results at 1p32-pter, we verified the CGH data by FISH using a probe mapping to 1p36. Our observations indicate that some PCCs indeed exhibit a partial loss of chromosome 1p. This correlates well with the results of others,^{23,37} who found evidence for a potential PCC tumor suppressor locus in the middle of the short arm of chromosome 1. There are multiple candidate TSGs in this region, including *TGF β -R3*, *p18INK4C*, and *PTPRF*, none of which has been proven to be relevant in PCC tumorigenesis. One should also consider the possibility that more than a single TSG on 1p contributes to the development of PCCs.

Another interesting finding of our study is the frequent loss of chromosome 3q in PCCs, which is more often encountered in benign than in malignant tumors. This might point to diverging pathways in the development of benign and malignant PCCs, although it is known that in malignant tumors duplications of chromosome arms can occur.³⁸ In that case, LOH will not be detected by CGH. However, it is certainly conceivable that loss of chromosome 3q is an early genetic event in the pathogenesis of PCCs. Thus far, frequent allelic loss of chromosome 3q has been demonstrated only in osteosarcoma, with a putative TSG localized to a region between 3q26.2 and 3q26.3.³⁹ The possible relationship of this candidate TSG for PCC development however, is not clear.

Data on the genetic events that determine the malignant potential of PCCs are scarce. We observed a strong association of losses of chromosome 6q with advanced disease stage. Frequent allelic imbalances at 6q have also been reported in other malignancies^{40,41} and appear to be related to poor prognosis or metastatic disease in certain tumors.^{42,43} Although these data are suggestive of the existence of several TSGs at 6q, none has been identified so far. In addition, underrepresentations of chromosome 17p were often observed in metastasizing lesions. 17p13 contains the *TP53* gene, the TSG most commonly involved in human carcinogenesis. Fourteen of 29 PCCs evaluated in the present study have previously been immunohistochemically investigated for *TP53*

Figure 1. Summary of all DNA copy number changes detected by CGH in 19 benign and 10 malignant sporadic PCCs (**A**). The vertical green lines on the right side of the chromosome ideograms indicate gains, the red on the left losses of the corresponding chromosome region. **B:** Individual representative examples of CGH digital images (**left**) and fluorescent ratio profiles (**right**) illustrating genomic alterations of chromosomes 1 (loss of 1p11–32 with gain of the C-G rich telomeric region), 3 (loss of 3q), 6 (loss of 6q), and 9 (gain of 9q33–34). **C:** Results of FISH analysis. I and II: interphase touch preparations of tumor number 8 analyzed using a centromeric probe for chromosome 1 (red signals) in combination with a 1p22–31-specific probe (green signals) (I) or a 1p36-specific probe (II), demonstrating aneuploidy of this tumor with loss of the 1p22–31 locus (I) but not of the 1p36 locus (II). III: Monosomy of chromosome 3 in case 4. IV: Diploid malignant tumor, showing the two copies of centromere 6 (red signals) and only one copy of the 6q22-specific locus (green signal), indicating loss of 6q.

accumulation.²⁰ Comparison of these results with our CGH results (Table 1), indicates that *TP53* alterations may play a role in a subset of these tumors. Involvement of another TSG on 17p, however, cannot be ruled out and one candidate gene could be the putative TSG at the medulloblastoma locus 17p13.3.⁴⁴ Interestingly, three malignant PCCs exhibited simultaneous gains of chromosomes 5p, 7p and 12q, similar to findings for malignant endocrine pancreatic tumors.⁴⁵

In conclusion, our results indicate that the development of PCCs is associated with specific genetic alterations, of which deletions of chromosome 1p (1p22-32) and 3q are early genetic events. Losses involving chromosome 3q (minimal overlapping region, 3q21-qter) and 6q (6q22-qter) and over-representations of chromosome 9q (9q33-34) and 17q point to new regions that may contain PCC TSGs and oncogenes in addition to the previously reported TSG loci 1p, 3p, 11q, 17p, and 22q.

Further studies are required to narrow down the critical regions in each identified chromosome and to characterize the putative genes involved in tumor initiation and progression of sporadic PCCs.

Acknowledgments

We thank S. Franke, Center for Human Genetics, University of Leuven, Belgium and J.R. Testa, Fox Chase Cancer Center, Philadelphia, PA, for providing FISH probes. We also thank Monica Seijbel, University of Rotterdam, for excellent secretarial assistance; Claudia Matter, Seraina Muletta-Feurer, and Alexander Scheidweiler, University of Zürich, for outstanding technical support and Norbert Wey and Ida Schreiter for assistance in the preparation of the figures. Finally, we wish to thank Dr. Michael Ashworth for critically reading the manuscript.

References

- Schlumberger M, Gicquel C, Lumbroso J, Tenenbaum F, Comoy E, Bosq J, Fonseca E, Ghilani PP, Aubert B, Travagli JP, Gardet P, Parmentier C: Malignant pheochromocytoma: clinical, biological, histological and therapeutic data in a series of 20 patients with distant metastases. *J Endocrinol Invest* 1992, 15:631-642
- Ponder BAJ, Smith D: The MEN2 syndromes and the role of the ret proto-oncogene. *Adv Cancer Res* 1996, 70:179-222
- Neumann HPH, Lips CJM, Hsia YE, Zbar B: Von Hippel-Lindau syndrome. *Brain Pathol* 1995, 5:181-193
- Bader JL: Neurofibromatosis and cancer. *Ann NY Acad Sci* 1986, 486:57-65
- Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnicliffe A, Ponder BAJ: Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993, 363:458-460
- Latif F, Tork K, Gnara J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choque P, Walther MM, Weng Y, Duan DR, Dean M, Glavac D, Richards FM, Crossey PA, Ferguson-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinault CA, Maher ER, Linehan WM, Zbar B, Lerman MI: Identification of the von Hippel-Lindau tumour suppressor gene. *Science* 1993, 260:1317-1320
- Richards FM, Webster AR, McMahon R, Woodward ER, Rose S, Maher ER: Molecular genetic analysis of von Hippel-Lindau disease. *J Intern Med* 1998, 243:527-533
- Crossey PA, Richards FM, Foster K, Green JS, Prowse A, Latif F, Lerman MI, Zbar B, Affara NA, Ferguson-Smith MA, Maher ER: Identification of intragenic mutations in the von Hippel-Lindau disease tumor suppressor gene and correlation with disease phenotype. *Hum Mol Genet* 1994, 3:1303-1308
- Ponder B: Human genetics: neurofibromatosis gene cloned. *Nature* 1990, 346:703-704
- Harst E van der, Krijger RR de, Bruining HA, Lamberts SWJ, Bonjer HJ, Dinjens WNM, Proye C, Koper JW, Bosman FT, Roth J, Heitz PU, Komminoth P: Prognostic value of *RET* proto-oncogene point mutations in malignant and benign, sporadic pheochromocytomas. *Int J Cancer* 1998, 79:537-540
- Harst E van der, Krijger RR de, Dinjens WNM, Weeks LE, Bonjer HJ, Bruining HA, Lamberts SWJ, Koper JW: Germline mutations in the *vhl* gene in patients presenting with pheochromocytomas. *Int J Cancer* 1998, 77:337-340
- Eng C, Crossey PA, Mulligan L, Healey CS, Houghton C, Prowse A, Chew SL, Dahia PLM, O'Riordan JLH, Toledo SPA, Smith DP, Maher ER, Ponder BAJ: Mutations in the *RET* proto-oncogene and the von Hippel-Lindau disease tumour suppressor gene in sporadic and syndromic pheochromocytomas. *J Med Genet* 1995, 32:934-937
- Gutmann DH, Geist RT, Rose K, Wallin G, Moley JF: Loss of neurofibromatosis type I (NF1) gene expression in pheochromocytomas from patients without NF1. *Genes Chromosomes Cancer* 1995, 13: 104-109
- Eng C, Foster KA, Healey CS, Houghton C, Gayther SA, Mulligan LM, Ponder BAJ: Mutation analysis of the c-mos proto-oncogene and the endothelin-B receptor gene in medullary thyroid carcinoma and pheochromocytoma. *Br J Cancer* 1996, 74:339-341
- Aguiar RCT, Dahia PLM, Sill H, Toledo SPA, Goldman JM, Cross NCP: Deletion analysis of the p16 tumour suppressor gene in pheochromocytomas. *Clin Endocrinol* 1996, 45:93-96
- Herfarth KK, Wick MR, Marshall HN, Gartner E, Lum S, Moley JF: Absence of TP53 alterations in pheochromocytomas and medullary thyroid carcinomas. *Genes Chromosomes Cancer* 1997, 20:24-29
- Yoshimoto T, Naruse M, Zeng Z, Nishikawa T, Kasajima T, Toma H, Yamamori S, Matsumoto H, Tanabe A, Naruse K, Demura H: The relatively high frequency of p53 gene mutations in multiple and malignant pheochromocytomas. *J Endocrinol* 1998, 59:247-255
- Castilla-Guerra L, Moreno AM, Fernández-Moreno MC, Utrilla JC, Fernández E, Galera-Davison H: Expression and prognostic value of c-*erbB-2* oncogene product in human pheochromocytomas. *Histopathology* 1997, 31:144-149
- Krijger RR de, Harst E van der, Ham F van der, Stijnen T, Dinjens WNM, Koper JW, Bruining HA, Lamberts SWJ, Bosman FT: Prognostic value of p53, bcl-2, and c-*erbB-2* protein expression in pheochromocytomas. *J Pathol* 1999, 188:51-55
- Goto K, Ogo A, Yanase T, Haji M, Ohashi M, Nawata H: Expression patterns of the c-myc gene in adrenocortical tumors and pheochromocytomas. *J Clin Endocrinol Metab* 1990, 70:353-357
- Tsutsumi M, Yokota J, Kakizoe T, Koiso K, Sugimura T, Terada M: Loss of heterozygosity on chromosomes 1p and 11p in sporadic pheochromocytoma. *J Natl Cancer Inst* 1989, 81:1097-1101
- Khosla S, Patel VM, Hay ID, Schaid DJ, Grant CS, Van Heerden JA, Thibodeau SN: Loss of heterozygosity suggests multiple genetic alterations in pheochromocytomas and medullary thyroid carcinomas. *J Clin Invest* 1991, 87:1691-1699
- Moley JF, Brother MB, Fong CT, White PS, Baylin SB, Nelkin B, Wells SA, Brodeur GM: Consistent association of 1p loss of heterozygosity with pheochromocytomas from patients with multiple endocrine neoplasia type 2 syndrome. *Cancer Res* 1992, 52:770-774
- Vargas MP, Zhuang Z, Wang C, Vortmeyer A, Linehan WM, Merino MJ: Loss of heterozygosity on the short arm of chromosomes 1 and 3 in sporadic pheochromocytoma and extra-adrenal paraganglioma. *Hum Pathol* 1997, 28:411-415
- Tanaka N, Nishisho I, Yamamoto M, Miya A, Shin E, Karakawa K, Fujita S, Kobayashi T, Rouleau GA, Mori T, Takai S: Loss of heterozygosity on the long arm of chromosome 22 in pheochromocytoma. *Genes Chromosomes Cancer* 1992, 5:399-403
- Richter J, Jiang F, Gorog JP, Sartorius G, Egenter C, Gasser TC, Moch H, Mihatsch MJ, Sauter G: Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer Res* 1997, 57:2860-2864

27. Cooke HJ, Hindley J: Cloning of human satellite III DNA: different components are on different chromosomes. *Nucleic Acids Res* 1979, 6:3177-3197
28. Lee WC, Balsara B, Liu Z, Jhanwar SC, Testa JR: Loss of heterozygosity analysis defines a critical region in chromosome 1p22 commonly deleted in human malignant mesothelioma. *Cancer Res* 1996, 56:4297-4301
29. Buroker N, Bestwick R, Haight G, Magenis RE, Litt M: A hypervariable repeated sequence on human chromosome 1p36. *Hum Genet* 1987, 77:175-181
30. Wayne JS, Willard HF: Chromosome specificity of satellite DNAs: short- and long-range organization of a diverged dimeric subset of human alpha satellite from chromosome 3. *Chromosoma* 1989, 97:475-480
31. Jabs EW, Wolf SF, Migeon BR: Characterization of a cloned DNA sequence that is present at centromeres of all human autosomes and the X chromosome and shows polymorphic variation. *Proc Natl Acad Sci USA* 1984, 81:4884-4888
32. Ozaki K, Nagata M, Suzuki M, Fujiwara T, Miyoshi Y, Ishikawa O, Ohigashi H, Imaoka S, Takahashi E, Nakamura Y: Isolation and characterization of a novel human pancreas-specific gene, *pancpin*, that is down-regulated in pancreatic cancer cells. *Genes Chromosomes Cancer* 1998, 22:179-185
33. Saito S, Okui K, Tokino T, Oshimura M, Nakamura Y: Isolation and mapping of 68 RFLP markers on human chromosome 6. *Am J Hum Genet* 1992, 50:65-70
34. Görtz B, Roth J, Speel EJM, Krähenmann A, De Krijger RR, Matias-Guiu X, Muletta-Feurer S, Rütimann K, Saremaslani P, Heitz PU, Komminoth P: MEN 1 gene mutations in sporadic adrenocortical lesions. *Int J Cancer* 1999, 80:373-379
35. Zhao J, Speel EJM, Muletta-Feurer S, Rütimann K, Saremaslani P, Roth J, Heitz PU, Komminoth P: Analysis of genomic alterations in sporadic adrenocortical lesions: gain of chromosome 17 is an early event in adrenocortical tumorigenesis. *Am J Pathol* 1999, 155:1039-1045
36. Fong CT, Dracopoli NC, White PS, Merrill PT, Griffith RC, Housman DE, Brodeur GM: Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastomas: correlation with N-myc amplification. *Proc Natl Acad Sci USA* 1989, 86:3753-3757
37. Yang KP, Nguyen CV, Castillo SG, Samaan NA: Deletion mapping on the distal third region of chromosome 1p in multiple endocrine neoplasia type IIA. *Antioncogene Res* 1990, 10:527-534
38. White VA, McNeil BK, Horsman DE: Acquired homozygosity (isodisomy) of chromosome 3 in uveal melanoma. *Cancer Genet Cytogenet* 1998, 102:40-45
39. Kruzelock RP, Murphy EC, Strong LC, Naylor SL, Hansen MF: Localization of a novel tumor suppressor locus on human chromosome 3q important in osteosarcoma tumorigenesis. *Cancer Res* 1997, 57:106-109
40. Gaidano G, Hauptschein RS, Parsa NZ, Offit K, Rao PH, Lenoir G, Knowles DM, Chaganti RS, Dalla-Favera R: Deletions involving two distinct regions of 6q in B-cell non-Hodgkin lymphoma. *Blood* 1992, 80:1781-1787
41. Bell DW, Jhanwar SC, Testa JR: Multiple regions of allelic loss from chromosome arm 6q in malignant mesothelioma. *Cancer Res* 1997, 57:4057-4062
42. Healy E, Belgaid C, Takata M, Harrison D, Zhu NW, Burd DA, Rigby HS, Matthews JN, Rees JL: Prognostic significance of allelic losses in primary melanoma. *Oncogene* 1998, 16:2213-2218
43. Speel EJM, Richter J, Moch H, Egenter C, Saremaslani P, Rütimann K, Zhao J, Barghorn A, Roth J, Heitz PU, Komminoth P: Genetic differences in endocrine pancreatic tumor subtypes detected by comparative genomic hybridization. *Am J Pathol* 1999, 155:1787-1794
44. Cogen PH, Daneshvar L, Metzger AK, Duyk G, Edwards MS, Sheffield VC: Involvement of multiple chromosome 17p loci in medulloblastoma tumorigenesis. *Am J Hum Genet* 1992, 50:584-589
45. Terris B, Meddeb M, Marchio A, Danglot G, Flejou JF, Belghiti J, Ruzniewski P, Bernheim A: Comparative genomic hybridization analysis of sporadic neuroendocrine tumors of the digestive system. *Genes Chromosomes Cancer* 1998, 22:50-56