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RESEARCH

Molecular Cytogenetic Characterization in Four Pediatric Pheochromocytomas and Paragangliomas

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Abstract Pheochromocytomas (PCCs) are rare tumors among children and adolescents and therefore are not genetically well characterized. The most frequently observed chromosomal changes in PCC are losses of 1p, 3q and/or 3p, 6q, 17p, 11q, 22q, and gains of 9q and 17q. Aberrations involving chromosome 11 are more common in malignant tumors. Unfortunately information about gene aberrations in childhood PCC's is limited. We used comparative genomic hybridization (CGH) and array comparative genomic hybridization (aCGH) to screen for copy number changes in four children suffering from pheochromocytoma or paraganglioma. Patients were diagnosed at the age 13 or 14 years. Bilateral pheochromocytoma was associated with von Hippel-Lindau syndrome (VHL). Multiple paraganglioma was associated with a germline mutation in SDHB. We found very good concordance between the results of CGH and aCGH techniques. Losses were observed more frequently than gains. All cases had a loss of chromosome 11 or 11p. Other aberrations were loss of chromosome 3 and 11 in sporadic pheochromocytoma, and loss of 3p and 11p in pheochromocytoma, which carried the VHL mutation. The deletion of chromosome 1p and other changes were observed in paragangliomas. We conclude that both array CGH and CGH analysis identified similar chromosomal regions involved in tumorigenesis of pheochromocytoma and paragangliomas, but we found 3 discrepancies between the methods. We didn't find any, of the proposed, molecular markers of malignancy in our benign cases and therefore we speculate that molecular cytogenetic examination may be helpful in separating benign and malignant forms in the future.

Keywords Pheochromocytoma · Paraganglioma · Comparative genomic hybridization · Pediatric · Microarray

Abbreviations

CGH	Comparative genomic hybridization
aCGH	Array comparative genomic hybridization
PCCS	Pheochromocytomas
VHL	Von Hippel-Lindau syndrome

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PGL	Paragangliomas
NF1	Neurofibromatosis type 1 gene
SDHB, SDHD and SDHC	The genes encoding the succinate dehydrogenase of mitochondrial subunits B, D, and C
VMA	Vanillylmandelic acid

Introduction

Pheochromocytomas (PCCs) are rare tumors among children and adolescents, despite being the most common pediatric endocrine tumor [1]. They're derived from chromaffin cells that originate from the neural crest. These cells are located in the adrenal medulla (pheochromocytes) and in the paraganglia along the sympathetic chain, and near the aorta. Tumors from extra-adrenal chromaffin tissue are referred to as extra-adrenal pheochromocytomas or paragangliomas (PGL). These two types often share the same clinical course and are histologically equivalent [2, 3]. Twenty percent of all pheochromocytomas occur in childhood [4]. In studies describing pediatric patients, multifocal disease, extra-adrenal disease, and familial association are more frequently described compared to adults. The majority (95%) of pediatric pheochromocytomas are intra-abdominal [5], 40% are bilateral, and 70% are multifocal [4–6]. Although the prevalence of malignancy is commonly cited to be about 10%, other estimates suggest rates of between 3% and 36% of pheochromocytomas/paragangliomas are malignant [7, 8]. Among pediatric patients, approximately 40% of pheochromocytomas are associated with known genetic mutations [9]. At present, the *RET*, *von Hippel-Lindau gene* (*VHL*), *neurofibromatosis type 1 gene* (*NF1*), *succinate dehydrogenase complex assembly factor 1*(*SDHAF1*), and the genes encoding the B, D, and C subunits of mitochondrial succinate dehydrogenase (*SDHB, SDHD* and *SDHC*) are known to be responsible for tumor formation. The chromosomal locations of these genes are summarized in Table 1. Germline mutations in these genes increase the risk of developing pheochromocytomas and/or paragangliomas, which variably associate with other tumors and characterize different clinical syndromes such as Multiple Endocrine Neoplasia 2 (usually MEN 2A, rarely MEN 2B), von Hippel-Lindau (VHL), and NF 1, or the PGL syndromes. The *SDHB* mutation predisposes patients to extra-adrenal locations and metastatic disease and has been more frequently reported in children [1, 10–12].

Due to the frequency of PCCs in childhood, it has been hypothesized that germline mutations in *RET*, *VHL*, *SDHB*, and *SDHD* cause PCCs more frequently among pediatric patients [9, 13, 14].

Table 1 Characteristics of genes associated with familial forms of pheochromocytoma

Gene	Chromosome	Protein
VHL	3p25-26	pVHL19 and pVHL30
SDHB	1p36.13	Catalytic iron-sulfur protein
SDHD	11q23	CybS(membrane-spanning subunit)
SDHC	1q21	CybL (Large subunit)
SDHAF1	19q13.12	Assembly factor 1
RET	10q11.2	Tyrosine-kinase receptor
NF1	17q11.2	Neurofibromin

A major problem in PCC relates to the unpredictability of clinical outcomes. Presently there are no defined histological markers to differentiate between benign and malignant PCCs. Features which arouse suspicion of malignancy include large tumor size, small tumor cells, extensive necrosis, vascular invasion, and aneuploidy [15–17]. Only the presence of distant metastases, derived from large pleomorphic chromaffin cells, is widely accepted as a criterion of malignancy [18]. The most frequently observed chromosomal changes in PCC are losses of 1p, 3q and/or 3p, 6q, 17p, 11q, 22q, and gains of 9q and 17q. Aberrations involving chromosome 11 are more common in malignant tumors. Unfortunately, information about gene aberrations in childhood PCC's is limited [19–22].

Applying chromosome comparative genomic hybridization (CGH), we first screened tumor specimens from four pediatric patients to identify genomic aberrations. Next, we validated these findings using array comparative genomic hybridization (aCGH) to increase mapping resolution. This was done because CGH resolution is limited to 10–20 Mb. When we compared results from both techniques, we found some discrepancies. These discrepancies can't be completely explained by limited resolution, indicating that tumor heterogeneity played a role in the discrepancies observed in our results.

Patients and Methods

A total of four cases were diagnosed and treated in the Department of Pediatric Hematology and Oncology of Motol University Hospital, 2nd Medical Faculty of Charles University, Prague, CZ, between 2003 and 2005.

Case Histories

Case 1

An asymptomatic 13-year-old boy was referred to our hospital for hypertension (blood pressure 190/110 mmHg). The

physical examination was entirely normal except for hypertension. A computed tomography (CT) scan examination revealed bilateral adrenal masses (left, 4 cm×3.5 cm×5 cm; right, 5 cm×4 cm×4.5 cm). Biochemical investigation showed an elevated 24-hour urine vanillylmandelic acid (VMA) level of 52.2 mmol/mol of creatinine per day (normal range 0.4–4.0 mmol/mol of creatinine per day). Fundoscopic examination revealed a capillary angioma.

A bilateral resection of the adrenal glands was performed and histological examination of the tissues confirmed pheochromocytoma. Von Hipple-Lindau syndrome was confirmed, DNA sequence analysis revealed a novel germline, heterozygous transversion M_000551:c.374A>C (p.His125Pro) in exon 2 of the *VHL* gene. The mother of the patient was negative for the *VHL* mutation; the father was not examined. The family history was negative for VHL syndrome and PCCs. The patient remains in complete remission (CR) 79 months after diagnosis.

Case 2

A 14-year-old boy presented to the emergency department with a history of a single, 2 min, episode of syncope with trismus. He was found, incidentally, to be hypertensive (blood pressure, 160/100 mmHg) with a history of headaches, fatigue, and vomiting. An abdominal CT scan revealed a mass on the right adrenal gland (4 cm× 4.2 cm×4 cm). Biochemical investigation showed an elevated 24-hour urine VMA level of 19.2 mmol/mol of creatinine per day (normal range 0.4–4.0 mmol/mol of creatinine per day). A right adrenal gland resection was performed. Histopathological examination of the tissue confirmed pheochromocytoma.

A germline mutation of the *VHL* gene wasn't identified in this case; however, we found a new somatic heterozygous mutation in the second exon of the *VHL* gene c.389 T>G (p. Val130Gly). We use the Multiplex Ligation-dependent Probe Amplification (MLPA) method to screen for large gene deletions in the VHL and SDH genes. This method was used because large gene deletions account for a considerable proportion of PCC syndromes. A large deletion in the VHL and SDH gene wasn't identified in this case. The family history was negative for VHL syndrome and PCCs. The patient remains in CR 57 months after diagnosis.

Case 3

A routine, preventive care, examination by a local pediatrician of a 13-year old boy revealed palpated resistance in the abdomen. A CT scan showed a spherical tumor on the left side of the abdomen (10 cm×8 cm×10 cm) with small local calcifications and hemorrhagic necrosis. Blood pressure was 95/50 mmHg. Twenty-four hour urine VMA was within normal range. A total surgical resection of the tumor was performed. Pathological examination of the tumor tissue confirmed paraganglioma. No germline or somatic mutation of *VHL*, *RET*, *SDHB*, or *SDHD* were found. MLPA was used for detecting large gene deletions in the VHL and SDH genes. While a large deletion in the VHL and SDH genes wasn't identified in peripheral blood, we found loss of one copy of SDHB, and gain of SDHC in the tumor tissue. The patient remains in CR 53 months after diagnosis. The family history was negative for PCCs.

Case 4

A 13-year-old girl was examined for a 3 year history of, unilateral (right side), sweating. Over the last 2 years she had suffered from headaches with vomiting two to three times per month. She was found to have hematuria and proteinuria, anisocoria, acute hypertension retinopathy, and hypertension (blood pressure, 223/153 mmHg). The child was referred to pediatric oncology with hypertension and a palpable abdominal mass. CT scan of chest and abdomen revealed a mediastinal mass (4.5 cm×4.5 cm×4.5 cm) and a left retroperitoneal mass (3.5 cm×3 cm×3.5 cm). A total surgical resection of both tumors was performed. The histopathological examination of the two lesions confirmed paraganglioma in both tumors. A germline heterozygous mutation in the *SDHB* gene was identified as *SDHB* 6 c.589 600 dup (p.Cys 196 Cys 200 dup) [23]. The same four-codon duplication was found in her older sister, her father, her paternal uncle, and the uncle's children. All of them were without history of any neoplastic disease. The patient remains in CR 49 months after diagnosis.

Methods

Comparative Genomic Hybridization

Tested DNA was extracted from fresh frozen samples to reference DNA came from 20 male to 20 female peripheral blood samples of healthy volunteers. Isolated DNA was mixed together (male or female). DNA was labeled with different fluorochromes using a commercially available kit and carried out according to the manufacturer's instructions (Abbott Molecular; Abbott Park, Illinois, U.S.A.), with a minor modification [24]. Fluorescence imaging and analysis were performed using an Olympus BX51 microscope (Olympus; Tokyo, Japan) and ISIS software (MetaSystems; Altlussheim, Germany). Thirty metaphases were captured and analyzed from each sample. Chromosomal regions were considered to be over-represented if the average green-to-red fluorescence ratio exceeded a cutoff of 1.25

(again) and as under-represented if the ratio was below a cutoff of 0.75 (a loss).

Array Comparative Genomic Hybridization

We used a commercially available genomic DNA microarray kit (GenoSensor Array 300; Abbott Molecular), which contained DNA representing 287 genes from the BAC, PAC, to P1 libraries. Each cloned DNA was spotted on slides, in triplicate. CGH was performed according to manufacturer's instructions (Abbott-Molecular) and analyzed with a microarray reader and analysis software (GenoSensor Array 300 system, Abbott-Molecular). Spots with G/R ratios more than the mean plus two standard deviations (≈1.2) were considered as gains, while spots with G/R ratios less than the mean minus two standards deviations (0.8) were considered as losses in copy number.

Gene Analyses

Genomic DNA was extracted from peripheral leukocytes or tumor cells using a salting out method modified according to Miller et al. (1988). We amplified exons 10, 11, and 13 through 16 of the *RET* proto-oncogene according to Neumann et al. (2002). Mutation analysis was carried out using DGGE (Denaturing Gradient Gel Electrophoresis) as previously described [25].

The six exons of the *RET* were amplified in a 25 μl reaction volume with 0.5 μM of each primer, 1x PCR buffer, 1.5 mM $MgCl_2$, 100 μM of each dNTP, between 50 and 300 ng of genomic DNA (as a template), and 1 unit of Taq DNA polymerase. PCR was performed for 35 cycles (30 s; 94°C, 45 s; 57–62°C, 40 s; 72°C) with a final extension of 10 min at 72°C. DGGE conditions are available on request. DNA fragments with an aberrant shift on DGGE were analyzed on an automatic fluorescent ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystems) according to the manufacturer's instructions.

Exons 1 to 3 of the *VHL* gene and exon-intron boundaries were amplified (primer sequences available on request). PCR was performed in a 30 μl reaction containing 1x PCR buffer, 1.0 mM $MgCl_2$, 100 μM of each dNTP, 0.5 μM of each primer, and 1 unit of Taq DNA polymerase (Fermentas, Lithuania). The PCR conditions were as follows: 32 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 58°C and extension for 2 min at 72°C, followed by a final extension for 7 min at 72°C. PCR products were then purified using Quick-Clean purification solution (Bioline), and both forward and reverse strands were sequenced using the appropriate PCR-primer and BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed on a ABI Prism 3100 Avant Genetic Analyzer (PE BioSystems).

Eight *SDHB* exons and four *SDHD* exons were screened using DGGE. Primers were designed based on GenBank sequences using Primer 3 software (available at: http://www.hgmp.mrc.ac.uk/GenomeWeb/nuc-primer.html) including intron-exon boundaries. The melting profile of DNA fragments, location of primers and GC clamps were analyzed using MacMelt™ software (Bio-Rad, California). The PCR reaction mixture (50 μl) contained 1x PCR buffer (MBI Fermentas), between 50 and 300 ng of genomic DNA (as template), 1.5 mM $MgCl_2$ (MBI Fermentas), 25 pmol of each primer, 200 μM of each deoxynucleotide triphosphate (Promega, USA), and 1.0 units of *Taq*DNA polymerase (MBI Fermentas). The amplification conditions included an initial denaturation at 94°C for 3 min, followed by 35 cycles of 45 s at 94°C, 45 s at 55–65°C (optimal annealing temperature according to the primers conditions), 1 min at 72°C and final extension step running for 5 min at 72°C. DNA fragments exhibiting aberrant band shifts were re-amplified and sequenced in both directions using an automatic fluorescent ABI Prism™ 310 Genetic Analyzer (PE Applied Biosystems) according to the manufacturer's instructions.

MLPA

Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was used to detect deletions or duplications in the *VHL* or *SDHB, SDHC, SDHD* and *SDHAF1* genes. The SALSA MLPA P016B *VHL* and the P226-B1 SDHD probe kits (MRC-Holland, Amsterdam, Netherlands) were used. The P016B kit contains eight probes to the *VHL* gene (four in exon 1, two in exon 2 and two in exon 3), additional probes to other genes on 3p and control probes to regions telomeric and centromeric from *VHL*. The P226-B1 kit contains nine probes to *SDHB,* seven probes to *SDHC,* five probes to *SDHD* and one probe to *SDHAF1*. Detailed information on probe sequences, gene loci and chromosome locations can be found at www.mlpa.com.

Genomic DNA (50–200 ng) was denatured and the probes were allowed to hybridize (16 h at 60°C). PCR was performed on the samples in a volume of 50 μl containing 10 μl of the ligation reaction mixture and using a thermal cycler Mastercycler ep gradient (Eppendor, Hamburg, Germany). Aliquots of 1 μl of the PCR reaction were combined with 0.5 μl ROX-labelled internal size standard (Applied Biosystems, Foster City, CA, USA) and 12 μl deionized formamide. Fragments were separated by electrophoresis on an Applied 3130XL capillary sequencer and quantified using GeneMarker version 1.6 software (SoftGenetics). For copy-number detection, normal control DNA samples were included in each set of MLPA experiments. Interpretation was based on the comparison

of peak heights between the control DNA and the tumor sample. Cut-off levels for loss of relative copy number were set at 0.75.

Results

Clinical data and CGH/aCGH results are summarized in Table 2 and Fig. 1. Chromosomal imbalances were observed in all 4 cases. The average amount of genetic aberrations was CGH/aCGH 2.75 and 2.5 changes, respectively (range 2–4) per case. Losses were as common as gains. A comparison of our CGH/aCGH data with data from adult to pediatric patients reported in the literature, together with the Progenetix CGH database (http://www.progenetix.net/progenetix/;14.9. 2009) showed high concordance of the aberration pattern [19–21].

A deletion on chromosome 11 was found in all cases (3×11p, 1×11). Patients with pheochromocytoma showed an aberration pattern distinct from paraganglioma patients. In both pheochromocytomas, there were losses of chromosome 3 or 3p. Paragangliomas showed losses of 1p. In case 4, aCGH detected a 1p deletion, while CGH only detected a partial deletion (1p31.3-ter). Patient 2 had a deletion on chromosome 3 and 11; we didn't find a constitutive *VHL* point mutation, using DNA sequencing, in the DNA extracted from peripheral blood leukocytes. Therefore, we sequenced the DNA from the tumor tissue to detected a new *VHL* mutation, *VHL* c.389 T>G (p. Val130Gly). The patient with the largest tumor, 416 cm^3 (case 3), showed the most extensive genetic changes including deletion of 17p11.2-pter and gain of 1q11-qter. In this case, we didn't find a constitutive or somatic mutation. In case 4, we found a discrepancy between CGH and aCGH results on chromosome 17. Therefore, fluorescence in situ hybridization (FISH) was used for validation of these results. We used ON *p53* (17p13)/*MPO* (17q22) ISO 17q″ probe (Kreatech Diagnostic) and *RARA* (17q21.1) probes. Testing of nuclei showed diploid status in 45% of *RARA* and 37% of *p53/MPO*. In the majority of nuclei there was aneuploidy, tetraploidy (*RARA* 39%, *p53/MPO* 20.5%), and triploidy (*RAR* 5%, *p53/MPO* 12%). An imbalance was detected in only 25% of nuclei (*MPO/p53*). This is probably due to the heterogeneity of the tumor cell population [26]. Another discrepancy between the techniques utilized in this study was found on chromosome 13, where CGH detected a gain of 13q13-q24, which was at odds with aCGH results. The difference might be explained by a low density of genes, on the chip, at chromosome 13.

We did not observe any correlation between genomic changes and prognosis of the disease. All patients are in the first complete remission following surgical treatment with a median follow up of 55 months.

Table 2 Clinical and genetic data

Case	Patient data			Tumor characteristic		CGH/aCGH imbalances			MLPA	Follow- up (year)
	Sex/age (year)	Germinal mutation	somatic mutation	size (cm^3)	Origin	Loss		Gain		
1	m/13	Het.-*VHL* c.374A>C	n.d	36+47	pheochromocytoma	CGH	3p12-pter;11p11-pter	–	n.d.	CR (6.58)
						aCGH	3p14.2-p26; 11p15.5-pter	–		
2	m/14	neg.	Het.-VHL c.389 T>G	35	pheochromocytoma	CGH	3;11	–	GGA. negative	CR (4.75)
						aCGH	3;11	–		
3	m/13	neg.	neg.	416	paraganglioma	CGH	1p11-pter;11p11-pter;17p12-pter	1q11-qter	GGA negative	CR (4.41)
						aCGH	1p13.1-pter;11p13-pter; 17p11.2-pter	1q11-qter	SGA del SDHB; gain SDHC	
4	f/13	Het.- *SDHB* 6 c.589-600dup. AGC ACC AGC TGC	n.d	47+19	paraganglioma	CGH	1p31.3-pter; 11p15.1-pter;17p11.2-ter[a]	13q13-q24	n.d.	CR (4.00)
						aCGH	1p12-pter; 11p13-pter	17p[a]		

n.d. not done, *neg.* negative, *GGA* germinal gene ateration, *SGA.* somatic gene alteration, *CR* complete remission, *m* male, *f* female [a] FISH found heterogeneity in status of 17p, *Het.* heterozygous

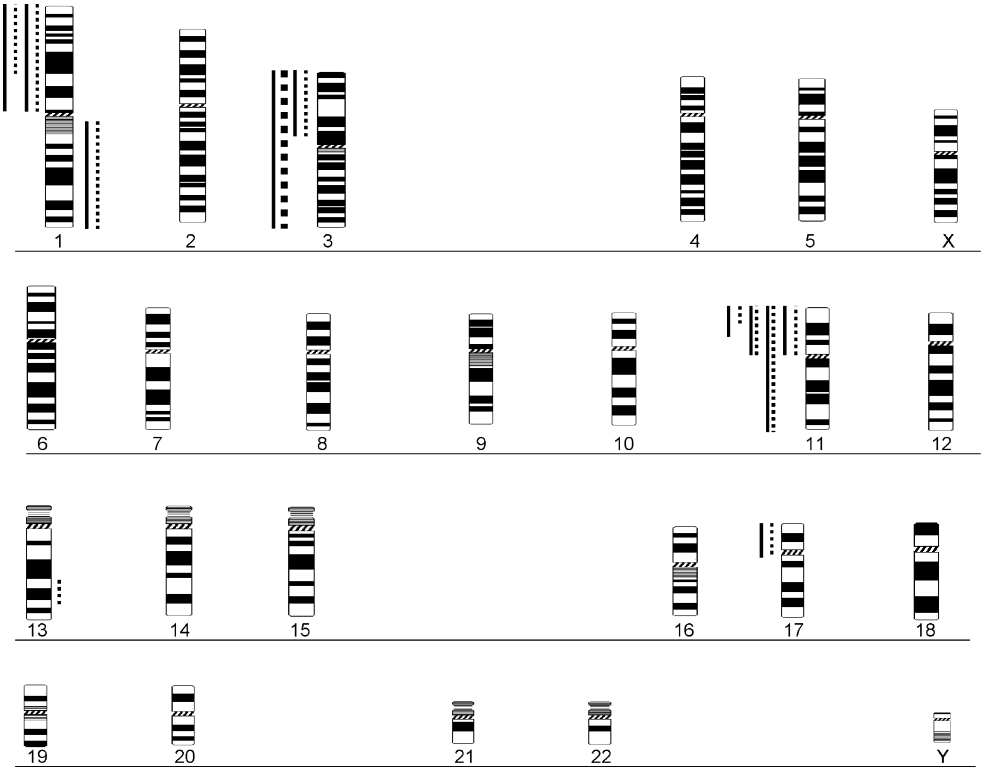

Fig. 1 Frequency plot of genetic changes for all 4 PCC. Loss and gain of chromosomal material are depicted by vertical bars to the left (loss) and right (gain) of chromosomes, respectively. *Dashed lines* indicate chromosomal CGH and normal lines aCGH. A deletion on chromosome 11 was found in all cases (3×11p, 1×11). Patients with pheochromocytoma showed an aberration pattern distinct from paraganglioma patients. In both pheochromocytomas, there were losses of chromosome 3 or 3p. Paragangliomas showed losses of 1p

Discussion

In agreement with other studies [4, 19–22], we found unbalanced chromosomal aberrations in PCCs, using CGH. This suggests that chromosomal changes might be an important tumorigenic event. Data from the literature shows that the most common copy number changes in PCC include loss of chromosomes 1p, 3q, 3p, 11p,11q, 6q, 17p, 22 and gain of chromosome 9q, 17q, and 20q [19–22, 27]. In our pediatric study, the most commonly observed chromosomal imbalances in PCCs included 1p, 3p, and 11p. All cases had more than one unbalanced change. These findings support the hypothesis of Koshla et al. [28] regarding involvement of multiple genes in the pathogenesis of these tumors.

Lui et al. [22], in a study of adult patients with PCC, reported a strong association between *VHL* mutations and loss of chromosomes 3 and 11. Hering's and our data suggest that mutations in *VHL*, which are either hereditary or somatic in origin, are also associated with 3p and 11p deletions. Hering et al. [21] identified a combined deletion of 3p and 11p in only 40% and combined deletion 3 and 11 in the remaining cases of VHL-associated PCC. In our study, we found loss of chromosome 11 or 11p in all cases (*VHL*-related pheochromocytoma and also in paraganglioma). Dannenberg et al. [19] detected the loss of 11p in two out of nine sporadic paragangliomas using CGH. Furthermore, loss of 11p has been reported in 5 of 11 sporadic abdominal paragangliomas [20]. We also detected deletion of 11p in a case of abdominal paraganglioma involving a mutation of the SDHB gene.

Numerous cases of deletion of chromosome 11 or 11p support the hypothesis that genes, relevant to PCC, are on the p arm of chromosome 11. Potential candidate genes are numerous and include WT1, IGF2, BW1, CDKN1C, H19 and others. Imprinting effects are important in some of these genes [21, 29].

Malignant pheochromocytomas represent very rare childhood tumors. Older age, absence of genetic syndromes in the family history, and DNA diploid tumors are favorable, relative to outcomes in pediatric PCC. The distinction between benign and malignant PCC cannot be made on the basis of clinical, biochemical, or histopathologic characteristics [4]. Data on genetic events, which could determine the malignant potential of PCCs are, so far, unsatisfactory, but some chromosomal changes (deletion 11q22-qter, deletion 6q) and aneuploidy are found more often in malignant tumors.

Edström et al. [20] showed that the main difference between benign and malignant tumors was partial deletion or gain of chromosome 11, as observed in 9 out of 12 malignant cases and 3 of 16 benign tumors. Among nine patients which developed metastasis, eight showed involvement of chromosome 11. Loss of 11q22-23 was significantly more common in malignant tumors than in benign ones [20]. Deletion of 11q22-23 has been described, by Hering, in patients with metastatic disease, which might strongly suggest the malignant potential of PCCs [21].

None of the tumors in our study showed loss of 11q22-qter as a solo aberration.

Frequent allelic imbalances at 6q have been reported in other malignancies and appear to be related to a poor prognosis or metastatic disease [30–32]. Dennenberg et al. [19] detected a loss of 6q in 34% of sporadic pheochromocytomas. These deletions were strongly associated with metastatic disease, although, Lemeta et al. [3] found that 72% of pheochromocytomas, including tumors classified as either benign or borderline, showed allele loss at 6q in two commonly affected regions (6q14 and 6q23-24). All cases were sporadic PCCs and the authors didn't find any significant difference in the allele loss between benign and borderline tumors. August et al. [33] was unable to confirm that a loss of 6q was an important event in tumor progression. CGH and aCGH did not revealed chromosomal changes on chromosome 6 in our cases.

Gain of genetic material is more frequently associated with malignant courses. The total number of genetic aberrations is higher in malignant tumors compared to benign tumors. Edström et al. [20] found a wide range in a number of genetic aberrations in both malignant tumors (mean = 6) and benign tumors (mean = 2.5). Dannenberg et al. [19] observed only a marginal association between the mean number of chromosomal alterations and malignancy (5.3±2.7 versus 8.2±6.1). August et al. [33] showed that tumors with 10 or more copy number changes were always associated with the development of metastases at a later stage, the presence of 8 chromosomal aberrations was associated with the occurrence of metastases in 85% of cases, while 60% of metastatic tumors showed less than 6 chromosomal aberrations. In our study the average of genetic aberrations, as revealed by CGH/aCGH, was 2.75 and 2.5 (range 2–4) per case, respectively. Additionally, we didn't find any of the 'supposed' molecular markers of malignancy in our patients.

In conclusion, our results showed which copy number changes, were the most common copy number changes in PCC's. Regarding the most common changes (1p, 3, 3p, 11, 11p), both techniques yielded similar results, however, we found 3 discrepancies between the methods. These discrepancies can't be completely explained by limited resolution, indicating that tumor heterogeneity played a role in the discrepancies observed in our results.

Our observations lead us to suggest that the incidence of deletion of chromosome 11 or 11p is more common in childhood PCC, than in adult PCC. These copy number alterations may play a significant role in PPC tumorigenesis.

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