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ORIGINAL PAPER

Mutations and polymorphisms in the SDHB, SDHD, VHL, and RET genes in sporadic and familial pheochromocytomas

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Abstract The prevalence of germ line mutations within the RET-protooncogene and the tumor suppressor genes SDHB, SDHD, and VHL in pheochromocytomas (PC) varies in recent studies from 12 to 24%, if one look at them collectively. DNA was extracted from frozen tumor tissue as well as from blood leukocytes of 36 PC (26 sporadic/10 MEN2). Exons 1-8 of the SDHB-gene, 1-4 of the SDHD-gene, 1-3 of the VHL-gene, and exons 10, 11, 13, 14, 16 of the RET-gene were amplified by PCR and analyzed by DHPLC with the Transgenomic WAVE®-System. Samples with aberrant wave profiles were subjected to direct sequencing. Genetic aberrations were correlated to clinical characteristics. Germ line mutations in sporadic PC were identified in four patients (11%) whereas somatic mutations were observed in two (5%) patients. Nine coding polymorphisms (PM) were identified in seven (19%) patients. Intronic variants were observed in six (17%) patients and were all located in the SHDB gene. Patients with wild type alleles in all assessed genes were older (53 vs. 37 years, $P = 0.007$) and presented with an increased tumor size (49 vs. 32 mm, $P = 0.003$) compared to patients with mutations. Malignant PC revealed multiple (>2) genetic alterations more frequently than benign PC (4/7 vs. 4/29, $P = 0.03$). Interestingly intronic variants of the SDHB gene occur more frequently in malignant than in benign PC (3/7 vs. 2/29, $P = 0.04$). The frequency of germ line mutations in sporadic pheochromocytomas was lower in our cohort than previously reported. Polymorphisms of the RET gene are common (17%) and occur in familial and sporadic PC. Multiple genetic alterations including mutations, polymorphisms and intronic variants are more frequently observed in malignant PC.

Keywords Pheochromocytoma · Genetics · Mutations

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Introduction

The traditional role that pheochromocytoma (PC) is called the "10%"-tumor is no longer tenable. About 24% of PC are caused by mutations of the RET-protooncogene, the succinate dehydrogenase subunit B (SDHB)-, the succinate dehydrogenase subunit D (SDHD)-, the Von-Hippel-Lindau (VHL)-, and the NF-1 gene according to a study which has included 271 patients with sporadic PCs [1]. However, the cumulative incidence of germ line mutations in several recent studies intended to be lower: RET (0.4%), SDHB (8.5%), SDHD (2.1%), and VHL (2.5%) [2–13]. Germ line mutations and hereditary PCs are associated with an increased risk of malignant PC, of recurrent tumors and bilateral disease [14]. These findings emphasize the importance of genetic testing in every patient with PC. Predictive genetic screening and regular screening of members with confirmed mutations in the context of hereditary PC intend an early diagnosis and treatment. Despite the high frequency of germ line mutations, somatic mutations of the genes responsible for hereditary PC and Paraganglioma (PG) have been rarely observed and occur in about 8% [7, 9, 10]. Recently the first somatic SDHB

mutation was reported [15]. A part from mutations which predispose for PC secondary genetic alterations may be needed to develop PC. Chromosomal loss of 1p, 3p, 3q, and 11q is frequently observed in sporadic and hereditary PC [8, 16–19]. The LOH of VHL, RET, and SDHD mutation is reported in hereditary PC, following the idea of Knudson's "two-hit" hypothesis. Several pathways have been recently identified which are activated in PC as the hypoxia-driven pathway HIF1-alpha, or the ras-mediated MAPK-pathway but the oncogenesis of PC is still not completely understood [20, 21]. An alternative mutation scanning method based on denaturing high performance liquid chromatography (DHPLC) has been introduced about 10 years ago. The high accuracy and specificity of this technology to identify mutations was documented by numerous reports [22, 23].

In this study we assessed the frequency of germ line and somatic mutations as well as polymorphisms and intronic variants in patients with PC and PG in the RET, VHL, SDHB, and SDHD gene.

Patients and methods

Patients

A total of 36 of 58 patients with pheochromocytoma, who underwent surgery in our hospital since 1993 were included. Ten of these 36 patients were known to have Multiple Endocrine Neoplasia Type 2 (MEN2) (8 MEN2A, 2 MEN2 B). Twenty-six patients had no family history of PC or PG and were classified as sporadic cases. Twenty-nine PC were classified histologically benign. Four patients showed PC with distant metastases and in another three patients histological criteria as invasion of the tumor capsule or lymphatic and vascular-invasion were recorded.

Diagnostic work-up

A 24-h urine sample was assessed for epinephrine, norepinephrine, metanephrine, and normetanephrine. All patients underwent a CT scan or MRI of the abdomen and a Metaiodobenzoguanidyl (MIBG)-scan.

Surgery

The standard procedure was a laparoscopic transabdominal adrenalectomy. If radiological findings suspected malignancy or distant metastases were present at diagnosis we choose an open adrenalectomy through a thoraco-abdominal approach.

Histology

The tumor size was measured. Invasion of the capsule as well as the invasion of lymphatic and blood vessels were determined. Immunohistochemistry for chromogranin A, synaptophysin, and Ki-67 was obtained in most samples. In all tumor samples a cellularity of at least 85% tumor was confirmed by AR based on HE staining before DNA was extracted.

Analysis of sequence variations

DNA was extracted from frozen tumor tissue as well as from whole blood leukocytes using standard procedures in all patients. Primers were selected and polymerase chain reactions (PCR) for every amplicon were established (see Tables 1 and 2). Exons 1-8 of the SDHB-gene, 1-4 of the SDHD-gene, 1-3 of the VHL-gene, and exons 10, 11, 13, 14, 16 of the RET-gene were amplified by PCR using PanTag polymerase. All PCRs were carried out on a Eppendorf Mastercycler. We focused on the letter RET exons as they have been reported to be hot spots for MEN2 mutations associated with PC and in regard to cost-effectiveness of mutation screening.

As recommended the melting profile of each amplicon was predicted using the computer program WAVE Maker 5.1 (Transgenomic, Omaha, Nebraska, USA). In some amplicons GCT-clamped primers were needed to prevent complete melt. Occasionally if the calculated conditions failed to produce well outlined peaks they were empirically modified. All analyzing conditions are displayed in Table 3. Prior to the WAVE-analysis hetero-duplex formation of an equal amount of the patients sample and a wild type sample was obtained in a PCR system: 95°C for 5 min, followed by a declining temperature T–1°C for 1 min until the sample reaches a temperature of 60°C. Samples with aberrant wave profiles were subjected to direct sequencing with the ABI310 sequencing analyzer according to standard procedures.

This study was performed according to the guidelines of the local ethic committee.

Results

Patients

The median age of the 36 patients was 46 years (20–78) and the median tumor size was 35 mm (9–130). The 10 MEN2 A patients were diagnosed by a median age of 31 (21–62) and therefore at younger age compared to non-syndromic patients. Three of ten patients with MEN2A

Table 1 Primer sequences of each gene with the size of amplicons and GCT-clamps, if used are encountered

Gene	Exon	Primer sequence (5′–3′)	Amplicon (bp)	GCT-Clamp
RET	10	ACA CTG CCC TGG AAA TAT GG	254	GCT
		CTC AGA TGT GCT GTT GAG AC		
RET	11	ATG AGG CAG AGC ATA CGC A	338	
		GAA ATG GGG GCA GAA CAC A		
RET	13	AAC TTG GGC AAG GCG ATG C	231	
		AGA ACA GGG CTG TAT GGA GC		
RET	14	AAG ACC CAA GCT GCC TGA C	296	
		GCT GGG TGC AGA GCC ATA T		
RET	16	AGG GAT AGG GCC TGG CCT T	184	
		TAA CCT CCA CCC CAA GAG		
VHL	1	CCT CGC CTC CGT TAC AAC GGC CTA	603	
		GCA GGG ACG ATA GCA CGG TC		
VHL	2	CAC CGG TGT GGC TCT TTA ACA A	264	
		CCA GTT CTC AAT TTT TGC CTG ATG T		
VHL	3	CTG AGA CCC TAG TCT GCC ACT GAG	266	
		TAC ACT GTT TCA TCT CAG CTT TTG		
SDHD	1	TGA CCT TGA GCC CTC AGG AAC G	98	
		TCA GGG TGG GAA GAC CCC T		
SDHD	2	GAT CAT CCT AAT GAC TCT TTC C	208	GCT
		AGC AGC AGC GAT GGA GAG AA		
SDHD	3	CTT TTA TGA ATC TGG TCC TTT TTG	236	GCT
		CAA CTA TAT TTG GAA TTG CTA		
SDHD	4	TGA TGT TAT GAT TTT TTC TTT TTC T	224	
		CAA TTC TTC AAA GTA TGA AGT CA		
SDHB	1	CGG AGA GCG ACC TCG GGG T	185	
		CAT CAG CTC CAG GCA GTC T		
SDHB	2	GTT TAT ATC CAG CGT TAC ATC	297	
		GGA TGT GAA AAG CAT GTC CCT		
SDHB	3	CTG AGA AGA CCA AAT GGA TAA G	240	
		GAC CAC AAG TAT CTG GAG CC		
SDHB	4	GGA GGA TCC AGA AGA AAG TA	281	
		GTA ACA CAC ATA GCA CTG CC		
SDHB	5	GCT GAG GTG ATG ATG GAA TCTG	248	
		CAC ACT CCT GGC AAT CAT CT		
SDHB	6	CAC TGA CCC CAA AGT AAC A	200	
		CCT CAG AAT GGC TGG CTT AC		
SDHB	7	GAG CTT TGA GTT GAG CCA GG	243	
		GCG TGT CAG CTC TGA GGC AG		
SDHB	8	GGA CAC TGA ACC AGC TGA GG	219	
		GCT CTG AGC TGG TTA TAA ATC		

bp base pairs

present with bilateral PC. In total three patients presented with sporadic paraganglioma.

Mutations

Fourteen germ line mutations were detected in 13 patients: 12 patients with RET mutations, one with a VHL mutation (L188P), and one MEN2A patient with an additional SDHB mutation (S163P). Two somatic mutations were identified in the VHL (G144X) and the SDHD (M1V) gene. All 10 patients with formerly known RET mutations were identified by DHPLC and confirmed by direct sequencing. In two patients with sporadic PC, RET mutations were identified. A 59-years-old female patient (P21) with a M918T mutation in exon 16 of the RET gene was presented with a relatively small tumor (40 mm) on the

Table 2 Cycle conditions for the amplification of genomic PCR fragments for the assessed genes are shown

Amplicon	DN 96°C	DN 96°C	Annealing	Ext 72°C	Ext 72°C	Cycles
RET Ex 10	5	0.5	66	0.5	10	35
RET Ex 11	5	1	61	1	10	35
RET Ex 13	5	0.5	62	0.5	10	30
RET Ex 14	5	1	62	1	10	35
RET Ex 16	5	0.5	62	0.5	10	30
VHL Ex 1	5	0.5	61	0.5	10	35
VHL Ex 2	5	0.5	61	0.5	10	35
VHL Ex 3	5	0.5	61	0.5	10	35
SDHB Ex 1	5	0.5	58	0.5	10	35
SDHB Ex 2	5	0.5	56	0.5	10	35
SDHB Ex 3	5	0.5	60	0.5	10	35
SDHB Ex 4	5	0.5	60	0.5	10	35
SDHB Ex 5	5	0.5	60	0.5	10	35
SDHB Ex 6	5	0.5	55	0.5	10	35
SDHB Ex 7	5	1	63	1	10	30
SDHB Ex 8	5	0.5	58	0.5	10	35
SDHD Ex 1	5	1	60	1	10	35
SDHD Ex 2	5	1	63	1	10	35
SDHD Ex 3	5	1	62	1	10	35
SDHD Ex 4	5	1	58	1	10	35

DN denaturing, *Ext* extension

Table 3 Details of the DHPLC analysis are listed regarding the temperature, the percentage of buffer B, the estimated running time, and the time shift

Amplicon	Temp (°C) DHPLC	Runtime	Time shift
RET Ex 10	64.7	2.5	0
RET Ex 11	64.1	2.5	0
RET Ex 13	62.3	2.5	0
RET Ex 14	62.0/65.8	2.5	−0.5/+1.5
RET Ex 16	58.6	2.5	0
VHL Ex 1	65.9	2.5	0
VHL Ex 2	58.2	2.5	+0.5
VHL Ex 3	63.9	2.5	0
SDHB Ex 1	63.4	2.5	0
SDHB Ex 2	55.5	2.5	0
SDHB Ex 3	56.5	2.5	0
SDHB Ex 4	58.2	2.5	0
SDHB Ex 5	56.7	2.5	0
SDHB Ex 6	58.2	2.5	0
SDHB Ex 7	62.0	2.5	0
SDHB Ex 8	56.8	2.5	0
SDHD Ex 1	65.8	2.5	0
SDHD Ex 2	61.8	2.5	0
SDHD Ex 3	62.0	2.5	0
SDHD Ex 4	60.0	2.5	+1

temp temperature

right side and had a negative family history. A 33-years-old male (P36) revealed a RET mutation in exon 11 (S649P). He had several operations due to a recurrent cervical paraganglioma which has metastasized to the lung and lymph nodes. In one patient we detected a VHL mutation L188P (P34). This male patient was diagnosed at the age of 20 years and presented with severe hypertension and a 40 mm PC of the right adrenal gland. The somatic VHL mutation G144X was detected in a 48-years-old male with a MEN1 syndrome which was reported previously [24, 25] with a germ line mutation in the Menin gene K119X. The somatic SDHD mutation M1V was found in the patient (P36) with the RET mutation (S649P) who was additionally harboring a RET PM (G691S). One male patient revealed a polymorphism in exon 5 of the SDHB gene S163P and interestingly was also harboring an RET germ line mutation (P15) (see Fig. 2). Although patient 21 displayed mild features of MEN2b, neither patient 21 nor patient 34 had a positive family history.

Polymorphisms in coding regions

We detected nine polymorphisms in seven patients: three times the PM G691S in RET exon 11, three times the L769L in RET exon 13, and the S836S in RET exon 14. One patient displayed both (P23). The patient (P34) with the VHL mutation had also the polymorphism S69S of the SDHD gene. All three patients with the PM G691S revealed also a RET germ line mutation in exon 11.

Intronic variants

These were only found in the SDHB gene in six patients in Introns 2, 4, and 5 (see Table 4, Figs. 1, 2).

Multiple genetic aberrations

Eight patients revealed more than one aberrant WAVE-profile which correspond to mutations, coding polymorphisms, and intronic variants in the direct sequencing as shown in Table 5.

Table 4 Result overview of the present study

Gene	*n* (G/S)	Mutations	*n* (G/S)	Polymorphism	*n* (G/S)	Intronic variants
RET	12/0	C634R, C634Y S649L	8/0	L769L, S836S G691S	0/0	
VHL	1/1	L168P/G144X	0/0		0/0	
SDHB	1/0	S163P	0/0		0/6	I2 G > A –33, I2 G > T +33, I4 T > C-38,-74, G > A –59, I5 G > A +33, I5 T > C +74
SDHD	0/1	M1V	1/0	S69S	0/0	
n	14/2		9/0		0/6	

G germ line, *S* somatic, *n* number

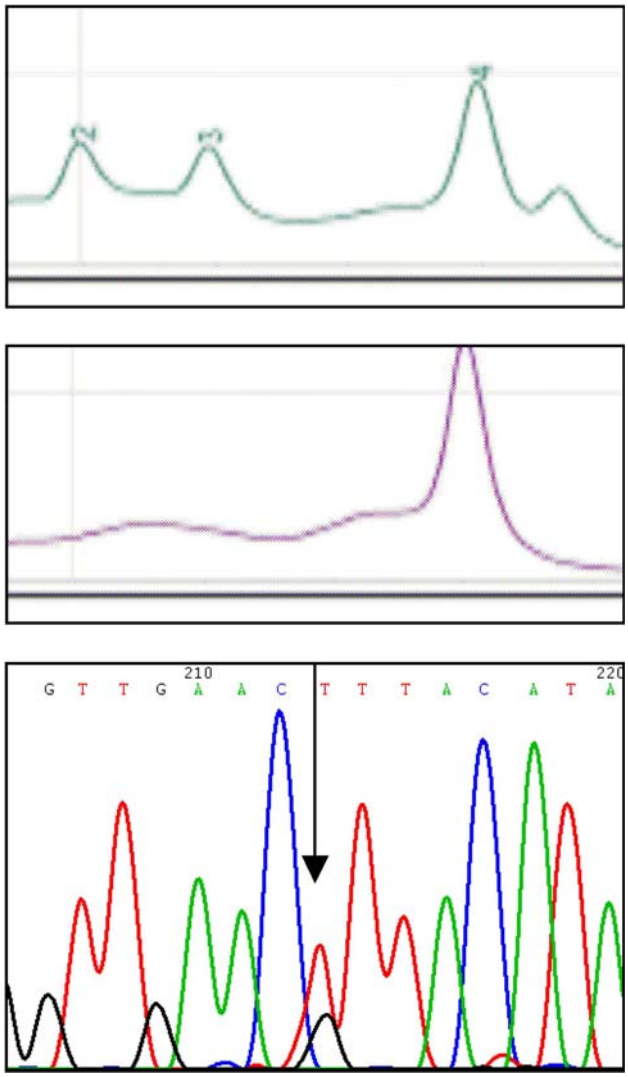

Fig. 1 Intronic variant ISV2-36 G > T was identified in five patients. On the top the DHPLC analysis is shown with the single peak of the wild type's electropherogram and the four peaks demonstrating the two homo- and two heteroduplexes of the mutant allele. The electropherogram shows the corresponding sequence

Prevalence of intronic SDHB variants

The intronic variants ISV4 an ISV5 were not identified in 30 healthy controls.

Correlation with clinical data

Fifteen patients had wild type alleles in all four genes screened for mutations. We compared them with either patients who revealed mutations (14), polymorphisms (9) or intronic variants (7).

Tumor size and age of diagnosis

Patients without any genetic aberration in the assessed genes were older (53 vs. 37 years, $P = 0.003$) and presented with larger tumors (49 vs. 32 mm, $P = 0.007$) when compared to patients with mutations. There were no differences between patients without mutations and polymorphisms. Patients with polymorphisms tend to have larger tumors (50 vs. 32, $P = 0.055$) and were older (51 vs. 37, $P = 0.10$) when compared to patients harboring germ line mutations.

Multiple tumors

All five patients (P4, P6, P14, P22, and P24) who demonstrated multiple tumors at histology harboring RET germ line mutations.

Ki-67

Twenty-nine PC were stained for Ki-67 by IHC. In most PC Ki-67 index was 1% (5) or lower (22). Two PC/PG showed higher Ki-67 indices with 10% (P36) and 5% in a sporadic PC in a 20-years-old patient (P34) revealing an SDHD PM S69S and a VHL mutation. Four of six malignant PC showed no increased Ki-67 indices.

Malignancy

Six of seven malignant PC were sporadic cases without a positive family history. Only one patient revealed a germ line mutation in the RET protooncogene (S649P + G691S) whereas genetic changes including polymorphisms and intronic variants were frequently found in four of seven malignant PC.

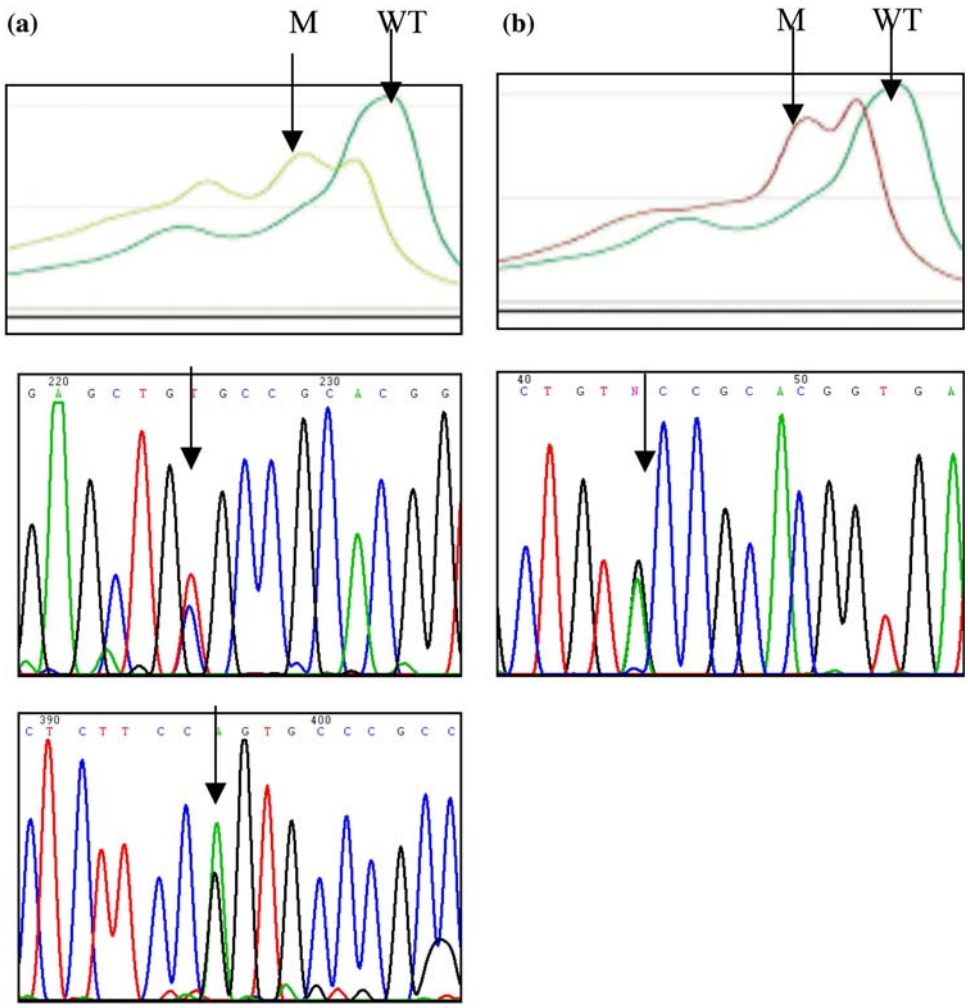

Fig. 2 **a** Mutation C634Y with the PM G691S; DHPLC electropherograms showing the Wild type (WT) and the Mutated amplicon (M). **b** Mutation C634R; DHPLC electropherograms showing the Wild type (WT) and the Mutated amplicon (M)

Interestingly three of seven patients with malignant PC revealed intronic variants in the SDHB gene, which was found to be less frequent in benign PC (3/7 vs. 2/29, $P = 0.04$).

Multiple aberrations (>2) were more frequently observed in malignant than in benign PC (4/7 vs. 4/29, $P = 0.03$).

There were no statistical differences between side, gender, and catecholamine concentration between the groups.

Discussion

The prevalence of RET mutations of 30% in our patients was higher than reported in the literature which relies on a selection bias due to our institution. If we exclude the 10 patients with family history we found an incidence of 7.8% which is similar to a mean incidence of 4.5% (0–21) reported the literature [1, 6, 9, 10]. Germ line mutations in the SDHB and SDHD gene were not detected which is consistent with the low frequency in these genes of approximately 4% (4–22) [1, 2, 6, 13]. In one patient with a paraganglioma we identified a somatic SDHD mutation M1V which is in order with the literature (1%) [26]. VHL mutations are found in 6.5% as a germ line and in 4.5% as a somatic mutation which is similar to our cohort having a frequency of 4% each [1, 6, 7, 9]. In recent studies single nucleotide polymorphisms (SNP) were described for RET, SDHB, SDHD, and VHL genes. The frequency of polymorphisms in a normal population and their role in tumorigenesis and clinical behavior of tumors, associated with the corresponding hereditary tumor syndromes, varies among these genes. Recently the polymorphism G691S in exon 11 of the RET gene was reported to be associated with earlier manifestation of medullary thyroid cancer in MEN2A patients and with familial C-cell hyperplasia [27, 28]. However, we identified the polymorphism G691S in 3/36 patients, all three had germ line mutations in RET exon 11 and were diagnosed by years of age 30, 33, and 59. Also the polymorphism L769L is reported to act as a modifier in medullary thyroid cancer [29]. Three of 36 (8%) or 3/10

Table 5 Clinical characteristics and results of the mutation analysis in 36 patients with pheochromocytoma

ID	D/Syndrome	Sex	FH	Age	Size/site	Mutation	Ki-67 (%)	Follow-up
1	Pheo/MEN 2a	f	pos	46	ND/l	RET C634Y	<1	NED
6	Pheo/MEN2a	f	pos	26	30,25/r;31/l	RET C634R	1	NED
14	Pheo/MEN-2a	m	pos	45	30,25,35/l	RET C634R	<1	NED
15	Pheo/MEN-2a	m	pos	31	40/l	RET C634R + G691S, SDHB S163P	<1	NED
20	Pheo/MEN2a	f	neg	62	30/r	RET C634R + G691S	<1	NED
24	Pheo/MEN2a	f	pos	59	15,20/l	RET C634R	<1	NED
26	Pheo/MEN2a	m	pos	21	25/r; 20/l	RET C634Y	<1	NED
27	Pheo/MEN2a	m	pos	36	24/r	RET C634R, RET Pol L769L	<1	NED
3	Pheo mal/MEN2b	m	pos	30	15,15,15/r	RET T M918T	<1	DED
22	Pheo/MEN2b	m	neg	28	15,10,12/l	RET M918T	<1	NED
4	Pheo/MEN1	m	pos	48	40/l	Menin K119X; VHL G144X (T)	ND	NED
8	Pheo mal	f	neg	78	50/r	SDHB ISV2 + 33 G > A (T), SDHB ISV236 G > T (T)	<1	DUC(MI)
10	Pheo mal	f	neg	65	90/l	SDHB ISV4 + 38, + 74 T > C (T); +59G > A (T)	ND	NED
23	Pheo mal	f	neg	44	45/l	RET Pol L769L, RET Pol S926S, SDHB ISV2 +33 G > A, ISV5 -74 T > C (T)	<1	NED
29	Pheo mal	f	neg	22	40/l	RET Pol S926S	<1	NED
33	Pheo mal	m	neg	42	75/l	WT	1	AWD
36	Paragangliom mal	m	neg	33		RET S649P + G691S, SDHD M1V (T)	10	AWD
32	Paragangliom	f	neg	47	30/l	WT	1	NED
35	Paragangliom	f	pos	48	35/r	WT	1	NED
2	Pheo	m	neg	40	50/l	WT	<1	Lost
5	Pheo	m	neg	54	45/l	WT	ND	NED
7	Pheo	f	neg	78	47/r	WT	<1	DUC
9	Pheo	f	neg	62	82/r	WT	<1	DUC
11	Pheo	f	neg	55	80/r	SDHB ISV5 -33 ho G > A (T)	<1	NED
12	Pheo	m	neg	63	35/r	WT	<1	NED
13	Pheo	f	neg	46	35/r	SDHB ISV5 -33 G > A (T)	<1	NED
16	Pheo	f	neg	60	60/l	WT	ND	NED
17	Pheo	f	neg	65	35/r	WT	ND	Lost
18	Pheo	m	neg	53	50/r	WT	ND	NED
19	Pheo	m	neg	37	35/r	WT	<1	NED
21	Pheo	f	neg	59	40/r	RET M918T	ND	NED
25	Pheo	m	neg	52	15/l	RET Pol S926S	<1	NED
28	Pheo	f	neg	71	60/l	WT	<1	NED
30	Pheo	f	neg	35	60/r	WT	1	Lost
31	Pheo	f	neg	54	39/r	WT	ND	NED
34	Pheo	m	neg	20	40/r	SDHD Pol S69S, VHL L188P	5	NED

T tumor, *Pheo* pheochromocytoma, *pos* positive, *neg* negative, *WT* wild type, *r* right, *l* left, *Mut* mutation, *Pol* polymorphism, *NED* no evidence for disease, *AWD* alive with disease, *DUC* died of unrelated causes, *ISV* intronic variant

(33%) MEN2 A patients revealed this polymorphism. The polymorphism S836S was detected in 3/26 (11%) patients with sporadic pheochromocytoma whereas recent studies report a frequency of only 3.7% [30] in a normal population. However, we do not know if these findings are randomly in this small cohort of 36 patients. Nevertheless, synonymous amino acid changes might not only cause altered rates of transcription but also, due to changed mRNA structure and stability, translational efficiency may be affected. The SNP (G691S) changes the nonpolar amino acid glycine into the polar amino acid serine. This alteration can substantially alter protein processing, folding, subcellular localization, or other functional properties. This principle has to kept in mind regarding the SDHB polymorphism S163P, which was detected in two patients: one with an MEN2 A syndrome and one with a malignant PC. Intronic variants have been found in six patients all within the SDHB gene (see Table 4). The frequencies in a normal

population are only known for ISV +33 (11%) and ISV-36 (3%) according to the Leiden Open Variation Database (http://chromium.liacs.nl). Despite being located in non-transcribed regions, these types of SNPs can have substantial functional effect by altering splice or branch sites or, if located in or near the promoter region, transcription. In this study intronic variants were more frequently in malignant than in benign PC (4/7 vs. 2/29, $P = 0.04$).

We showed that malignant PC tend to have multiple genetic changes when regarding mutations, polymorphisms and intronic variants (4/7 vs. 4/29, $P = 0.03$), which could be a hint for the genetic instability. This could be both either consequence or reason for the malignant behavior. The malignancy is only certain if distant metastases are present.

The prevalence of malignancy in PC ranges from 2.4% to 26%, depending on how malignancy is defined. The highest rate of malignancy is observed in patients with SDHB mutations and reaches 50%. However, the histologic distinction of benign from malignant PC is not reliable. Malignancy is only proven by the presence of distant or lymph node metastases. Efforts have been made to establish molecular markers for malignancy as overexpression of HSP90, human telomerase reverse transcriptase, HIF-2 alpha, tenascin, Ncadherin, COX-2, and others. Comparative genomic hybridization experiments in malignant and benign PC revealed losses of 1p, 3q, 6q and gains of 9q and 17q. Progression to malignant PC was associated to deletion of 6q and 17p [16].

As malignant pheochromocytomas tend to develop multiple genetic alterations, especially intronic variants in the SDHB gene, could be used as an additional marker for malignancy.

In conclusion we confirmed the frequency of germ line mutations in the RET, SDHB, SDHD, and VHL gene in sporadic pheochromocytomas. Polymorphisms in the RET gene are common and occur in about 30% of patients with MEN2A and in 15% of patients with sporadic PC. Multiple genetic alterations including mutations, polymorphisms, and intronic variants are more frequently observed in malignant PC.

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