Systematic comparison of sporadic and syndromic pancreatic islet cell tumors

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Abstract

Pancreatic islet cell tumors (ICTs) occur as sporadic neoplasias or as a manifestation of multiple endocrine neoplasia type 1 (MEN1) and von Hippel–Lindau disease (VHL). Molecular classification of ICTs is mandatory for timely diagnosis and surveillance. Systematic comparison of VHL-ICTs and sporadic ICTs has been lacking. Our registry-based approaches used the German NET-Registry with 259 patients with neuroendocrine tumors (NETs), who were primarily

diagnosed with NETs, and the German VHL-Registry with 485 molecular genetically confirmed patients who had undergone magnetic resonance imaging or computed tomography of the abdomen. All patients provided blood DNA for testing of the *MEN1* and *VHL* genes for intragenic mutations and large deletions. In the NET-Registry, 9/101 patients (8.9%) with ICTs had germline mutations, 8 in *MEN1* and 1 in *VHL*. In the VHL-Registry, prevalence of NETs was 52/487 (10.6%), and all were ICTs. Interestingly, of those with *VHL* p.R167W, 47% developed ICTs, compared to 2% of those with p.Y98H. In total, there were 92 truly sporadic, i.e. mutation-negative ICT patients. Comparing these with the 53 VHL-ICT patients, the statistically significant differences were predominance of female gender ($P=0.01$), multifocal ICTs ($P=0.0029$), and lower malignancy rate ($P<0.001$) in VHL-ICTs compared to sporadic cases. VHL was prevalent in $<0.5\%$ of NETs, while NETs occur in $\sim 10\%$ of VHL, virtually exclusively as ICTs, which are rarely the first presentation. Patients with NETs should not be subjected to genetic testing of the *VHL* gene, unless they have multifocal ICTs, other VHL-associated tumors, and/or a family history for VHL.

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Introduction

Islet cell tumors of the pancreas (ICTs) are neuroendocrine tumors (NETs), which are classified into three entities: 1) ICT as sporadic tumor; 2) ICT as a component of multiple endocrine neoplasia type 1 (MEN1; Chandrasekharappa *et al.* 1997, Brandi *et al.* 2001); and 3) ICT in patients with von Hippel–Lindau disease (VHL; Doherty 2005, Woodward & Maher 2006, Blansfield *et al.* 2007, Corcos *et al.* 2008, Toumpanakis & Caplin 2008). MEN1 and VHL are autosomal dominant diseases affecting several organs, whereas the etiological background in sporadic ICTs remains to be clarified. In contrast to the well-recognized sporadic and MEN1-associated ICTs, ICT as a component of VHL has not been systematically investigated. In this context, it may also be noteworthy that coincidence of extra-pancreatic NETs has rarely been reported in patients with VHL (Kees 1980, Cheng *et al.* 1997). Comprehensive studies, however, on the prevalence of VHL in patients with NETs and their characteristics have yet to be performed.

As with all heritable neuroendocrine neoplasia syndromes in the 21st century, recognition of VHL is mandatory because gene-based risk assessment and organ-specific surveillance and interventions reduce morbidity and mortality. Major manifestations of VHL are hemangioblastoma of the retina and the central nervous system (CNS), renal clear cell carcinoma, pheochromocytoma and paraganglioma, multiple pancreatic cysts, ICTs, endolymphatic sac tumor of the inner ear, and cystadenoma of the epididymis and broad ligament. The susceptibility gene, *VHL*, is located in 3p25–26 comprising three exons and encoding the 213-amino acid VHL protein. The germline mutations are scattered throughout the gene. Potentially, malignant VHL-associated tumors,

e.g. leading to distant metastases, are renal clear cell carcinoma, malignant pheochromocytoma, and ICTs (reviewed by Lonser *et al.* (2003)).

In this study, we sought to evaluate a population-based registry of patients with NETs for the prevalence of gene-defined VHL and MEN1. In parallel, we interrogated a population-based registry of VHL for the prevalence of NETs. Finally, we compared the demographic and clinical characteristics of truly sporadic ICTs and those with MEN1- and VHL-associated ICTs in order to help define guidelines for offering molecular genetic testing of the *VHL* gene in patients presenting with ICTs as well as the necessity of pancreatic screening in individuals with VHL.

Methods

Registries

The NET-Registry

Incepted in 2003, German endocrinology, gastroenterology, and oncology centers registered their NET patients into the NET-Registry. Patients were registered with demographic and clinical data including age at onset, gender, location, number and size of the tumor, and benign or malignant status (Plöckinger *et al.* 2010). All registrants from 1 November 2005 until 31 October 2008 ($n=259$) were offered mutation analysis of the *MEN1* and *VHL* genes. In addition, the NET-Registry was open to patients from other countries, which only represent about 3% of the total entries.

The VHL-Registry

Founded in 1983, the VHL-Registry based in Freiburg was established by a nation-wide networking of ophthalmologists, neurosurgeons, endocrinologists, and geneticists. Criteria for inclusion were a proven germline

mutation of the *VHL* gene. Since 1983, ascertainment and registration included >95% of all patients with *VHL* in Germany. In addition, as of 1998, the *VHL*-Registry was also open to patients from other countries, representing about 5% of the total entries.

Clinical data

The diagnosis of ICTs in NET-Registry patients was based on histopathological confirmation in all cases. In contrast, in the *VHL*-Registry, solid pancreatic tumors were diagnosed as ICTs by histological confirmation and/or imaging since no other solid pancreatic tumors have been described in *VHL*, and surgery is only recommended for patients with tumors of a considerable size (Hammel *et al.* 2000, Libutti *et al.* 2000, Lonser *et al.* 2003). Thus, the common phenotype of ICTs in both the registries is the radiologically solid tumor of the pancreas, but in contrast to the ICTs in the NET-Registry all of which were confirmed by histology, in *VHL*-associated ICTs, histology was not obligatory.

Clinical data included demographic data, personal and family history for neoplasias, tumor imaging, laboratory-measured hormonal status before surgery, histopathological findings, and follow-up data. Basic investigations included gadolinium-enhanced magnetic resonance imaging (MRI) of the abdomen and the set of gastrin, C-peptide, and insulin measurements using standard assays. The diagnosis of a malignant neuroendocrine pancreatic tumor was based on the presence of metastases.

Patients with *VHL* underwent additional clinical investigation, which included retinoscopy, gadolinium-enhanced MRI of the brain and spinal cord, as well as catecholamine measurement in the 24 h urine.

Molecular genetic analyses

All registrants provided 10 ml EDTA-anticoagulated blood for molecular genetic analyses. The analyses comprised testing of all exons including the splice sites of the *VHL* (RefSeq:NM_000551.2) and *MEN1* (RefSeq:NM_130803.1) genes for germline mutations. DNA extraction, primers, PCR conditions, and sequencing methods followed published protocols of international standards (Lemmens *et al.* 1997, Franke *et al.* 2009). The primers are available on request. Of note, analyses also included screening for large deletions or rearrangements of the *VHL* and *MEN1* genes. For screening for intragenic mutations, we used the PCR-based denaturing HPLC. For detection of large deletions/rearrangements, we used two commercially available kits for multiplex ligation-dependent probe

amplification, the SALSA MLPA KIT P016 *VHL*, and the SALSA MLPA KIT P017-B1 *MEN1* respectively (MLPA, MRC Holland, Amsterdam, The Netherlands). The primers are available on request. All analyses for the *VHL* gene and the analyses for intragenic mutations in the *MEN1* gene have been performed in Freiburg. The analyses for the large deletions and rearrangements of the *MEN1* gene have been performed in Madrid.

Statistical analysis

We compared the groups of *VHL* mutation as positive and sporadic ICT patients for differences in the variables age, gender, pancreatic location as first documented manifestation (yes or no), tumor biology (benign and malignant), multiple or single ICT, and family history for *VHL*, *MEN1*, or NETs. For comparison of means, we used the *t*-test. Two-tailed Fisher's exact test was used for analysis of categorical data. Missing data were excluded from the analysis.

Approval and consent

The study design was approved by the ethical committee of the University Medical Center of Freiburg and all participating centers accordingly. The study was performed in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 1983. For the NET-Registry, a positive ethical consent was acquired at the Charité, Berlin. In addition, official consent from the Berlin Commissioner for Data Protection and Freedom of Information was secured. All patients signed an informed consent form for participation with the registry and an additional form consenting to the genetic analysis.

Role of the funding source

The funding sources had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

The NET-Registry

The 259 unrelated index registrants in the NET-Registry from whom blood DNA was available for molecular genetic analyses formed the basis for our first set of studies addressing the prevalence of *VHL* and *MEN1* in unselected consecutive patients with NETs. Of the 259 subjects, 101 (38.9%) had ICTs, 42 females and 59 males, with mean age at diagnosis 55.7 years (range 12–82; s.d. 13.1). Of these 101 unrelated patients with

ICTs from the NET-Registry, 8 were found to carry germline mutations in the *MEN1* gene and 1 had a mutation of the *VHL* gene. The detected *MEN1* germline mutations were different in each of the eight cases, namely *MEN1* c.322 C>T, p.Arg108X, *MEN1* c.333 delT, p.Val112SerfsX7, *MEN1* c.640 del26, p.Gly214ProfsX9, *MEN1* c.673 G>A, p.G225R, *MEN1* c.740 T>C, p.I247T, *MEN1* c.758 C>T, p.S253L, *MEN1* c.824+1 G>A, and *MEN1* c.1546 dupC, p.Ala516ProfsX15. The only patient with VHL-ICT was found to carry the *VHL* c.349 dupT, W117LfsX15 mutation. Of these mutations, *MEN1* c.322 C>T, p.Arg108X*, *MEN1* c.673 G>A, p.G225R*, and *MEN1* c.824+1 G>A* have already been published. The published mutations are indicated by an * (Giraud et al. 1998, Hai et al. 1999, Crepin et al. 2003). In contrast, molecular genetic analyses of the 158 patients of the NET-Registry with extra-pancreatic NETs did not show any germline mutations of the *MEN1* or *VHL* gene. Major locations of these tumors were jejunum–ileum–cecum in 31% of the entire NET-Registry, colorectum 8%, appendix 4%, bronchus 3%, stomach 2%, and 12% were from other sites or unknown primary location. None of the 259 patients of the NET-Registry had signs of neurofibromatosis type 1 or tuberous sclerosis complex.

VHL-Registry

A total of 485 registrants from the VHL-Registry underwent pancreatic imaging. Of these 485 patients, 224 had pancreatic abnormalities. Multiple pancreatic cysts or cystadenoma were demonstrated in 171 subjects. ICT was present in 53 (11%) subjects, 10 of whom also had pancreatic cysts.

The 52 index cases, 34 female and 18 male, were diagnosed with ICTs at mean age of 36.8 years (range 13–75, s.d. 12.5). Forty (76%) subjects had a single ICT, whereas 12 subjects had multiple ICTs.

All 52 patients were diagnosed with VHL due to clinical symptoms caused by tumors in other organs; in addition, 11 VHL patients had symptoms due to the pancreatic tumor later in life. Malignant ICTs occurred in 13 of the 52 index cases. Of these 13 cases, 11 had liver metastases, 5 had lymph node metastases, and 2 had additional bone metastases.

Patients with mutations of the *VHL* gene

The germline *VHL* mutations of the 53 VHL patients with ICTs, 52 from the VHL-Registry and 1 from the NET-Registry, are shown in Table 1. These 53 patients harbored 30 different intragenic mutations, 21 missense, and 9 truncating mutations, and in addition

6 large deletions ranging from deletion of 1 to all 3 exons. Interestingly, while the VHL-Registry expectedly has a predominance of the Y98H founder mutation observed frequently in the Black Forest region (35%), only 1% of those with this mutation developed ICTs. In contrast, 47% of those with hot spot mutation R167W developed ICTs. Excluding the 158 registrants with the Y98H founder mutation, a clustering of mutations at codons 160–167 (19/53 ICT patients vs 34/296 VHL registrants) was present.

Associated tumors in the 53 VHL patients with ICTs included retinal hemangioblastomas in 34 (64%) cases, CNS hemangioblastomas in 44 (81%), renal clear cell carcinoma in 20 (37%), pheochromocytoma in 29 (53%), endolymphatic sac tumor of the inner ear in 1 (1.8%), cystadenomas of the epididymis in 3 (5.5%) and broad ligament tumor in 2 (3.7%) subject. These figures do not differ statistically from the 432 VHL patients in this study without ICTs from the VHL-Registry: retinal hemangioblastomas 213/394 (54%), CNS hemangioblastomas 246/400 (62%), renal clear cell carcinoma 88/416 (21%), pheochromocytoma 148/416 (36%), endolymphatic sac tumor of the inner ear 6/339 (2%), and cystadenomas of the broad ligament or the epididymis 21/344 (6%).

Comparison of demographic and clinical data in sporadic ICTs and VHL-associated ICTs

The demographic and clinical data of 53 patients with VHL-associated ICTs and 92 patients with apparently sporadic ICTs (those in whom germline mutations of the *MEN1* and *VHL* genes have been excluded by this study) are shown in Table 2. The mean age at diagnosis of ICTs was 37 (s.d. 12.5) years for the VHL group and 57 (s.d. 12.0) years for the sporadic group (<0.001). Patients with VHL-associated ICTs were more frequently female (VHL 34/54 vs sporadic 37/92, $P=0.01$), had more multifocal ICTs (VHL 13/54 vs sporadic 5/92; $P=0.0029$), and had fewer instances of malignant tumors (VHL 14/54 vs sporadic 68/92, $P=<0.001$) compared to sporadic ICT patients.

Comparison of demographic and clinical data in VHL- and *MEN1*-associated ICTs

Although based on small numbers of *MEN1*-related ICT patients ($n=8$), VHL-related ICTs had fewer instances of malignancy than those found in *MEN1* (14/53 vs 6/8, $P=0.012$). There were no significant differences in gender distribution, age at diagnoses, single vs multifocal tumors, and family histories between ICT patients with VHL and those with

Table 1 von Hippel–Lindau disease (VHL) germline mutations in VHL patients with islet cell tumors (ICTs)

Nucleotide change	Consequence	VHL patients with ICTs	Patients with the given mutation in this study	References
c.208 G>T ^a	p.E70X	1	1	Chen <i>et al.</i> (1995)
c.221 T/G ^a	p.V74G	1	4	Glavac <i>et al.</i> (1996)
c.440_442delTCT	p.F76CfsX83	1		
c.240 T>G ^a	p.S80R	1	2	Crossey <i>et al.</i> (1994)
c.266 T/C ^a	p.L89P	1	7	Crossey <i>et al.</i> (1994)
c.277 G>C ^a	p.G93R	1		Klein <i>et al.</i> (2001)
c.292 T>C ^a	p.Y98H	2	158	Crossey <i>et al.</i> (1994)
c.340 G>A ^a	p.G114S	1	1	Blanco <i>et al.</i> (2004)
c.349dupT	p.W117LfsX1	1	1	
c.357 C>G ^a	p.F119L	2	2	Chen <i>et al.</i> (1995)
c.362 A>G ^a	p.D121G	1	1	Whaley <i>et al.</i> (1994)
c.364_365 GC>AT	p.A122I	1	1	
c.393 C>A ^a	p.N131K	1		Gallou <i>et al.</i> (2004)
c.394 C>T ^a	p.Q132X	1		Glavac <i>et al.</i> (1996)
c.445_458del14	p.N150SfsX19	1	2	
c.453 C>G	p.I151M	1	1	
c.461 C>T ^a	p.P154L	1	4	Crossey <i>et al.</i> (1994)
c.464-2 A>G ^a	Splice	2	4	Glavac <i>et al.</i> (1996)
c.467 A>G ^a	p.Y156C	1		Neumann <i>et al.</i> (2002)
c.472 C>G ^a	p.L158V	1	1	Crossey <i>et al.</i> (1994)
c.478_479delGA	p.E160AfsX12	2	3	
c.482 G>A ^a	p.R161Q	5	8	Chen <i>et al.</i> (1995)
c.488 T>A ^a	p.L163H	1	2	Chen <i>et al.</i> (1995)
c.499 C>T ^a	p.R167W	7	15	Crossey <i>et al.</i> (1994)
c.500 G>A ^a	p.R167Q	4	5	Crossey <i>et al.</i> (1994)
c.533 T>C ^a	p.L178P	1	1	Crossey <i>et al.</i> (1994)
c.548 C>A ^a	p.S183X	1	4	Whaley <i>et al.</i> (1994)
c.583 C>T ^a	p.Q195X	1	3	Crossey <i>et al.</i> (1994)
c. 593 T>A ^a	p.L198Q	2		Neumann <i>et al.</i> (2002)
Large deletions ^b		6	63	

^aIndicates mutations that have been already published.^bIndicates deletions of 1, 2, or 3 exons of the *VHL* gene.

MEN1 ($P=0.2$ – 1.0 ; Table 3). The hormone status for blood levels of gastrin, C peptide, and insulin was known from all 8 (7 insulinomas) MEN1, 16 VHL, and 72 patients with sporadic ICTs. In the MEN1 group, seven patients had insulinomas; in the VHL group, all patients were without elevated hormone levels; in the patients with sporadic ICTs, four patients had insulinomas, three gastrinomas, three glucagonomas, two vipomas, and two Zollinger–Ellison syndrome.

Discussion

Both NETs and paraganglial tumors are of neural crest origin, but the pathogenesis appears to be only partially overlapping. With respect to NETs and paraganglial tumor predisposition, NETs are frequent tumors in MEN1 (Brandi *et al.* 2001), whereas paraganglial tumors are associated with VHL, MEN2, neurofibromatosis type 1, and the paraganglioma syndromes type 1, 2, 3, and 4 (Erlic & Neumann 2009). In contrast,

pancreatic ICTs are typical component tumors of MEN1 and VHL. Very rarely, ICTs have also been reported in neurofibromatosis type 1 and tuberous sclerosis complex (Perren *et al.* 2006, Dworakowska & Grossman 2009). However, studies on large series of patients with systematic molecular genetic analyses for germline mutations in the genes *MEN1* and *VHL* were pending.

In this study, we used a series of 744 patients from two population-based registries, the VHL-Registry based in Freiburg with 485 patients and the German NET-Registry with 259 patients, all of whom had DNA and relevant clinical data available. These two registries were selected for purposes of this study so that we are able to answer specific hypotheses. It is important to note that the two registries are completely different registries that, naturally enough, have different eligibility criteria for registration. The NET-Registry registers all comers with histologically confirmed NETs from any anatomic site, irrespective of other features or family history. For purposes of our study,

Table 2 Comparison between VHL-related and sporadic islet cell tumors

Variables	VHL	Sporadic	P value
Age (years)			
Mean	36.8	57.07	
S.D.	12.49	11.98	<0.001
Sex			
Female	34	37	
Male	19	55	0.01
Tumor number			
Single	41	87	
Multiple	12	5	0.0029
Tumor biology			
Benign	39	24	
Malignant	14	68	<0.001
Pancreatic tumor as first presentation			
Yes	2	92	
No	51	0	<0.001
Family history of NETs/VHL/MEN1			
Positive	32	1	
Negative	21	91	<0.001

P values considered to be significant are represented in bold. VHL, von Hippel–Lindau disease; MEN1, multiple endocrine neoplasia syndrome type 1; NETs, neuroendocrine tumors.

the database of this registry was then searched for only pancreatic ICTs. This allows us to ask the question among all comers with histologically documented pancreatic ICTs, what is the prevalent frequency of germline *MEN1* and *VHL* mutations. The second registry is the VHL-Registry whose entry criteria are noted in the section ‘Methods’, but in brief, registration requires VHL diagnostic criteria and/or germline *VHL* mutations. This registry notates patients who are operated on for ICTs and thus have histological diagnosis, but it also includes patients not operated on for pancreatic ICTs, mostly smaller than 3 cm in diameter. This registry allowed us to ask what is the prevalence of ICTs among those with *VHL* mutations. Owing to our practice guidelines, ICTs smaller than 3 cm are usually not operated on. These differences of ascertaining ICTs from the NET-Registry and from the VHL-Registry are slightly different, and may, in part, affect our results and conclusions. In particular, the relatively low prevalence of patients with mutations of the *MEN1* gene, perhaps in part due to the strict entry criteria of histological documentation, should initiate confirming studies.

Prevalence of ICTs in the NET-Registry was 101/259 (39%). While slightly under half of the 259 NET-Registry patients had ICTs, only 1 carried a germline *VHL* mutation and 8 an *MEN1* mutation. The very low frequency of occult germline *VHL* mutations in sporadic ICT series is confirming the poor involvement

of this pathway in the development of the sporadic ICTs (Chung *et al.* 1997, Giraud *et al.* 1998, Anlauf *et al.* 2006). While one could invoke a selection bias to explain these observations, this is unlikely as our registries are essentially population based.

When there is a relatively high frequency (usually >10%) of unexpected germline mutations in a single organ (apparently sporadic) presentation of a neoplasia, the usual practice recommendation is to offer genetic testing to all incident cases in the setting of genetic counseling. This is true for medullary thyroid cancer (Kloos *et al.* 2009) and pheochromocytoma/paraganglioma (Neumann *et al.* 2002, Benn *et al.* 2006, Burnichon *et al.* 2009, Erlic *et al.* 2009, Franke *et al.* 2009, Mannelli *et al.* 2009). Therefore, a clinically relevant question is whether *VHL* and/or *MEN1* testing should be offered to all presentations of ICTs. Given the very low frequency of occult mutations in *VHL* in the NET-Registry, whether it is an ICT presentation (one registrant) or an extra-pancreatic NET presentation (none), our data suggest that *VHL* testing is not warranted for every NET, even ICT, presentation in the absence of other VHL component features or family history. On the other hand, if an individual already has VHL especially with the R167W mutation, then careful surveillance for ICTs is warranted.

Our study has suggested two distinct, molecular genetically defined groups of patients with ICTs. It is not surprising that VHL-related and MEN1-related ICTs are very similar to each other, but distinct from sporadic ICTs, in regard to younger age at diagnosis and higher frequency multifocal tumors, both

Table 3 Comparison between VHL-related and MEN1-related islet cell tumors

Variables	VHL	MEN1	P value
Age (years)			
Mean	36.8	43.88	0.27
95% CI	12.49	16.21	
Sex			
Female	34	5	1.0
Male	19	3	
Tumor number			
Single	41	4	0.19
Multiple	12	4	
Tumor biology			
Benign	39	2	0.012
Malignant	14	6	
Family history of NETs/VHL/MEN1			
Positive	31	5	1.0
Negative	22	3	

P values considered to be significant are represented in bold. VHL, von Hippel–Lindau disease; MEN1, multiple endocrine neoplasia syndrome type 1; NETs, neuroendocrine tumors.

hallmarks of heritable neoplasia. Interestingly, there is a predominance of female gender and benign disease in VHL-related ICTs compared to sporadic ICTs. Although the sample size is small for MEN1-related ICTs, it is interesting to preliminarily note that VHL-related ICTs have a preponderance of benign disease even over MEN1-related ICTs. It is not clear whether the differences in the age at diagnosis and tumor biology are biased by the early and regular clinical check ups that VHL patients undergo, or whether it is a characteristic relevant to the VHL genotype. However, both hypotheses merit further investigation at different levels. In the first case, it could lend proof that VHL patients benefit from regular screening for ICTs, and in the second that VHL-associated pathways might present a protective role for ICT behavior. One hint derives from the preponderance of ICTs in individuals with VHL carrying the p.R167W mutation compared to the relative underrepresentation of ICTs in VHL individuals with the p.Y98H Black Forest founder mutation.

In conclusion, VHL is a rare cause of ICT and extra-pancreatic NET. Thus, *VHL* gene testing in individuals with ICTs or NETs is not indicated unless the patient and/or family have other clinical features of VHL. Surveillance for ICTs with known VHL, however, is likely indicated in patients, in particular those who are female and/or with the R167W mutation. All patients with VHL receive routine abdomino-pelvic surveillance, and so no extra imaging is required, but perhaps vigilance and awareness of ICTs in the VHL setting. In contrast to MEN1- and sporadic ICTs, VHL-related ICTs affect more females than males but are less often malignant.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

H P H Neumann, C Eng, Z Erlic, U Plöckinger, A Winter, R P Baum, and M Robledo designed the study, performed the registrations of the patients, performed the analyses, drafted,

revised, and approved the final manuscript. The molecular genetic analyses have been performed in the laboratories in Freiburg and Madrid by A Cascon, M M Hoffmann, J Bacher, M Sullivan, A Weber, and M Nunez. Important and detailed informations of patients were provided by B Isermann, L Fischer, A Raffel, W T Knoefel, M Schott, T Baumann, O Schaefer, T Keck, I Milos, M Muresan, M Peczkowska, A Januszewicz, K Cupisti, A Tönjes, M Fasshauer, J Langrehr, P von Wussow, A Agaimy, G Schlimok, R Lamberts, T Wiech, and K W Schmid.

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