Clinical Cancer Research

MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma

Nelly Burnichon, Alberto Cascon, Francesca Schiavi, et al.

Clin Cancer Res Published OnlineFirst March 27, 2012.

MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma.

Nelly Burnichon, Ph.D.[1,2,3], Alberto Cascón, Ph.D.[4,5], Francesca Schiavi, Ph.D.[6], Nicole Paes Morales, B.Sc.[7], Iñaki Comino-Méndez, M.Sc.[4], Nasséra Abermil, Ph.D.[1,2,3], Lucía Inglada-Pérez, M.Sc.[4,5], Aguirre A. de Cubas, M.Sc.[4], Laurence Amar, M.D.[2,3,8], Marta Barontini, M.D.[9], Sandra Bernaldo de Quirós, M.Sc.[10], Jérôme Bertherat, M.D.[2,11], Yves-Jean Bignon, M.D.[12], Marinus J. Blok, Ph.D.[13], Sara Bobisse, Ph.D.[6], Salud Borrego, M.D.[5,14], Maurizio Castellano, M.D.[15], Philippe Chanson, M.D.[16], María-Dolores Chiara, M.D.[10], Eleonora P.M. Corssmit, M.D.[17], Mara Giacchè, M.D.[15], Ronald R. de Krijger, M.D.[18], Tonino Ercolino, Ph.D.[19], Xavier Girerd, M.D.[20], Encarna B. Gómez-García, M.D.[13], Álvaro Gómez-Graña, B.Sc.[4], Isabelle Guilhem, M.D.[21], Frederik J. Hes, M.D.[22], Emiliano Honrado, M.D.[23], Esther Korpershoek, Ph.D.[18], Jacques W.M. Lenders, M.D.[24], Rocío Letón, B.Sc.[4], Arjen R Mensenkamp, Ph.D.[25], Anna Merlo, B.Sc.[10], Luigi Mori, Ph.D.[15], Arnaud Murat, M.D.[26], Peggy Pierre, M.D.[27], Pierre-François Plouin, M.D.[2,3,8], Tamara Prodanov, M.D.[28], Miguel Quesada-Charneco, M.D.[29], Nan Qin, M.Sc.[30], Elena Rapizzi, Ph.D.[31], Victoria Raymond, M.D.[32], Nicole Reisch, M.D.[33], Giovanna Roncador, M.Sc.[34], Macarena Ruiz-Ferrer, Ph.D.[5,14], Frank Schillo, M.D.[35], Alexander P.A. Stegmann, Ph.D.[13], Carlos Suarez, M.D.[10], Elisa Taschin, Ph.D.[6], Henri J.L.M. Timmers, M.D.[36], Carli M.J. Tops, Ph.D.[22], Miguel Urioste, M.D.[5,37], Felix Beuschlein, M.D.[33], Karel Pacak, M.D.[38], Massimo Mannelli, M.D.[31,39], Patricia L. M. Dahia, M.D.[7,40], Giuseppe Opocher, M.D.[41], Graeme Eisenhofer, Ph.D.[30], Anne-Paule Gimenez-Roqueplo, M.D. [1, 2, 3]and Mercedes Robledo, Ph.D. [4,5]

The following three groups of authors contributed equally to the article: Dr. Burnichon and Dr. Cascón; Drs. Schiavi, Paes Morales, Comino-Méndez, Abermil, and Inglada-Pérez; and Drs. Eisenhofer, Gimenez-Roqueplo and Robledo.

†Address correspondence to: Mercedes Robledo
Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme. Centro Nacional de Investigaciones Oncológicas, CNIO, Melchor Fernández Almagro 3, 28029 Madrid, Spain
Phone: +34-91-224 69 47; Fax: +34-91-224 69 23
e-mail: mrobledo@cnio.es

Affiliations:

1. Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, F-75015 Paris, France.
2. Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, F-75006 Paris, France.
3. INSERM, UMR970, Paris Cardiovascular Research Center, F-75015 Paris, France.
4. Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.
5. Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain.
6. Familial Cancer Clinic and Oncoendocrinology, Veneto Institute of Oncology, IRCCS, Padova, Italy.
7. Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA.
8. Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Médecine vasculaire et d'Hypertension artérielle, F-75015 Paris, France.

9. Center for Endocrinological Investigations-CEDIE. Hospital de Ninos Dr. Ricardo Gutierrez. Buenos Aires, Argentina.
10. Othorhinolaryngology Service. Hospital Universitario Central de Asturias. Instituto Universitario de Oncología del Principado de Asturias. Oviedo, Spain.
11. INSERM U1016, CNRS UMR 8104, Institut Cochin, Paris, France.
12. Oncogenetic Department. Centre Jean Perrin. Clermont-Ferrand, France.
13. Department of Clinical Genetics. Maastricht University Medical Center. Maastricht, The Netherlands.
14. Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal. Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain.
15. Clinica Medica, Endocrine and Metabolic Disease Unit and Molecular Medicine Laboratory. University of Brescia, Spedali Civili of Brescia, Brescia, Italy.
16. Assistance Publique Hôpitaux de Paris, Hôpital de Bicêtre Endocrinology Unit, Le Kremlin-Bicêtre, France.
17. Department of Endocrinology. Leiden University Medical Center. Leiden, The Netherlands.
18. Department of Pathology. Erasmus MC, University Medical Center Rotterdam. Rotterdam, The Netherlands.
19. Endocrinology Unit, Azienda Ospedaliera Universitaria Careggi. Florence, Italy.
20. Assistance Publique Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière, Unité de Prévention Cardiovasculaire, Pôle Endocrinologie, Paris, France.
21. Endocrinology Unit, Centre Hospitalier de Rennes, Rennes, France.
22. Department of Clinical Genetics. Leiden University Medical Center (LUMC). Leiden, The Netherlands.
23. Anatomical Pathology Service. Hospital de León. León, Spain.
24. Internal Medicine, Radboud University Nijmegen Medical Centre. Nijmegen, The Netherlands.
25. Human Genetics, Radboud University Nijmegen Medical Centre. Nijmegen, The Netherlands.
26. Endocrinology Unit. Hôpital Laënnec, Nantes, France.
27. Endocrinology Unit, Centre Hospitalier Régional Universitaire Bretonneau, Tours, France.
28. Program in Reproductive and Adult Endocrinology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, USA.
29. Endocrinology Service. Hospital Clínico Universitario San Cecilio. Granada, Spain.
30. Institute of Clinical Chemistry and Laboratory Medicine and Department of Medicine. University Hospital Dresden. Dresden, Germany.
31. Department of Clinical Pathophysiology. University of Florence. Florence, Italy.
32. Division of Molecular Medicine & Genetics. University of Michigan. Michigan, USA.
33. Endocrine Research Unit, Medizinische Klinik Campus Innenstadt, Klinikum der LMU, Ziemssenstr. 1, D-80336 Munich, Germany.
34. Monoclonal Antibodies Unit; Spanish National Cancer Research Centre (CNIO), Madrid, Spain.
35. Endocrinology Unit, Centre Hospitalier Universitaire de Besançon, Besançon, France.
36. Endocrinology, Radboud University Nijmegen Medical Centre. Nijmegen, The Netherlands.
37. Human Genetics Cancer Group; Spanish National Cancer Research Centre (CNIO), Madrid, Spain.
38. Section on Medical Neuroendocrinology, Program in Reproductive and Adult Endocrinology National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, USA.

39. Istituto Toscano Tumori. Florence, Italy.
40. Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA.
41. Department of Medical and Surgical Sciences, University of Padova, Italy.

Running title: *MAX* causes pheochromocytoma and paraganglioma.

Keywords: *MAX*, Pheochromocytoma, Paraganglioma, inherited susceptibility, gross deletion.

Corresponding author:

Mercedes Robledo
Hereditary Endocrine Cancer Group, Human Cancer Genetics Programme. Centro Nacional de Investigaciones Oncológicas, CNIO, Melchor Fernández Almagro 3, 28029 Madrid, Spain
Phone: +34-91-224 69 47; Fax: +34-91-224 69 23
e-mail: mrobledo@cnio.es

Word count: 3012
Total number of figures and tables: 2 main figures, 2 main tables, and 3 supplementary figures.

Translational Relevance.

MAX has been recently identified as the 10th susceptibility gene for pheochromocytoma (PCC). However, its clinical relevance was not addressed. This international study, based on an outstanding series of 1694 unrelated patients with PCC or paraganglioma (PGL), has been able to ascertain the prevalence of *MAX* mutations in PCC patients, extended the spectrum of MAX-related tumors to PGL, uncovered contributions of somatic *MAX* mutations to sporadic disease, and defined an intermediate catecholamine phenotype, that may guide testing of *MAX* gene in patients with PCC/PGLs. This study also confirms a preferential paternal mode of transmission with important consequences for genetic counseling. We establish here that *MAX* germline mutations are responsible for the disease in 1.12% of cases, similarly to the genes recently described, such as *TMEM127*, *SDHAF2* or *SDHA*, and now *MAX* should be considered in the genetic work-up of affected patients.

Abstract

Purpose: Pheochromocytomas (PCC) and paragangliomas (PGL) are genetically heterogeneous neural crest–derived neoplasms. Recently we identified germline mutations in a new tumor suppressor susceptibility gene, *MAX* (MYC associated factor X), which predisposes carriers to PCC. How *MAX* mutations contribute to PCC/PGL and associated phenotypes remain unclear. This study aimed to examine the prevalence and associated phenotypic features of germline and somatic *MAX* mutations in PCC/PGL.

Design: We sequenced *MAX* in 1694 patients with PCC or PGL (without mutations in other major susceptibility genes) from 17 independent referral centers. We screened for large deletions/duplications in 1535 patients using a multiplex polymerase chain reaction-based method. Somatic mutations were searched for in tumors from an additional 245 patients. The frequency and type of *MAX* mutation was assessed overall and by clinical characteristics.

Results: Sixteen *MAX* pathogenic mutations were identified in 23 index patients. All had adrenal tumors, including 13 bilateral or multiple PCCs within the same gland (P<0.001), 15.8% developed additional tumors at thoracic-abdominal sites, and 37% had familial antecedents. Age at diagnosis was lower (P=0.001) in *MAX* mutation carriers compared to non-mutated cases. Two patients (10.5%) developed metastatic disease. A mutation affecting *MAX* was found in five tumors, four of them confirmed as somatic (1.65%). MAX tumors were characterized by substantial increases in normetanephrine, associated with normal or minor increases in metanephrine.

Conclusions: Germline mutations in *MAX* are responsible for 1.12% of PCC/PGL in patients without evidence of other known mutations, and should be considered in the genetic work-up of these patients.

INTRODUCTION

Pheochromocytoma (PCC) has been referred to as "the 10 percent" tumor due in part to the belief that 10% are hereditary and usually associated with three well-known cancer syndromes: von Hippel-Lindau disease, multiple endocrine neoplasia type 2, and neurofibromatosis type 1 due to mutations in *VHL*,(1) *RET*,(2) and *NF1*(3) respectively. The 10 percent rule was challenged after identification of germline mutations in *SDHD*, *SDHB*, and *SDHC* as important causes of familial paraganglioma (PGL)(4-6) that led Neumann and colleagues to establish that up to a quarter of affected patients carried a PCC/PGL susceptibility gene mutation (7). Since then three additional susceptibility genes (*SDHAF2, SDHA* and *TMEM127*) (8-10) have been identified. Thus, the proportion of hereditary PCC/PGLs may exceed estimates of 30-40% (11, 12), rendering PCC/PGL one of the most inherited tumor entities in existence.

Findings of other patients with a clinical presentation of PCC/PGL that includes a positive family history, early age of presentation and bilateral adrenal or multiple tumors, but without known mutations, has suggested the presence of further susceptibility genes. With this observation in mind, we recently identified *MAX* (MYC associated factor X) as a new PCC tumor suppressor susceptibility gene in three independent patients with familial antecedents of the disease (13). Further analysis of 59 patients, selected because they had bilateral PCC and/or an early age of disease presentation, allowed detection of 5 additional cases with *MAX* mutations (13). Preliminary genotype-phenotype associations suggested *MAX* mutations were associated with bilateral PCC and an apparent paternal transmission of the disease (13).

Although little is known about genetic alterations in sporadic tumors, it has been proposed that mutations in PCC/PGL susceptibility genes are detrimental for neuronal precursor cells, explaining the apparent rarity of somatic mutations in these genes in apparently sporadic PCC/PGL (14, 15). Guided by transcriptome classification and loss of heterozygosity (LOH) profiles of a large series of 202 PCC/PGL, we nevertheless recently established that 14% of

sporadic tumors harbored somatic mutations in *VHL* or *RET* genes (16). Since deregulation of MYC is a prominent hallmark in numerous forms of cancer (17), with activation of *MYC* genes commonly detected in solid human tumors (18), it is plausible that *MAX* somatic mutations may also occur in sporadic PCC/PGL.

Establishing the above associations and the prevalence of *MAX* mutations among patients with PCC/PGL requires analysis of a larger cohort of patients. In a large international collaborative effort we therefore screened for the presence of germline mutations affecting *MAX* in 1694 patients without mutations in major PCC/PGL susceptibility genes. Somatic mutations were searched for in tumors from an additional 245 patients.

METHODS

Patients

The study population consisted of 1694 apparently unrelated index cases with PCC or PGL, from whom blood-leukocyte DNA samples were available and in whom familial antecedents, presence of metastasis and number of primary tumors are shown in table 1. Clinical variables collected for this study were: gender, number of PCC/PGLs, tumor location, age of diagnosis for each tumor (in patients with multiple tumors), the biochemical secretion when available, as well as other malignancies developed by probands. These clinical variables were collected electronically into preformatted forms provided to all contributors, and statistically analyzed in a single center. More detailed assessment of clinical data was further obtained for *MAX* mutation carriers. Mutations in *RET, VHL, SDHB, SDHC, SDHD* and *TMEM127* were excluded and there were no clinical features of neurofibromatosis type 1. Patients were referred from 15 participating centers of the European Network for the Study of Adrenal Tumours (ENS@T) consortium (Madrid, Oviedo and Seville in Spain (12), Paris, Marseille and Angers in France (19, 20), Leiden, Rotterdam, Nijmegen and Maastricht in The Netherlands, Padua, Florence and Brescia in Italy (11), Munich and Dresden in Germany), and 2 centers in the US (San Antonio and Bethesda). Diagnosis of pheochromocytoma and/or paraganglioma, including tumors of both sympathetic (thoracic or abdominal) and parasympathetic (head and neck) origin, was established following conventional procedures (including clinical, biochemical and imaging tests).

Written informed consent to collect phenotypic and genotypic data was obtained from all participants in accordance with institution review board (IRB) - approved protocols for each center. DNA from 400 unrelated and unaffected individuals was analyzed as controls.

Tumors

Frozen tumors obtained from a total of 245 apparently unrelated patients without known mutations in the mentioned susceptibility genes were collected through the Spanish National Tumor Bank Network in Madrid (Spain) (21), the Erasmus MC Tissue Bank in Rotterdam (Netherlands), Munich (Germany), the International Familial Pheochromocytoma Consortium of San Antonio and Bethesda (US) (22), Nijmegen Pheochromocytoma Tissue Bank, and the COMETE network in Paris (France) (Table 1) (16, 23). From these 245 samples, 106 belonged to patients included in the germline screening. The remaining 139 tumors represented independent cohort. For samples with identified *MAX* mutations, the corresponding mutation was assessed in constitutive DNA when available in order to classify them as germline or somatic.

Molecular genetic analyses

Complete genetic characterization of *MAX* included both point mutation and gross deletion/duplication analyses, the latter performed in 1535 cases with good DNA quality. Primers spanning the five exons and intron-exon boundaries of the *MAX* transcript 2 (ENST00000358664, NM_002382.3) were used as previously described (13). To assess for rearrangements, a semi-quantitative multiplex-PCR method using labeled primers was designed as previously described for other genes (24). PCR conditions and primers are available upon request. To assess the pathogenicity of variants we used Alamut® mutation interpretation software (http://www.interactive-biosoftware.com/software.html).

LOH was estimated by direct sequencing when tumor DNA was available. Uniparental disomy (UPD) or chromosomal loss was assessed by microsatellite analysis as previously described.(13)

Immunohistochemistry

Immunohistochemical staining was performed using 3 µm formalin-fixed paraffin embedded tumor sections from tumors carrying *MAX* mutations, as previously described (13). Normal

adrenal sections and tumors carrying mutations in other PCC susceptibility genes were used as controls. Only cases showing nuclear staining of stromal cells were considered as evaluable.

Biochemical test results and biological features

Biochemical test results available in patients with *MAX* mutations included urinary fractionated metanephrines in 16 patients measured as part of the routine diagnostic work-up at participating centers, either by liquid chromatography with electrochemical detection (LC-EC) or tandem mass spectrometry. Concentrations of catecholamines (epinephrine, norepinephrine, dopamine) in tumor tissue available from 7 patients with *MAX* mutations were quantified in frozen specimens by LC-EC as described elsewhere (25). Results were compared with historical data from 57 patients in one group with mutations of *VHL* (n=44), *SDHB* (n=10) and *SDHD* (n=3) and 36 patients in the other group with *RET* (n=31) and *NF1* (n=5) mutations, in all of whom tumor tissue catecholamine results were available (26). Transcriptomic data, involving two different microarray platforms (Affymetrix for the french series and Agilent for spanish series) (27, 28), was further used to determine the expression of mRNA for phenylethanolamine N-methyltransferase (*PNMT*).

Statistical Analysis

Statistical analyses were carried out using SPSS software package version 17.0 (SPSS, Inc., Chicago, IL). The four patients carrying variants of unknown significance (VUS) and a subject in whom it was not possible to establish the germline status were not considered for statistical purposes. Thus, only patients with mutations leading to truncated proteins or affecting conserved amino acids were included in the final analysis. Differences between mutation carriers and non-mutation carriers for gender, adrenal multiple tumors, familial history and malignancy were assessed using a $\chi 2$-test or Fisher's exact test, where appropriate. Since age, biochemical and gene expression data could not be established to be normally distributed,

non-parametric analysis by Mann-Whitney and Kruskal-Wallis tests were used to assess statistical significance of differences in these variables among the different groups examined.

RESULTS

Germline and somatic MAX variants

Among the 1694 patients with PCC and/or PGL and no evident germline mutations in *RET*, *VHL*, *SDHB*, *SDHC*, *SDHD,* and *TMEM127* genes (Table 1), we identified 16 different heterozygous variants affecting 23 subjects that spanned all five exons of the *MAX* gene (Figure 1; Table 2). In addition, we analyzed *MAX* in 245 tumors (Table 1) and found four cases (1.65%) carrying a mutation that was confirmed as somatic by the finding of no mutation in the germline DNA (Table 2). A fifth tumor with a *MAX* mutation (case 24, Table 2) was not considered in further analysis since it was not possible to establish its germline status. None of the variants were found in at least 400 controls, or in public databases (dbSNP132 and 1000 Genomes Project; www.1000genomes.org/).

Overall, taking into account the germline and the somatic findings, we identified 18 novel variants affecting *MAX* and two previously reported mutations, c.97C>T and c.223C>T (13). Seven mutations disrupted the MAX protein, because they affected the initial methionine (c.2T>A), created a premature stop codon (c.25del, c.97C>T, c.223C>T and c.244C>T) or affected a donor/acceptor splice site (c.171+1G>A and c.295+1G>T) (Figure 1). Additionally, two deletions were identified: the first caused an in-frame loss of six highly conserved amino acids within the first helix of the protein (c.140_157del), and the second, detected by multiplex-PCR (Supplementary Fig. S1), spanned the whole gene (c.1-?_483+?del). Immunohistochemical detection of MAX in tumor embedded paraffin slides demonstrated complete loss of the protein in all analyzed tumors that carried truncating mutations (Supplementary Fig. S2).

Amongst the 11 non-truncating variants, seven mutations (c.73C>T, c.103C>T, c.178C>T, c.212T>G, c.220A>G, c.269G>C and c.305T>C) changed conserved or highly conserved amino acids located within the basic helix-loop-helix leucine zipper (bHLH-Zip) domain of the MAX protein (Figure 1), and were classified as deleterious by the Alamut® software. The remaining

four non-truncating variants (c.25G>T, c.63G>T, c.414G>A, and c.-18C>T) were classified as VUS since these were predicted as benign through bioinformatic tools; it was also not possible to demonstrate their pathogenicity with further analyses (table 2). Positive immunohistochemistry staining was observed in all non-truncating variants assessed (Supplementary Fig. S2).

LOH of the *MAX* wild-type allele was found in 16/18 tumors analyzed (Table 2). *MAX* wild-type allele was present in two tumor associated with the variants c.25G>T/p.(Val9Leu), and c.63G>T / p.(=), both considered as VUS.

In summary, we found pathogenic germline *MAX* variants in the 1.12% of the 1694 index cases included in this analysis. All mutations, except one gross deletion, consisted of a single nucleotide substitution.

Clinical presentation of *MAX* carriers

Only those 19 patients who harbored a germline variant defined as pathogenic were considered for examination of phenotypic associations (Table 2). The presence of familial antecedents of disease was found in seven of the 19 patients (37%) and appeared in the paternal branch in the three pedigrees with more than one generation of affected members (Supplementary Fig. S3). These 19 patients developed at least one PCC, with 13 (68.4%) showing either bilateral PCC or multiple PCCs within the same gland, a 48-fold higher rate ($P<0.001$) than in *MAX*-negative cases (4.28%). Age at diagnosis was lower ($P<0.001$) in mutation carriers than in cases without mutations (median 34, range 13-58 years versus 48 range 3-88 years). Three of the 19 patients (15.8%) developed additional tumors at thoracic-abdominal sites at a median age of 48 years (range 44 to 64 years). Importantly, these tumors presented as PGLs, distinct from recurrences of the earlier adrenal tumors. Two patients (10.5%) developed metastatic disease.

Among four sporadic cases with *MAX* somatic mutations, the median age at diagnosis was 47.5 years (range 24 to 57 years), which was not significantly different from those with *MAX* negative sporadic tumors (median 52, range 11 to 83 years) (Table 1).

Biochemical test results and biological features

All patients with *MAX* mutations showed increased urinary outputs of normetanephrine that did not differ from patients in the group with *VHL* and *SDHB/D* mutations or the other with *RET* and *NF1* mutations (Figure 2A). In contrast, urinary outputs of metanephrine were either normal or moderated increased in patients with *MAX* mutations and showed an intermediate distribution, significantly (P<0.001) higher than in the VHL/SDH group, but lower than in the RET/NF1 group (Figure 2B). Similarly, tumor tissue concentrations of epinephrine also showed an intermediate distribution, representing 8.4% of total catecholamine contents in *MAX* tumors, a proportion 5.6-fold higher (P<0.001) than in VHL/SDH tumors, but a sixth (P=0.003) that in *RET/NF1* tumors (Figure 2C). Furthermore, levels of PNMT expression in *MAX* tumors were 14-fold higher (P<0.001) than in *VHL/SDH* tumors and a little under a half (P=0.05) that in *RET/NF1/TMEM127* tumors (Figure 2D).

DISCUSSION

It is widely accepted that MYC deregulation is not restricted to translocations and amplifications at the MYC locus, which suggests that the impact of its deregulation on human cancer incidence is higher than previously thought (18). We recently found *MAX* germline mutations in patients with PCC (13) suggesting that alterations in this most important regulator of the MYC/MAX/MXD1 network promote hereditary susceptibility to neoplasias. This study followed up on these observations, taking advantage of a large international collaborative network to determine the prevalence and the genotype-phenotype correlations of *MAX* mutations in 1694 PCC/PGL patients previously negative for 6 major PCC/PGL susceptibility genes. We establish here that *MAX* germline mutations are responsible for the disease in 1.12% of cases, a similar contribution to that of the recently reported *TMEM127* mutation (29). Furthermore, our findings reveal the presence of *MAX* somatic mutations in sporadic tumors, extend the spectrum of *MAX*-related tumors to PGLs, ascertain that *MAX* tumors are not particularly prone to malignancy, and demonstrate that *MAX* tumors produce predominantly norepinephrine, but with some capacity to also produce epinephrine.

Though it has been reported that somatic mutations in the known PCC susceptibility genes constitute an extremely rare event, we recently found 14% of sporadic PCC/PGL carrying somatic mutations in *VHL* or *RET* (16). The presence of somatic *MAX* mutations in 1.65% of sporadic tumors described here is in agreement with this latter finding, and highlights the importance of the MYC/MAX/MXD1 network in the development of neural crest tumors. It is well known that somatic amplification and over-expression of MYCN is a genetic hallmark in neuroblastoma (30), so ablation of *MAX* transcriptional repression of MYC in PCC could lead to the same oncogenic MYC dysregulation that occurs in neuroblastoma. Nevertheless, no meaningful trend for a contribution of *MAX* mutations to other neoplasms, including neuroblastoma, was found in the current series.

The identified variants were distributed along the gene, but were especially frequent in exons three and four, matching some of the most important residues within the conserved bHLH-Zip domain of MAX. The majority of mutations lead to truncated proteins, and the expected LOH affecting the remaining wild-type allele of the *MAX* tumor suppressor gene was further supported by the absence of the protein by immunohistochemistry.

The most frequently found mutation was the previously described c.97C>T variant(13) discovered in eight unrelated patients from five nations (Italy, Spain, USA, France, and The Netherlands). This recurrent mutation affects a CpG dinucleotide located contiguous to Glu32, the crucial residue for DNA binding, and represents the first hotspot mutation affecting *MAX*. In agreement with this, one of the c.97C>T mutation carriers was a *de novo* case, further suggesting the high mutability of this dinucleotide. The six missense variants that altered conserved MAX residues were predicted as deleterious by the Alamut® software (Table 2) and have been reported as critical for dimerization and DNA binding of HLH proteins or for interactions within the protein structure (31, 32). Mutations affecting highly conserved amino acids within the bHLH-Zip domain of MAX, involved in protein-protein interactions and DNA binding, can be expected to destroy the ability of MAX to antagonize MYC-dependent cell transformation leading to tumor development.

The absence of familial antecedents in more than 65% of individuals, as well as the paternal transmission identified in three pedigrees, further supports previous suggestions of a paternal mode of transmission (13). This mode of inheritance, with its consequence of generation-skipping, complicates identification of candidate mutation carriers. In general, the phenotypic characteristics of *MAX*-mutant patients overlap with clinical features observed for other PCC/PGL related hereditary disorders. For example, the presence of a significant proportion of bilateral/multiple PCC cases amongst *MAX* germline mutation carriers, representing 21% (13/63) of patients included in this cohort (Table 1), is in agreement with the high percentage (35-60%) of bilateral tumors found in patients with mutations in *VHL, RET* or *TMEM127* (7, 29,

33, 34). The age at diagnosis of PCCs in *MAX* mutation carriers (34 yr) was clearly lower than in negative cases (48 yr), also lower that the reported on average in patients with *TMEM127, RET,* and *NF1* mutations (38-42 yr), but higher to that for *VHL* and *SDH* mutation carriers (27-32 yr) (19, 20, 29, 33-35).

Extra-adrenal thoracic-abdominal PGL are relatively common compared to adrenal tumors in patients with *SDHB* and *SDHD* mutations, less common in *TMEM127* and *VHL* mutation carriers, and rarely associated with *RET* and *NF1* germline mutations (12, 33, 36). Interestingly, in all *MAX*-patients with PGL, the extra-adrenal tumor was diagnosed after the adrenal tumor. This contrasts with VHL patients with head and neck PGLs, 50% of whom showed no adrenal tumors (34). In the present study, only two patients developed metastasis, suggesting that unlike *SDHB* mutations, mutations of *MAX* are not associated with a high risk of malignancy.

The catecholamine-related information available from patients with *MAX*-mutations indicated a biochemical phenotype intermediate between the established phenotypes of epinephrine-producing tumors due to *NF1* and *RET* mutations and the predominantly norepinephrine-producing tumors due to *VHL* or *SDHB/D* mutations (26). This intermediate diagnostic phenotype, manifested by at least 3-fold larger tumor-associated increases in urinary outputs of normetanephrine than of metanephrine, was explained by a significant but limited capacity to produce epinephrine. This latter finding is supported by the intermediate tissue concentrations of epinephrine and expression of mRNA for PNMT, the enzyme responsible for conversion of norepinephrine to epinephrine. The intermediate biochemical phenotype associated with *MAX* mutations, together with lack of MAX immunohistochemical staining of tumor tissue, may prove useful for guiding testing of the *MAX* gene in patients with PCC/PGLs.

In summary, this study involving an unprecedented international effort to genotype and phenotype a substantial number of patients with PCC/PGLs reveals the importance of the MYC/MAX/MXD1 network in the development of both hereditary and sporadic forms of these

tumors. *MAX* is the 10th PCC/PGL susceptibility gene described to date, which now should be considered in the genetic work-up of affected patients.

Conflicts of interest

The authors have no conflict of interest to declare.

Acknowledgments

Brescia: *the authors wish to thank Franco Veglio, MD and Paolo Mulatero, MD, University of Turin, Luigi Bartalena, MD, University of Insubria Varese, Ermanno Rossi, MD, Reggio Emilia Hospital, Pietro Nicolai, MD, and Fabio Facchetti, MD, University of Brescia, who contributed to the recruitment of patients; Alessandra Panarotto for excellent technical assistance; Enrico Agabiti Rosei for continued support and encouragement.* ***Dresden****: The authors wish to thank Roland Därr who contributed to patient enrollment and evaluation.* ***France****: The authors wish to thank Annabelle Vénisse, Christophe Simian, Céline Loriot and Judith Favier (AP-HP, Hôpital européen Georges Pompidou, Genetics department and INSERM U970, Paris France) for technical assistance and the members of the COMETE (Cortico and Medullar Endocrine Tumors) and PGL.NET networks, of the GTE (Groupe des Tumeurs Endocrines) and of the INCA-COMETE and INCA-RENATEN reference centers. They are particularly grateful to Frédéric Illouz, Vincent Rohmer, Delphine Prunier-Mirebeau (CHU d'Angers Endocrinology and biochemical departments), Anne Barlier, Morgane Pertuit (CHU de Marseille, Molecular Biological department), Catherine Genestie, Julie Rigabert, Charlotte Lepoutre (APHP-Hôpital de la PItié Salpétrière, Endocrinology and nuclear medicine departments); Serge Guyetant (CHU de Tours, Pathological department,); Nathalie Rioux-Leclercq, Elisabeth Tarasco, Catherine Dugast (CHU de Rennes, Endocrinology and Genetics departments); Jihad Samrani, Philippe Thieblot, Igor Tauveron (CHU de Clermont Ferrand, Endocrinology department); Sylvette Helbert-Davidson (APHP Hôpital Henri Mondor, Nuclear Medicine department); Séverine Valmary-Degano, Bernadette Kantelip (Tumorothèque régionale de Franche-Comté), Bruno Heyd, Gabriel Viennet, Franck Monnien, Alfred Penfornis (CHU de Besançon, Surgical, pathological and endocrinology departments); Xavier Bertagna, Rossella Libé, Frédérique Tissier (APHP, Hôpital Cochin, Endocrinology and pathological departments); Annick Rossi, Thierry Frébourg (CHU de Rouen, Genetic department); Michel Krempf, Anne Moreau, Maelle Lebras (CHU de Nantes, Endocrinology department); Cécile Badoual, Claudia de Toma, Plateforme de ressources biologiques de l'HEGP (APHP, Hôpital européen Georges Pompidou, Pathological department), Sophie Ferlicot, Annonciade Biaggi (CHU de Bicêtre, Pathological department) for their helpful assistance in this study.* ***NIH, Bethesda****: The authors wish to thank Karen Adams who contributed to the patient enrollment and evaluation.* ***Nijmegen****: the authors wish to thank Neeltje Arts and Erik Jansen for excellent technical assistance.* ***Padova****: the authors are grateful to the following colleagues who contributed to the recruitment of patients and/or clinical information: Adele Nardecchia, MD, Domenico Meringolo, MD, Giuseppe Picca, MD, Paola Loli, MD, Erika Grossrubatscher, MD, Maurizio Iacobone, MD, Antonio Toniato, MD, Ambrogio Fassina, MD, Isabella Negro, MD, GianPaolo Rossi, MD, Massimo Terzolo, MD, Serena Demattè, MD, Maria Vittoria Davì, MD, Giorgio Bertola, MD,* Luca Persani, MD, Uberta Verga, MD, Iacopo Chiodini, MD, Gilberta Giacchetti, MD, Giorgio Arnaldi, MD. ***San Antonio****: we thank the members of the Familial Pheochromocytoma Consortium for their support and earlier contributions. We are grateful to the following colleagues who contributed to the recruitment of patients and/or clinical information: Elizabeth King, MD, Jan Bruder, MD, Neil Aronin, MD, Kathy Schneider, MPH, and Stephen Gruber, MD.* ***Spain****: the authors wish to thank Sara Cristina Hernández for excellent technical assistance, to Cristina Álvarez-Escolá, MD, Carmen Bernal, MD, Amparo Meoro, MD, José Ángel Díaz, MD, María Teresa Calvo, MD, José María de Campos, MD, María García-Barcina, MD, Sharona Azriel, MD, Marcos Lahera, MD, who contributed to the recruitment of patients and clinical information, to María Jesús Artiga, PhD, and Manuel Morente, MD, for contributing to the recruitment of tumor samples, as well as to*

Biobanco del Sistema Sanitario Público de Andalucía and the Tumor Bank Network coordinated by CNIO.

Grant support

The ENS@T consortium received funding from the Seventh Framework Programme (ENS@T-CANCER; HEALTH-F2-2010-259735). The ENS@T registry is supported by a grant of the European Science Foundation (ESF-ENS@T).

This work was supported in part by the Fondo de Investigaciones Sanitarias (projects PI11/01359, PS09/00942, PI10/01290, PI08/0531 and P108/0883), Mutua Madrileña (AP2775/2008), Consejería de Innovación Ciencia y Empresa de la Junta de Andalucía (CTS-2590), Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0034).

The French COMETE network is supported in part by the Programme Hospitalier de Recherche Clinique grant COMETE 3 (AOM 06 179) , by grants from INSERM and Ministère Délégué à la Recherche et des Nouvelles Technologies and by the Institut National du Cancer.

This work was also funded by grants from the Agence Nationale de la Recherche (ANR 08 GENOPATH 029 MitOxy) and by the national program "Cartes d'Identité des Tumeurs" funded and developed by the "Ligue Nationale contre le Cancer" (http://cit.ligue-cancer.net).

This research was supported, in part, by the Intramural Research Program of the NIH, NICHD.

This work received funding support from the Voelcker Fund to P.L.M.D.

This work was also supported in part by grants from the Fondazione Comunità Bresciana and the Fondazione Guido Berlucchi.

Author contributions

Dr Robledo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

(a) The conception and design of the study:

Nelly Burnichon, Alberto Cascón, Lucía Inglada-Pérez, Maurizio Castellano, María-Dolores Chiara, Ronald R. de Krijger, Frederik J. Hes, Jacques W.M. Lenders, Arjen R Mensenkamp, Carlos Suarez, Henri J.L.M. Timmers, Felix Beuschlein, Karel Pacak, Massimo Mannelli, Patricia L. M. Dahia, Giuseppe Opocher, Graeme Eisenhofer, Anne-Paule Gimenez-Roqueplo, and Mercedes Robledo.

(b) Acquisition, analysis, and interpretation of data:

Nelly Burnichon, Alberto Cascón, Francesca Schiavi, Nicole Paes Morales, Iñaki Comino-Méndez, Nasséra Abermil, Lucía Inglada-Pérez, Laurence Amar, Marta Barontini, Sandra Bernaldo de Quirós, Jérôme Bertherat, Yves-Jean Bignon, Marinus J. Blok, Salud Borrego, Maurizio Castellano, Philippe Chanson, Ronald R. de Krijger, Tonino Ercolino, Xavier Girerd, Encarna B. Gómez-García, Álvaro Gómez-Graña, Isabelle Guilhem, Emiliano Honrado, Esther Korpershoek, Jacques W.M. Lenders, Rocío Letón, Arjen R Mensenkamp, Anna Merlo, Luigi Mori, Arnaud Murat, Peggy Pierre, Pierre-François Plouin, Tamara Prodanov, Miguel Quesada-Charneco, Nan Quin, Elena Rapizzi, Nicole Reisch, Giovanna Roncador, Macarena Ruiz-Ferrer, Frank Schillo, Alexander P.A. Stegmann, Carlos Suarez, Elisa Taschin, Henri J.L.M. Timmers, Carli Tops, Miguel Urioste, Eleonora P.M. Crossmit, Felix Beuschlein, Karel Pacak, Massimo Mannelli, Patricia L. M. Dahia, Giuseppe Opocher, Graeme Eisenhofer, Anne-Paule Gimenez-Roqueplo, and Mercedes Robledo.

(c) The drafting of the article or critical revision for important intellectual content:

Nelly Burnichon, Alberto Cascón, Lucía Inglada-Pérez, Maurizio Castellano, Ronald R. de Krijger, Frederik J. Hes, Jacques W.M. Lenders, Arjen R Mensenkamp, Felix Beuschlein, Karel Pacak, Massimo Mannelli, Patricia L. M. Dahia, Giuseppe Opocher, Graeme Eisenhofer, Anne-Paule Gimenez-Roqueplo, and Mercedes Robledo.

(d) Figures:

Nelly Burnichon, Alberto Cascón, Graeme Eisenhofer, Anne-Paule Gimenez-Roqueplo, and Mercedes Robledo.

(e) Study supervision:

Graeme Eisenhofer, Anne-Paule Gimenez-Roqueplo, and Mercedes Robledo.

(f) Final approval of the version to be published: all the authors.

References

1. Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 1993; 260:1317-20.
2. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993; 363:458-60.
3. Wallace MR, Andersen LB, Saulino AM, Gregory PE, Glover TW, Collins FS. A de novo Alu insertion results in neurofibromatosis type 1. Nature 1991; 353:864-6.
4. Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 2000; 287:848-51.
5. Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 2001; 69:49-54.
6. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet 2000; 26:268-70.
7. Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, et al. Germ-line mutations in nonsyndromic pheochromocytoma. N Engl J Med 2002; 346:1459-66.
8. Hao HX, Khalimonchuk O, Schraders M, Dephoure N, Bayley JP, Kunst H, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 2009; 325:1139-42.
9. Burnichon N, Briere JJ, Libe R, Vescovo L, Riviere J, Tissier F, et al. SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 2010; 19:3011-20.
10. Qin Y, Yao L, King EE, Buddavarapu K, Lenci RE, Chocron ES, et al. Germline mutations in TMEM127 confer susceptibility to pheochromocytoma. Nat Genet 2010; 42:229-33.
11. Mannelli M, Castellano M, Schiavi F, Filetti S, Giacche M, Mori L, et al. Clinically guided genetic screening in a large cohort of italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas. J Clin Endocrinol Metab 2009; 94:1541-7.
12. Cascon A, Pita G, Burnichon N, Landa I, Lopez-Jimenez E, Montero-Conde C, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab 2009; 94:1701-5.
13. Comino-Mendez I, Gracia-Aznarez FJ, Schiavi F, Landa I, Leandro-Garcia LJ, Leton R, et al. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet 2011; 43:663-7.
14. Lee S, Nakamura E, Yang H, Wei W, Linggi MS, Sajan MP, et al. Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. Cancer Cell 2005; 8:155-67.
15. Maher ER, Eng C. The pressure rises: update on the genetics of phaeochromocytoma. Hum Mol Genet 2002; 11:2347-54.
16. Burnichon N, Vescovo L, Amar L, Libe R, de Reynies A, Venisse A, et al. Integrative Genomic Analysis Reveals Somatic Mutations in Pheochromocytoma and Paraganglioma. Hum Mol Genet 2011.
17. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74.
18. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008; 8:976-90.
19. Amar L, Bertherat J, Baudin E, Ajzenberg C, Bressac-de Paillerets B, Chabre O, et al. Genetic testing in pheochromocytoma or functional paraganglioma. J Clin Oncol 2005; 23:8812-8.

20. Burnichon N, Rohmer V, Amar L, Herman P, Leboulleux S, Darrouzet V, et al. The succinate dehydrogenase genetic testing in a large prospective series of patients with paragangliomas. J Clin Endocrinol Metab 2009; 94:2817-27.
21. Rodriguez-Antona C, Pallares J, Montero-Conde C, Inglada-Perez L, Castelblanco E, Landa I, et al. Overexpression and activation of EGFR and VEGFR2 in medullary thyroid carcinomas is related to metastasis. Endocr Relat Cancer 2010; 17:7-16.
22. Dahia PL, Ross KN, Wright ME, Hayashida CY, Santagata S, Barontini M, et al. A HIF1alpha regulatory loop links hypoxia and mitochondrial signals in pheochromocytomas. PLoS Genet 2005; 1:72-80.
23. Favier J, Gimenez-Roqueplo AP. Pheochromocytomas: the (pseudo)-hypoxia hypothesis. Best Pract Res Clin Endocrinol Metab 2010; 24:957-68.
24. Cascon A, Montero-Conde C, Ruiz-Llorente S, Mercadillo F, Leton R, Rodriguez-Antona C, et al. Gross SDHB deletions in patients with paraganglioma detected by multiplex PCR: a possible hot spot? Genes Chromosomes Cancer 2006; 45:213-9.
25. Eisenhofer G, Lenders JW, Timmers H, Mannelli M, Grebe SK, Hofbauer LC, et al. Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. Clin Chem 2011; 57:411-20.
26. Eisenhofer G, Pacak K, Huynh TT, Qin N, Bratslavsky G, Linehan WM, et al. Catecholamine metabolomic and secretory phenotypes in phaeochromocytoma. Endocr Relat Cancer 2011; 18:97-111.
27. Favier J, Briere JJ, Burnichon N, Riviere J, Vescovo L, Benit P, et al. The Warburg effect is genetically determined in inherited pheochromocytomas. PLoS ONE 2009; 4:e7094.
28. Lopez-Jimenez E, Gomez-Lopez G, Leandro-Garcia LJ, Munoz I, Schiavi F, Montero-Conde C, et al. Research resource: Transcriptional profiling reveals different pseudohypoxic signatures in SDHB and VHL-related pheochromocytomas. Mol Endocrinol 2010; 24:2382-91.
29. Yao L, Schiavi F, Cascon A, Qin Y, Inglada-Perez L, King EE, et al. Spectrum and prevalence of FP/TMEM127 gene mutations in pheochromocytomas and paragangliomas. Jama 2010; 304:2611-9.
30. Kohl NE, Kanda N, Schreck RR, Bruns G, Latt SA, Gilbert F, et al. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 1983; 35:359-67.
31. Ferre-D'Amare AR, Prendergast GC, Ziff EB, Burley SK. Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. Nature 1993; 363:38-45.
32. Voronova A, Baltimore D. Mutations that disrupt DNA binding and dimer formation in the E47 helix-loop-helix protein map to distinct domains. Proc Natl Acad Sci U S A 1990; 87:4722-6.
33. Mannelli M, Castellano M, Schiavi F, Filetti S, Giacche M, Mori L, et al. Clinically guided genetic screening in a large cohort of Italian patients with pheochromocytomas and/or functional or non-functional paragangliomas. J Clin Endocrinol Metab 2009.
34. Cascon A, Pita G, Burnichon N, Landa I, Lopez-Jimenez E, Montero-Conde C, et al. Genetics of pheochromocytoma and paraganglioma in Spanish patients. J Clin Endocrinol Metab 2009.
35. Eisenhofer G, Timmers HJ, Lenders JW, Bornstein SR, Tiebel O, Mannelli M, et al. Age at diagnosis of pheochromocytoma differs according to catecholamine phenotype and tumor location. J Clin Endocrinol Metab 2011; 96:375-84.
36. Boedeker CC, Erlic Z, Richard S, Kontny U, Gimenez-Roqueplo AP, Cascon A, et al. Head and neck paragangliomas in von Hippel-Lindau disease and multiple endocrine neoplasia type 2. J Clin Endocrinol Metab 2009; 94:1938-44.

Figure legends

Figure 1.

Schematic representation of *MAX* [transcript ENST00000358664] mutations identified in this study. The bottom panel shows the conservation of the amino acids altered by pathogenic missense changes (underlined). Double arrows delimit protein domains between the first and the last missense mutation.

Figure 2.

Dot-box plots illustrating urinary outputs of normetanephrine (A) and metanephrine (B), tumor tissue contents of epinephrine (C) and expression of PNMT mRNA (D) for patients with *MAX* mutations compared to those with *VHL* and *SDHB/D* mutations or *RET*, *NF1* and *TMEM127* mutations.

Table 1. Clinical features of the entire Pheochromocytoma and Paraganglioma cohort.

Table 2. Genetic and Clinical Features of the 28 *MAX* positive patients[a] and tumors*

Table 1. Clinical features of the entire Pheochromocytoma and Paraganglioma cohort.

Analysis	Clinical features	n	single PCC	single PGL H&N/TA	mPGL H&N/TA/both	PCC&PGL H&N/TA/both	bPCC/mPCC
	Total	**1694***	**1252**	**315** 110/205	**29** 1/23/5	**35** 1/34/0	**63**
Index cases	With familial antecedents¥ (%)	37 (2.18%)	24 (1.91%)	5 (1.58%)	0	2 (5.71%)	6 (9.52%)
	Malignant₩ (%)	129 (7.61%)	79 (6.30%)	27 (8.57%)	2 (6.89%)	15 (42.85%)	6 (9.52%)
	Gender (female/ male/ unknown)	1003/682/9					
	Median age first tumor (range)	48(3-88)					
	Total	**245****	**216**	**29**			
Tumors	Gender (female/ male)	150/95					
	Median age (range)	52 (11-83)					

PCC – adrenal pheochromocytoma; PGL – paraganglioma; mPGL – multiple paragangliomas; bPCC- bilateral adrenal pheochromocytoma; mPCC - multiple pheochromocytomas within the same gland; H&N – Head and Neck; TA- Thoraco-abdominal; Both – H&N and TA PGL; * Index cases origin (n): France (664), Italy (428), Spain (245), The Netherlands (166), USA (152), Germany (39). ** Tumor origin (n): France (106), USA (75), The Netherlands (39), Spain (17), Germany (8). ¥. A hereditary cause of PCC/PGL was considered likely when disease affected at least two family members. ₩. Malignancy was defined as the presence of metastases where chromaffin cells are normally absent.

Author Manuscript Published OnlineFirst on March 27, 2012; DOI:10.1158/1078-0432.CCR-12-0160
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Table 2. Genetic and Clinical Features of the 28 *MAX* positive patients[a] and tumors[Ω]

ID	Gender/ age	Fam	PCC	PGL	Mets	Other disease[d]	cDNA mutation[b]/ Protein alteration[b]	Predicted Pathogenicity[c]	LOH	IHC
1	M/27	no	PCC	no	no	no	c.1-?_483+?del / p.?	n.a.	yes	neg
2	F/46	no	bPCC	1 TA	no	no	c.2T>A / p.?	n.a.	yes[UPD]	neg
3	F/43	no	bPCC	4 TA	no	BrC, RO	c.73C>T / p.(Arg25Trp)	Y	-	-
4	M/23	yes	bPCC	no	no	no	c.97C>T / p.(Arg33*)	n.a.	-	-
5	M/27	yes[p]	bPCC & mPCC	no	no	no	c.97C>T / p.(Arg33*)	n.a.	yes	-
6	M/34	no[dn]	bPCC	no	no	no	c.97C>T / p.(Arg33*)	n.a.	yes	neg
7	F/58	yes	mPCC	no	no	no	c.97C>T / p.(Arg33*)	n.a.	yes	neg
8	F/26	yes[p]	PCC	no	no	no	c.97C>T / p.(Arg33*)	n.a.	yes	neg
9	M/38	no	PCC	no	no	SCCT	c.97C>T / p.(Arg33*)	n.a.	-	-
10	M/24	yes	bPCC	no	no	CCH	c.97C>T / p.(Arg33*)	n.a.	-	-
11	F/43	no	PCC	1TA	no	no	c.97C>T / p.(Arg33*)	n.a.	yes	-
12	F/18	no	bPCC	no	no	no	c.171+1G>A / p.?	n.a.	yes	-
13	F/55	no	bPCC	no	no	no	c.178C>T / p.(Arg60Trp)	Y	-	-
14	F/34	no	bPCC	no	no	no	c.212T>G / p.(Ile71Ser)	Y	yes[UPD]	pos
15	M/57	no	mPCC	no	no	PA	c.220A>G / p.(Met74Val)	Y	yes	pos
16	M/18	no	bPCC	no	no	1ºHPT	c.223C>T / p.(Arg75*)	n.a.	-	-
17	F/18	yes	PCC	no	yes	no	c.244C>T / p.(Gln82*)	n.a.	-	-
18	F/40	no	bPCC	1 TA	yes	no	c.295+1G>T / p.?	n.a.	yes	neg
19	M/13	yes[p]	PCC	no	no	no	c.305T>C / p.(Leu102Pro)	Y	-	-
20[#]	F/48	no	-	1 H&N	no	no	c.-18C>T / p.(=)	N	-	-
21[#]	M/13	no	PCC	1 TA	no	no	c.25G>T / p.(Val9Leu)	N	no	pos
22[#]	M/22	no	PCC	no	no	no	c.63G>T / p.(=)	N	no	-
23[#]	F/80	no	PCC	no	no	no	c.414G>A / p.(=)	N	-	-
24[Ω¥]	F/29	no	PCC	no	no	no	c.269G>C / p.(Arg90Pro)	Y	yes	-
25[Ω]	M/39	no	PCC	no	no	no	c.223C>T / p.(Arg75*)	n.a.	yes	neg
26[Ω]	F/57	no	PCC	no	no	RC	c.103C>T / p.(Arg35Cys)	Y	yes	pos
27[Ω]	M/24	no	PCC	no	no	no	c.140_157del / p.(Arg47_Ser52del)	n.a.	yes	neg
28[Ω]	F/56	no	PCC	no	no	no	c.25del / p.(Val9Trpfs*56)	n.a.	yes[UPD]	neg

[a] Only data from probands are shown in the table.

[b] All cDNA and protein nomenclature is based on reference sequence ENST00000358664. All MAX variants were named following Human Genome Variation Society, and checked using Mutalyzer Name Checker (www.mutalyzer.nl).
[c] Pathogenicity potential of missense variants was examined by Alamut® mutation interpretation software (version 2.5), which provides variant interpretation according to several prediction methods (AlignGVGD, Polyphen, SIFT, ESEfinder, GeneSplicer, RESCUE-ESE).
[d] other tumors in the proband.
[p] Paternal familial antecedents.
[dn] *de novo* case.
[#] Variant of Unknown Significance (VUS), not considered for examination of phenotypic associations.
[Ω] Somatic mutation.
[¥] This tumor was not considered in further analysis since it was not possible to establish its germline status.
Abbreviations. Gender: F, female; M, male. Fam: familial antecedents; PCC, adrenal pheochromocytoma; bPCC-bilateral adrenal pheochromocytoma; mPCC - multiple pheochromocytomas within the same gland; PGL – paraganglioma; H&N – Head and Neck; TA- Thoraco-abdominal; Mets, presence of metastases where chromaffin cells are normally absent; Other disease: BrC, Breast Cancer; RO, Renal oncocytoma; SCCT, Squamous cell carcinoma of the tongue; CCH, C-cell hyperplasia; PA, Pituitary adenoma; 1ºHPT, Primary hyperparathyroidism; RC, Renal carcinoma; n.a., not applicable; -, not available; LOH, loss of heterozygosity; UPD, uniparental disomy; IHC, Immunohistochemistry: pos, positive; neg, negative.

Figure 1

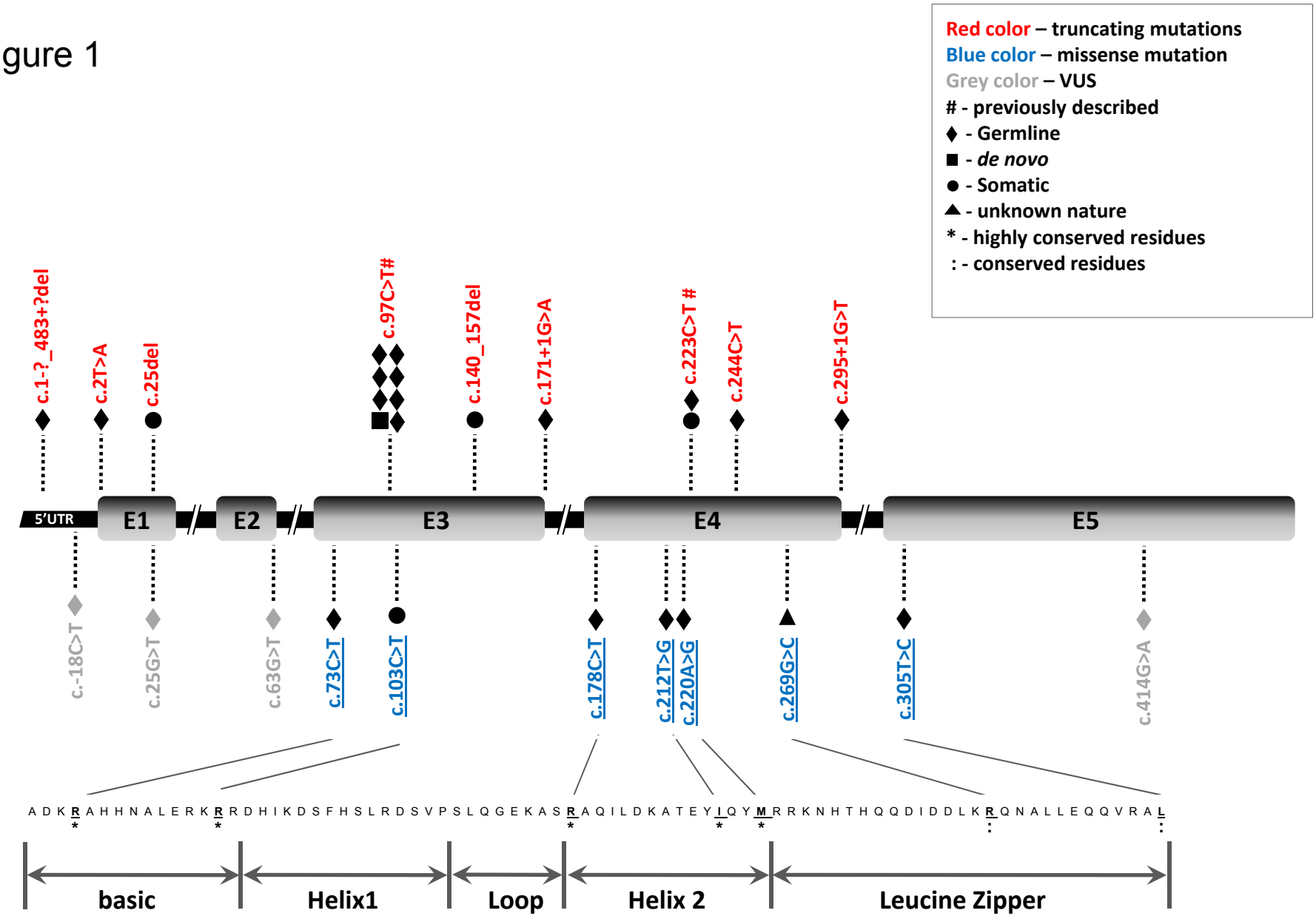

Figure 2