MAX mutations cause hereditary and sporadic pheochromocytoma and paraganglioma.


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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 \( VHL \), \( RET \), and \( NF1 \) respectively. The 10 percent rule was challenged after identification of germline mutations in \( SDHD \), \( SDHB \), and \( SDHC \) as important causes of familial paraganglioma (PGL) that led Neumann and colleagues to establish that up to a quarter of affected patients carried a PCC/PGL susceptibility gene mutation. Since then three additional susceptibility genes (\( SDHAF2 \), \( SDHA \) and \( TMEM127 \)) 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 χ2-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 \textit{RET}, \textit{VHL}, \textit{SDHB}, \textit{SDHC}, \textit{SDHD}, and \textit{TMEM127} genes (Table 1), we identified 16 different heterozygous variants affecting 23 subjects that spanned all five exons of the \textit{MAX} gene (Figure 1; Table 2). In addition, we analyzed \textit{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 \textit{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 \textit{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, 28).
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.

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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:

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

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References


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

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Clinical features</th>
<th>n</th>
<th>single PCC</th>
<th>single PGL</th>
<th>mPGL</th>
<th>PCC&amp;PGL</th>
<th>bPCC/mPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1252</td>
<td>315</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>With familial antecedents ¥ (%)</td>
<td></td>
<td>37</td>
<td>24 (1.91%)</td>
<td>5 (1.58%)</td>
<td>0</td>
<td>2 (5.71%)</td>
<td>6 (9.52%)</td>
</tr>
<tr>
<td>Malignant H (%)</td>
<td></td>
<td>129</td>
<td>79 (6.30%)</td>
<td>27 (8.57%)</td>
<td>2</td>
<td>6 (6.89%)</td>
<td>6 (9.52%)</td>
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<tr>
<td>Gender (female/ male/ unknown)</td>
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<td>1003</td>
<td>682/9</td>
<td></td>
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<tr>
<td>Median age first tumor (range)</td>
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<td>48</td>
<td>3-88</td>
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<tr>
<td>Tumors</td>
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<td>245</td>
<td>216</td>
<td>29</td>
<td></td>
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<td></td>
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<tr>
<td>Total</td>
<td></td>
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<tr>
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<td>95</td>
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<tr>
<td>Median age (range)</td>
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<td>52</td>
<td>11-83</td>
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</table>

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. H. Malignancy was defined as the presence of metastases where chromaffin cells are normally absent.
Table 2. Genetic and Clinical Features of the 28 MAX positive patients and tumors

<table>
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<tr>
<th>ID</th>
<th>Gender/age</th>
<th>Fam</th>
<th>PCC</th>
<th>PGL</th>
<th>Mets</th>
<th>Other disease</th>
<th>cDNA mutation</th>
<th>Protein alteration</th>
<th>Predicted Pathogenicity</th>
<th>LOH</th>
<th>IHC</th>
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<td>PCC</td>
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<td>no</td>
<td>no</td>
<td>c.1?-_483+?del</td>
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<tr>
<td>2</td>
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<td>no</td>
<td>c.2T&gt;A / p.?</td>
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<tr>
<td>3</td>
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<td>BrC, RO</td>
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<td>c.73C&gt;T / p.(Arg25Trp)</td>
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<td>-</td>
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<td>no</td>
<td>no</td>
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<td>-</td>
<td>-</td>
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<td>neg</td>
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<td>no</td>
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<td>no</td>
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<td>no</td>
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<td>c.212T&gt;G / p.(Ile71Ser)</td>
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<td>PA</td>
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<td>c.220A&gt;G / p.(Met74Val)</td>
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<td>yes</td>
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<td>no</td>
<td>c.244C&gt;T / p.(Gln82*)</td>
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<tr>
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<td>c.295+1G&gt;T / p.?</td>
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<td>neg</td>
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<td>no</td>
<td>c.305T&gt;C / p.(Leu102Pro)</td>
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<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1 H&amp;N</td>
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<td>c.-18C&gt;T / p.(=)</td>
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<td>-</td>
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<tr>
<td>21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M/13</td>
<td>no</td>
<td>PCC</td>
<td>1 TA</td>
<td>no</td>
<td>no</td>
<td>c.25G&gt;T / p.(Val9Leu)</td>
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<td>no</td>
<td>pos</td>
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<tr>
<td>22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M/22</td>
<td>no</td>
<td>PCC</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>c.63G&gt;T / p.(=)</td>
<td>N</td>
<td>no</td>
<td>-</td>
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<td>23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F/80</td>
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<td>PCC</td>
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<td>no</td>
<td>no</td>
<td>c.414G&gt;A / p.(=)</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>24&lt;sup&gt;av&lt;/sup&gt;</td>
<td>F/29</td>
<td>no</td>
<td>PCC</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>c.269G&gt;C / p.(Arg90Pro)</td>
<td>Y</td>
<td>yes</td>
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<tr>
<td>25&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>no</td>
<td>PCC</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>c.223C&gt;T / p.(Arg75*)</td>
<td>n.a.</td>
<td>yes</td>
<td>neg</td>
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<td>F/57</td>
<td>no</td>
<td>PCC</td>
<td>no</td>
<td>no</td>
<td>RC</td>
<td>c.103C&gt;T / p.(Arg35Cys)</td>
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<td>yes</td>
<td>pos</td>
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<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>PCC</td>
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<td>no</td>
<td>c.140_157del / p.(Arg47_Ser52del)</td>
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<td>no</td>
<td>c.25del / p.(Val9Trpfs*56)</td>
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<td>yes&lt;sup&gt;UPD&lt;/sup&gt;</td>
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</table>

* Only data from probands are shown in the table.
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). 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).

Other tumors in the proband.

Paternal familial antecedents.

de novo case.

Variant of Unknown Significance (VUS), not considered for examination of phenotypic associations.

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

- Red color – truncating mutations
- Blue color – missense mutation
- Grey color – previously described
- # - de novo - unknown nature
- * - highly conserved residues
- : - conserved residues

- c.223C>T
- c.97C>T
- c.103C>T
- c.178C>T
- c.212T>G
- c.220A>G
- c.269G>C
- c.305T>C
- c.414G>A
- c.140_157del
- c.171+1G>A
- c.2T>A
- c.1-?_483+?del
- c.25del
- c.25G>T
- c.244C>T
- c.244C>T
- c.295+1G>T
- c.25G>T
- c.178C>T
- c.223C>T
- c.244C>T
- c.295+1G>T
- c.25del

- 5'UTR
- E4 E5
- E3
- E2
- E1

- basic Helix1 Loop
- Helix1
- Loop
- Helix2
- Leucine Zipper

- A D K
- R A H H N A L E R K
- R A H D H I K D S F H S L R D S V P S L Q G E K A S
- A Q I L D K A T E Y
- R R K N H T H Q Q D I D D L K
- Q N A L L E Q Q V R A
- L

- "-- basic Helix1 Loop Helix 2 Leucine Zipper"
Figure 2

A. Not significant

B. P<0.001

C. P<0.001

D. P<0.001

Upper limits of reference intervals
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.

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Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.