HRAS Mutation Prevalence and Associated Expression Patterns in Pheochromocytoma

Adam Stenman,1,2* Jenny Welander,3 Ida Gustavsson,3 Laurent Brunaud,4 Martin Backdahl,5 Peter Soderkvist,3 Oliver Gim,3,6 C. Christofer Juulini,1,2 and Catharina Larsson2

1Department of Oncology and Pathology, Karolinska Institutet, Stockholm, SE-17176, Sweden
2Cancer Center Karolinska, CCK, Karolinska University Hospital Solna, Stockholm, SE-171 76, Sweden
3Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, SE-581 85, Sweden
4Department of Digestive, Hepato-Biliary and Endocrine Surgery, CHU Nancy ^ Hospital Brabois Adultes, University De Lorraine, Vandoeuvre-les-Nancy, F-5451 1, France
5Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, SE-171 76, Sweden
6Department of Surgery, Region Östergötland, Linköping, SE-58185, Sweden

Introduction

Pheochromocytomas (PCCs) and abdominal paragangliomas (PGLs), together abbreviated PPGL, are neuroendocrine tumors of the adrenal medulla and extra-adrenal paraganglia, respectively, displaying a highly diverse genetic background (Dahia, 2014). Although the majority of cases are benign, significant subsets of PGLs are malignant and often associated with inactivating SDHB gene mutations. Recent studies have revealed that approximately 40% of PGL patients carry a constitutional mutation in a susceptibility gene, and somatic mutations are found in an additional 30% of the tumors (Dahia, 2014). The currently known susceptibility genes include NF1, RET, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2, EGLN1, EPAS1, FH (Letouzé et al., 2013), KIF1Bb (Schlisio et al., 2008), MAX (Comino-Mendez et al., 2011), and TMEM127 (Dahia, 2014). Single families with PPGL and a constitutional mutation in one of the genes RAP1 (Wadt et al., 2012) and MDH2 (Cascon et al., 2015) have also been reported. The known genetic background of PGL further includes a set of genes that are recurrently mutated in PPGL tumors such as ATRX (Fishbein et al., 2015), KMT2D (Juilin et al., 2015), MET (Castro-Vega et al., 2015), BRAF (Luchetti et al., 2015), the TERT promoter (Liu et al., 2014), and HRAS (Yoshimoto et al., 1992; Crona et al., 2013). Expression profiling studies of PPGL have recently been verified in sporadic PGLs. In order to further establish the HRAS mutation frequency and to characterize the associated expression profiles of HRAS mutated tumors, 156 PPGLs for exon 2 and 3 hotspot mutations in the HRAS gene were screened, and compared with microarray-based gene expression profiles for 93 of the cases. The activating HRAS mutations G13R, Q61R, and Q61K were found in 1/14 PGL (7.1%). All HRAS mutated cases included in the mRNA expression profiling grouped in Cluster 2, and 21 transcripts were identified as altered when comparing the mutated tumors with 91 HRAS wild-type PPGL. Somatic HRAS mutations were not revealed in cases with known PPGL susceptibility gene mutations and all HRAS mutated cases were benign. The HRAS mutation prevalence of all PGL published up to date is 5.2% (49/950), and 8.8% (48/548) among cases without a known PPGL susceptibility gene mutation. The findings support a role of HRAS mutations as a somatic driver event in benign PGL without other known susceptibility gene mutations. HRAS mutated PPGL cluster together with NF1- and RET-mutated tumors associated with activation of kinase-signaling pathways. © 2016 The Authors Genes, Chromosomes & Cancer Published by Wiley Periodicals, Inc.
shown that tumors fall into two main clusters depending on their genetic composition (Dahia et al., 2005; Burnichon et al., 2011). Cluster 1 with VHL, SDHx and EPAS1 mutated tumors is characterized by a pseudo-hypoxic response and Cluster 2 includes tumors with mutations in MAX, NF1, RET, and TMEM127 that are associated with active kinase-signaling pathways (Dahia et al., 2005).

Somatic mutations in the Harvey rat sarcoma viral oncogene homolog (HRAS) gene were first reported in a single pheochromocytoma (Yoshimoto et al., 1992), and HRAS was more recently verified as a recurrently mutated gene in PCC. However, the two other members of the RAS family, that is, NRAS and KRAS have not been reported to be mutated in PPGL. Crona et al. identified HRAS mutations via exome sequencing and reported 3 mutated PCCs and 1 PGL (Crona et al., 2013). Oudijk and co-workers subsequently detected HRAS mutations in 5.2% of cases (14/271 PCCs) and proposed that the mutations are restricted to sporadic PCCs (10%, 14/140) (Oudijk et al., 2014) and Luchetti et al. published HRAS mutations in 6/65 PPGL (9.2%) (Luchetti et al., 2015). Recently, in a multiomics study by Castro-Vega et al. the authors screened 193 PPGL for HRAS mutations and found 10 mutated cases, all in benign, sporadic PPGL (Castro-Vega et al., 2015). Additionally, de Cubas et al. have mentioned 4 HRAS-mutated PPGL among 156 cases screened, whereof one mutation was found in a metastatic PPGL (de Cubas et al., 2015). Mutations at the hotspots codons 13 and 61 activate the transforming properties of various tumor types, and hence these recurrent mutations are thought to propagate PPGL tumorigenesis for a subset of cases. Germ-line HRAS mutations have been associated with the Costello syndrome, but to date no co-occurrence of this syndrome and PPGL has been reported (Crona et al., 2013; Luchetti et al., 2015). In this study, we aimed to further establish the HRAS mutation prevalence as well as its possible impact on global mRNA expression profiles in HRAS mutated PPGL.

MATERIALS AND METHODS

Phaeochromocytoma and Paraganglioma (PPGL) Tumor Samples

A total of 156 PPGL (142 PCCs and 14 PGLs) were collected from Karolinska University Hospital, Stockholm, Sweden (Series A; n = 75), University de Lorraine, Vandoeuvre-les-Nancy, France (Series B, n = 60), Linköping University Hospital, Sweden (Series C, n = 12), and Haukeland University Hospital in Bergen, Norway (Series D, n = 9), (Supporting Information Table 1). Samples were obtained with informed patient consent and with approval from the local ethics committee of the respective centers. Tumors were classified as benign or malignant following the WHO criteria (DeLellis et al., 2004). For Series A, a subset of the tumors (n = 54) had been characterized for mutations in 14 proposed PPGL susceptibility genes (EGLN1, EPAS1, KIF1Bb, MAX, MEN1, NF1, RET, SDHA, SDHB, SDHC, SDHD, SDHAF2, TMEM127, and VHL) (Welander et al., 2014a) and the remaining tumors (n = 21) were screened for mutations in 8 of these genes (EPAS1, MAX, NF1, SDHB, SDHD, RET, TMEM127, and VHL) (Welander et al., 2014b) (Supporting Information Table 1). Furthermore, all tumors in Series C and D were previously analyzed for mutations in the 8 genes (EPAS1, MAX, NF1, SDHB, SDHD, RET, TMEM127, and VHL) (Welander et al., 2014b) (Supporting Information Table 1). For Series B, a subset of patients exhibited established PPGL syndromes with associated mutations (Supporting Information Table 1).

HRAS Mutation Analysis

Genomic DNA isolated from fresh frozen tumor samples was used for amplification of fragments of exon 2 and 3 covering codons 13 and 61 of the HRAS gene (NM_001130442) with primer sequences available upon request. Sanger sequencing was carried out at the KIGene core facility at Karolinska Institutet for 113 cases and at Linköping University for 42 cases using previously described methodology (Welander et al., 2014a). All samples showing chromatogram alterations were re-analyzed with the reverse primer. One HRAS mutation (case 88) has been previously reported and was found via whole-exome sequencing (Supporting Information Table 1) (Juhlin et al., 2015).

Gene Expression Profiling

Total RNA was extracted from 53 PPGLs from Series A (Supporting Information Table 1), using the mirVana Isolation Kit (Ambion, Austin, TX) and subsequently analyzed in an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA). As previously reported, RNA preparations from all cases were of sufficient quality as measured by RIN values (Andreasson et al., 2013a,b). RNA samples (250 ng) were used for whole-transcriptome analysis with GeneChip Human Gene 1.0 ST arrays (Affymetrix),
covering approximately 29K annotated genes as previously described (Welander et al., 2014b). Tumor RNA from 40 cases in Series A–C (detailed in Supporting Information Table 1) had previously been analyzed with the GeneChip Human 1.0 ST array (Affymetrix) (Welander et al., 2014b). HRAS mutation status from the current study was implemented into the dataset and after normalization using the robust multiarray average (RMA) algorithm, hierarchical clustering of the microarray expression data for all 93 PPGLs was performed as previously described (Welander et al., 2014b) using a set of genes that has been shown to separate the clusters (Burnichon et al., 2011). These genes overlapped with 454 of the probe sets on the array were compared between the 7 HRAS mutated cases and the 91 HRAS wild-type cases included. Given their involvement in PPGL, normalized signal intensities for the HRAS, vascular endothelial growth factor A (VEGFA) and phenylethanolamine N-methyltransferase (PNMT) genes were exported for separate statistical analysis.

Within the cohort, tumors with known somatic mutations in EPAS1, KIF1Bb, MAX, NF1, RET, SDHA, SDHB, TMEM127, and VHL were included (Supporting Information Table 1). Additionally, five cases from patients with known PPGL syndromes (2 MEN2, 1 NF1, 1 PGL5, and 1 VHL) were included as internal controls and were also included in the hierarchical clustering. One identical sample was analyzed at both time points in (Welander et al., 2014b) and in the current study as an internal control between the GeneChip arrays. This sample did not show any difference in clustering behavior as evaluated with a principal component analysis quality control in the GeneSpring software (data not shown).

**Statistical Analyses**

Transcriptome-wide statistical analyses and clustering were performed as previously described.

---

*Figure 1. Detection of a HRAS Q61L mutation, hierarchical clustering of PPGLs and PNMT gene expression in relation to HRAS mutation status. (A) Chromatogram of case 227 (PGL) showing the Q61L mutation (c.182A>T, COSM498), which has previously not been reported in PPGL. A vertical arrow shows the heterozygous missense variant. (B) Hierarchical clustering of 93 tumors (indicated by their case numbers) and 5 control cases (indicated as MEN2, NF1, SDHA, and VHL) based on their expression levels for 454 genes according to Burnichon et al. 2011. The dendrogram shows separation of tumors into two distinct groups (Cluster 1 to the left and Cluster 2 to the right). The PPGL mutation status is indicated below. All 7 HRAS-mutated cases clustered together with the tumors endowed with mutations in the NF1- and RET genes. (C) RNA levels of the PNMT gene compared between the PPGL with (HRAS mutated n = 7) and without (HRAS wild-type n = 86) HRAS mutations. Horizontal bars represent mean values and the gene expression has been normalized to the mean value of cases endowed with constitutional NF1- and RET mutations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]*
(Welander et al., 2014b) using the GeneSpring GX v. 12.6 (Agilent, Santa Clara, CA) software and the Benjamini–Hochberg method (Benjamini and Hochberg, 1995) was used to control for multiple testing. When comparing the gene expression profiles between the 7 HRAS-mutated cases and the 91 HRAS wild-type cases based on the entire probe sets on the array, a Benjamini–Hochberg corrected false discovery rate (FDR) of less than 0.1 was applied. Gene expression levels for HRAS, VEGFA, and PNMT were compared between sporadic HRAS-mutated and HRAS wild-type tumors using two-tailed Student’s t-test. Two-tailed Mann–Whitney U or Fisher’s exact tests were used to analyze potential significant correlations between the clinical parameters and HRAS mutational status. P-values of less than 0.05 were considered as statistically significant.

RESULTS

Detection of HRAS Mutations

A HRAS mutation was found in 11 out of 156 tumors screened (142 PCCs and 14 PGLs), equaling a total frequency of 7.1% (11/156) in our cohort (Table 1). One mutation was found in exon 2
and ten mutations were found in exon 3 (six Q61R, three Q61K, and one Q61L) (Table 1, Fig. 1A).

The HRAS mutation frequency in apparently sporadic PPGL (non-familial and without known susceptibility gene mutation) was 10.9% (11/101; Table 1). The HRAS mutation status was compared with clinical and genetic characteristics of the present cohort and in combination with published studies (Table 1). No HRAS mutation was found in any PPGL endowed with a known PPGL susceptibility gene mutation (Table 1, Supporting Information Table 1). Hence, HRAS mutations were associated with the PPGL group without a known susceptibility gene mutation both in our study (Fisher’s exact test, \( P = 0.017 \)) (Table 1) and in all available studies combined (Fisher’s exact test, \( P < 0.0001 \)) (Table 2). Regarding clinical parameters, no mutations were found in PPGLs classified as malignant according to the current WHO criteria and the patients endowed with a HRAS mutation tended to have higher age at diagnosis (mean 63 ± 10 years) compared with those without HRAS mutation (mean 54 ± 16 years) however this association did not reach statistical significance (two-tailed Mann–Whitney \( U \)-test, \( P = 0.08 \)). In our series of 11 mutated PPGLs there were four men and seven women, and no gender-related difference in HRAS

| TABLE 2. Summary of HRAS Mutation Studies in PPGL |
|-----------------------------------------|-----------------|-----------------|-----------------|-----------------|
| HRAS gene status | PPGL susceptibility gene |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| HRAS mutated | Codon 13 | G13R | Codon 61 | Q61R | Codon 61 | Q61K | Codon 61 | Q61L | Wild-type codon 13/61 |
| This study a | | 9 | 1 | 5 | 3 | 0 | 132 | 48 | 93 |
| Moley et al. 1991 | | 1 | 0 | 0 | 0 | 1 | 13 | 7 | 7 |
| Yoshimoto et al. 1992 | | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| Crona et al. 2013 b | | 3 | 1 | 1 | 1 | 0 | 69 | 22 | 50 |
| Oudijk et al. 2013 | | 1 | 0 | 1 | 0 | 0 | 8 | 3 | 6 |
| Luchetti et al. 2015 c | | 4 | 1 | 2 | 1 | 0 | 77 | 25 | 56 |
| Castro-Vega et al. 2015 d | | 1 | 0 | 1 | 0 | 0 | 55 | 31 | 24 |
| de Cubas et al. 2015 | | 14 | 1 | 12 | 1 | 0 | 257 | 107 | 164 |
| Moley et al. 1991 | | 0 | 0 | 0 | 0 | 0 | 54 | 16 | 44 |
| Yoshimoto et al. 1992 | | 0 | 0 | 0 | 0 | 0 | 8 | 3 | 5 |
| Crona et al. 2013 b | | 3 | 1 | 1 | 1 | 0 | 69 | 22 | 50 |
| Oudijk et al. 2013 | | 1 | 0 | 1 | 0 | 0 | 8 | 3 | 6 |
| Luchetti et al. 2015 c | | 4 | 1 | 2 | 1 | 0 | 77 | 25 | 56 |
| Castro-Vega et al. 2015 d | | 1 | 0 | 1 | 0 | 0 | 55 | 31 | 24 |
| de Cubas et al. 2015 | | 14 | 1 | 12 | 1 | 0 | 257 | 107 | 164 |

\[ a \text{One PCC with a Q61R HRAS mutation has been previously published (Juhlin et al., 2015) and is excluded.} \]

\[ b \text{One head and neck paraganglioma is excluded.} \]

\[ c \text{Twenty head and neck paragangliomas are excluded.} \]

\[ d \text{Six metastases and 3 thoracic paragangliomas are excluded. Three HRAS mutations from this study are not reported in the table: G12R (n = 1), S145L (n = 1), and A146T (n = 1).} \]
TABLE 3. Genes with Altered Expression in HRAS Mutated Tumors (n = 7) Compared with HRAS Wild-Type Cases (n = 91) using a Benjamini–Hochberg Corrected FDR of 10 %

| Transcript cluster id | Corrected P-value | Fold change | Fold change log | Gene symbol |
|-----------------------|-------------------|-------------|----------------|-------------|
| **Up-regulated in HRAS mutated vs. wild-type** | | | | |
| 8135774               | 0.0632            | 2.7526      | 1.4608         | PTPRZ1      |
| 8138337               | 0.0000            | 2.0660      | 1.2424         | TMEM195     |
| 7928907               | 0.0253            | 2.0682      | 1.0484         |             |
| 8000963               | 0.0025            | 1.9834      | 0.9880         | STX1B       |
| 7921852               | 0.0532            | 1.7622      | 0.8174         | MPZ         |
| 8107518               | 0.0532            | 1.6164      | 0.6928         |             |
| 8103374               | 0.0889            | 1.5410      | 0.6239         |             |
| 8152863               | 0.0253            | 1.5150      | 0.5993         |             |
| 8156110               | 0.0253            | 1.5148      | 0.5991         |             |
| 8000757               | 0.0005            | 1.4531      | 0.5391         | DOC2A       |
| 8157027               | 0.0253            | 1.4506      | 0.5367         | NIPSNAP3B   |
| 7998053               | 0.0854            | 1.3622      | 0.4459         |             |
| 7948037               | 0.0253            | 1.3206      | 0.4012         |             |
| 7965838               | 0.0300            | 1.1981      | 0.2608         |             |
| 8040672               | 0.0832            | 1.1767      | 0.2347         | DRC1        |
| **Down-regulated in HRAS mutated vs. wild-type** | | | | |
| 8036483               | 0.0909            | −1.3839     | −0.4687        | TIP1B       |
| 8098705               | 0.0604            | −1.3403     | −0.4225        | MTRF1L      |
| 8061542               | 0.0832            | −1.3147     | −0.3948        | HM13        |
| 7989619               | 0.0419            | −1.3038     | −0.3827        | PP1B        |
| 7983290               | 0.0253            | −1.3018     | −0.3805        | SERF2       |
| 7924230               | 0.0832            | −1.2333     | −0.3025        | ABHD17A     |

**DISCUSSION**

In this study we aimed to further establish the *HRAS* mutation frequency in PPGL and examine the impact on global expression profiles in *HRAS* mutated tumors. We consequently screened 156 PPGLs for mutations in the *HRAS* gene and compared the results with microarray-based gene expression profiles for 93 (60%) of the cases.

Eleven out of 156 cases were found endowed with *HRAS* mutations equaling a total frequency of 7.1%. This prevalence is in line with previously published results (Table 2) (Yoshimoto et al., 1992; Crona et al., 2013; Oudijk et al., 2014; Castro-Vega et al., 2015; Luchetti et al., 2015).

One single mutation was found in exon 2 (G13R) and ten mutations were found in exon 3 (six Q61R, three Q61K and one Q61L). The Q61L mutation at c.182 A>T (COSM498), which has previously not been reported in PPGL, was the only mutation found in a PGL in our cohort. This alteration has previously been reported in cutaneous squamous cell carcinoma (Su et al., 2012) and in penile cancer (Andersson et al., 2008).

No *HRAS* mutations were found in PPGLs classified as malignant according to the current WHO criteria, which is in line with previous findings (Yoshimoto et al., 1992; Crona et al., 2013; Oudijk et al., 2014; Castro-Vega et al., 2015), however one single metastatic case with a *HRAS* mutation has been previously reported (de Cubas et al., 2015). The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.

The observed male:female proportion is in line with the results shown in two studies (Oudijk et al., 2014; Castro-Vega et al., 2015), but conflicting the results shown in an earlier study (Crona et al., 2013) where 4/4 patients with *HRAS* mutations were men.

The mean tumor size of 7.1%.
None of the HRAS mutated tumors in our cohort were malignant according to the WHO criteria, suggesting a role of HRAS mutations as a somatic driver event in benign PPGL. As with the other genes associated with kinase signaling pathways in PPGL, HRAS mutations appear to be associated with a benign phenotype overall, although characterization and long term follow-up in additional cohorts will be required to determine if they may be used as predictive markers.

Activating mutations in the HRAS gene are known to affect MAPK signaling (Balmain and Pragnell, 1983), and as might be expected, all seven HRAS mutated cases included in the microarray-based profiling were grouped in Cluster 7 (Pragnell, 1983), and as might be expected, all may be used as predictive markers.

Hypoxia inducible factor-2 alpha (HIF-2α) has also been suggested (Wang et al., 2013) to play a role in the conversion (methylation) between norepinephrine and epinephrine. This finding is in line with a previous study showing that Cluster 2 tumors have increased PNMT expression and hence higher epinephrine levels in the patient (Eisenhofer et al., 2004). Interestingly, several tumors without mutations in any of the so far known susceptibility genes appear to form a group within Cluster 1 (Fig. 1B). An underlying somatic VHL mutation was excluded in these cases based on previous mutation screenings in all cases included in the microarray (Supporting Information Table S1). One may speculate that these tumors might share unknown underlying genetic mechanisms that potentially involve regulation of the hypoxia response, which may be an interesting subject for future studies.

Based on the analyses using the entire probe set on the array, PTPRZ1 was found to be the most up-regulated gene in HRAS mutated PPGL compared with HRAS wild-type tumors (Table 3). This gene has been shown to regulate glioblastoma cell motility (Müller et al., 2003) and activation of PTPRZ1 via hypoxia inductive factor-2 alpha (HIF-2α) has also been suggested (Wang et al., 2010).

To summarize, we were able to establish a low HRAS mutation frequency (7.1%) in PPGL. Taken together with all other studies published up to date, the overall HRAS mutation prevalence in PPGL is 5.2% (49/950) and 8.8% (48/548) among apparently sporadic cases without a known PPGL susceptibility gene mutation. HRAS mutated cases were grouped into Cluster 2 and somatic HRAS mutations did not occur in patients with a known PPGL susceptibility gene mutation or in patients with malignant PPGL. Somatic HRAS mutations thus represent a possible driver event for a subset of benign PPGLs.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Lisa Ånfalk, Karolinska University Hospital, for excellent tissue handling and Professor Michael Brauckhoff at the Haukeland University Hospital, Norway, for providing material and clinical information regarding series D samples used in the study. The authors also want to thank Professor Anne-Paule Gimenez-Roqueplo and Dr. Luis-Jaime Castro-Vega for kindly providing the mutational data for the cases in the Paris study.

REFERENCES

Andresson P, Karlsdottir B, Karlsson MG. 2008. PIK3CA, HRAS and KRAS gene mutations in human penile cancer. J Urol 179:2030–2034.
Andresson A, Kiss NB, Caramuta S, Sulaiman L, Svahn F, Ackdahl M, Haukeland University Hospital, Norway, for providing the microarray-based profiling.
Andreasson P, Kolaric A, Windahl T, Kirrander P, Soderkvist P, Karlsson MG. 2008. PIK3CA, HRAS and KRAS gene mutations in human penile cancer. J Urol 179:2030–2034.
Balmain A, Pragnell IB. 1983. Mouse skin carcinomas induced in vivo by chemical carcinogens have a transforming Harvey-ras oncogene. Nature 303:72–74.
Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc B Met 57:289–300.
Burnichon N, Vescovo L, Amar L, Libé R, de Reynies A, Venisse A, Jouanno E, Laurendeau I, Parfait B, Bertherat J, Plouin PF, Jeune MA, Favier J, Gimenez-Roqueplo AP. 2011. Integrative genomic analysis reveals somatic mutations in pheochromocytomas and paraganglioma. Hum Mol Genet 20:3974–3985.
Cascón A, Comino-Méndez I, Currrás-Freixes M, de Cubas AA, Contreras L, Richter S, Peitsch M, Mancikova V, Inglađa-Pérez L, Pérez-Barrios A, Galatayud M, Aznél S, Villar-Vicente R, Aller J, Setién F, Moran S, García JF, Río-Machín A, Lecón R, Gómez-Graña A, Apellániz-Ruiz M, Roncador G, Esteller M, Rodríguez-Antonio G, Satrustegui J, Eschenhofer U, Urbante M, Robledo M. 2015. Whole-exome sequencing identifies MDH2 as a new familial paraganglioma gene. J Natl Cancer Inst 117:107.
Castro-Vega IJ, Letouzé E, Burnichon N, Buffet A, Diéderot PH, Khâfif E, Lortot G, Elarouci N, Monin A, Menara M, Leportre-Lussey C, Badosal C, Sibony M, Dousset B, Libé R, Zimindohoue F, Plouin PF, Bertherat J, Amar L, de Reynies A, Favier J, Gimenez-Roqueplo AP. 2015. Multi-omics analysis defines core genomic alterations in pheochromocytomas and paragangliomas. Nat Commun 6:6044.
Comino-Méndez I, Gracia-Aznárez FJ, Schiavi F, Landi I, Leandro-García IJ, Léotin R, Healey E, Ramos-Medina R, Caronia D, Pita G, Gómez-Grana A, de Cubas AA, Inglađa-Pérez L, Maliszewska A, Taschin E, Bobisse S, Pica G, Loli P, Hernández-Lavado R, Díaz JA, Gómez-Morales M, González-Neva A, Roncador G, Rodríguez-Antonio C, Benetjé J, Manneli
M. Opojer G, Robledo M, Cascón A. 2011. Exome sequencing identifies MAX mutations as a cause of hereditary pheochromocytoma. Nat Genet 43:663–667.

Crona J, Delgado Verdugo A, Mahatran R, Ställberg P, Granberg D, Hellman P, Björklund P. 2013. Somatic mutations in H-RAS in sporadic pheochromocytoma and paraganglioma identified by exome sequencing. J Clin Endocrinol Metab 98:E1266–E1271.

de Calpti AA, Korpershoek E, Ingledew WE, Lenzú E, Curtás-Freixes M, Fernández AF, Comino-Méndez I, Schiavi F, Mancikova V, Eisenhofer G, Mannelli M, Opojer G, Timmers H, Beuschlein F, de Krjrger K, Cascón A, Rodriguez-Antonia C, Praga MF, Favier J, Gimenez-Roqueplo AP, Robledo M. 2015. DNA methyltransferase profiling in pheochromocytoma and paraganglioma reveals diagnostic and prognostic markers. Clin Cancer Res 21:3020–3030.

Dahia PLM. 2014. Pheochromocytoma and paraganglioma pathogenesis: Learning from genetic heterogeneity. Nat Rev Cancer 14:108–119.

Dahia PLM, Ross KN, Wright ME, Hayashida CY, Santagata S, M, Opocher G, Robledo M, Cascón A, Rodríguez-Antonia C, Praga MF, Favier J, Gimenez-Roqueplo AP, Robledo M. 2015. DNA methylation profiling in pheochromocytoma and paraganglioma identified by targeted next generation sequencing analysis. Int J Endocrinol 2015:138573.

Moley JF, Brother MB, Wells SA, Spengler BA, Biedler JL, Brodeur GM. 1991. Low frequency of ras gene mutations in neuroblastomas, pheochromocytomas, and medullary thyroid cancers. Cancer Res 51:1596–1599.

Müller S, Kunkel P, Lamszus K, Ulbricht U, Lorente GA, Nelson AM, von Schack D, Chin DJ, Loehr SC, Westphal M, Melcher T. 2003. A role for receptor tyrosine phosphatase zeta in glioma cell migration. Oncogene 22:6661–6668.

Oudshoorn L, de Krjrger K, Beuschlein F, de Cubaas AA, Dois AP, Djinjins WN, Koppershoek E, Mancikova V, Mannelli M, Papotti M, Varatano S, Robledo M, Volante M. 2014. H-RAS mutations are restricted to sporadic pheochromocytomas lacking specific clinical or pathological features: Data from a multi-institutional series. J Clin Endocrinol Metab 99:E1376–E1380.

Schiloso S, Kenchappa RS, Vedeveeld LC, George RE, Stewart R, Greulich H, Shachter K, Nguyen NV, Pigpy P, Duhia PL, Pomery SL, Maris JM, Look AT, Meyerson M, Peeper DS, Carter BD, Kaelin WG, Jr. 2008. The kinesin KIF1Bbetta acts downstream from EglN3 to induce apoptosis and is a potent 1p36 tumor suppressor. Genes Dev 22:884–893.

Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spielis O, Reis-Filho JS, Kong X, Koyc RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Han JS, Noe J, Lambros M, Geyer F, Dhmone N, Niculescu-Duvaz i, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Gdoufing, hereditary and sporadic pheochromocytomas: Activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. Endocr Relat Cancer 11:897–911.

Frischlein L, Khsue S, Wuubenhorst B, DeSloover D, D’Andrea K, Eisenhofer G, Huynh T-T, Pacak K, Brouwers FM, Walther MM, Dei Tos AP, Dinjins WN, Korpershoek E, Mancikova V, Mannelli M, Papotti M, Varatano S, Roblebo M, Volante M. 2014. H-RAS mutations are restricted to sporadic pheochromocytomas lacking specific clinical or pathological features: Data from a multi-institutional series. J Clin Endocrinol Metab 99:E1376–E1380.

Schiloso S, Kenchappa RS, Vedeveeld LC, George RE, Stewart R, Greulich H, Shachter K, Nguyen NV, Pigpy P, Duhia PL, Pomery SL, Maris JM, Look AT, Meyerson M, Peeper DS, Carter BD, Kaelin WG, Jr. 2008. The kinesin KIF1Bbetta acts downstream from EglN3 to induce apoptosis and is a potent 1p36 tumor suppressor. Genes Dev 22:884–893.

Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spielis O, Reis-Filho JS, Kong X, Koyc RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Han JS, Noe J, Lambros M, Geyer F, Dhmone N, Niculescu-Duvaz i, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte MJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Gdoufing, hereditary and sporadic pheochromocytomas: Activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. Endocr Relat Cancer 11:897–911.

Frischlein L, Khsue S, Wuubenhorst B, DeSloover D, D’Andrea K, Merrill S, Cho NW, Greenberg RA, Else T, Montone K, LVolsy V, Fraker D, Daher R, Cohen DL, Nathanson KL. 2015. Whole-exome sequencing defines the mutational landscape of pheochromocytoma and identifies KMT2D as a recurrently mutated gene. Genes Chromosomes Cancer 54:6140.

Juhlin CC, Stenman A, Huglund F, Clark VE, Brown TC, Baranasko PJ, Bilguvar K, Geh G, Welander J, Svahn F, Rubinstein JC, Caramuta S, Yasunoo K, Günsel M, Bäckdahl M, Gimm O, Söderkvist P, Prasad ML, Korah R, Lifton RP, Martin M, Yang H, Wang Q, Hilton H, Han JS, Noe J, Lambros M, Geyer F, Dhmone N, Niculescu-Duvaz i, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte MJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Gdoufing, hereditary and sporadic pheochromocytomas: Activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. Endocr Relat Cancer 11:897–911.

Frischlein L, Khsue S, Wuubenhorst B, DeSloover D, D’Andrea K, Merrill S, Cho NW, Greenberg RA, Else T, Montone K, LVolsy V, Fraker D, Daher R, Cohen DL., Nathanson KL. 2015. Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. Nat Commun 6:6140.

Juhlin CC, Stenman A, Huglund F, Clark VE, Brown TC, Baranasko PJ, Bilguvar K, Geh G, Welander J, Svahn F, Rubinstein JC, Caramuta S, Yasunoo K, Günsel M, Bäckdahl M, Gimm O, Söderkvist P, Prasad ML, Korah R, Lifton RP, Martin M, Yang H, Wang Q, Hilton H, Han JS, Noe J, Lambros M, Geyer F, Dhmone N, Niculescu-Duvaz i, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte MJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Gdoufing, hereditary and sporadic pheochromocytomas: Activation of hypoxia-driven angiogenic pathways in von Hippel-Lindau syndrome. Endocr Relat Cancer 11:897–911.

Frischlein L, Khsue S, Wuubenhorst B, DeSloover D, D’Andrea K, Merrill S, Cho NW, Greenberg RA, Else T, Montone K, LVolsy V, Fraker D, Daher R, Cohen DL., Nathanson KL. 2015. Whole-exome sequencing defines the mutational landscape of pheochromocytoma and identifies KMT2D as a recurrently mutated gene. Genes Chromosomes Cancer 54: 542–554.

Letouré E, Martinelli G, Loriot C, Burnichon N, Avermil N, Ottolenghi G, Janin M, Menara M, Nguyen AT, Benit P, Buffet A, Marcaillou G, Bertherat J, Amor L, Rustin P, De Reyniés A, Gimenez-Roqueplo AP, Favier J. 2013. SDH mutations establish a hypermethylator phenotype in paraganglioma. Cell Melanoma Res 25:815–818.

Liu T, Brown TG, Juhlin CC, Andreasson A, Wang N, Bäckdahl M, Healy JM, Prasad ML, Korah R, Carling T, Xu D, Larsson C. 2014. The activating TRIFT promoter mutation C225T is recurrent in subsets of adrenal tumors. Endocr Relat Cancer 21: 427–434.

Luchetti A, Walsh D, Rodger F, Clark G, Martin T, Irving R, Sanna M, Yao M, Roblebo M, Neumann HP, Woodward ER, Latt F, Abbs S, Martin H, Maher ER. 2015. Profiling of somatic mutations in paragangliomas lacking specific clinical or pathological features: Data from a multi-institutional series. J Clin Endocrinol Metab 99: E1376–E1380.