Composite pheochromocytoma/paraganglioma-ganglioneuroma: analysis of SDH and ATRX status, and identification of frequent HRAS and BRAF mutations

Jingci Chen1,*, Yan Wu1,*, Pengyan Wang1,*, Huanwen Wu1, Anli Tong2 and Xiaoyan Chang1

1Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
2Department of Endocrinology, Key Laboratory of Endocrinology, National Health Commission of the People’s Republic of China, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Correspondence should be addressed to X Chang: changxy@pumch.cn
*(J Chen, Y Wu and P Wang contributed equally to this work)

Abstract

Introduction: Composite pheochromocytoma/paraganglioma (CP) is a rare neoplasm with most cases presented as single reports. Little is known about its pathogenesis and relationship with ordinary pheochromocytoma (PCC) or paraganglioma (PGL). Our study is aimed at analyzing the status of SDH and ATRX and identifying novel genetic changes in CP.

Methods: Eighteen CP cases were collected. SDH and ATRX status was screened by immunohistochemistry. Targeted region sequencing (TRS) was successfully performed on formalin-fixed paraffin-embedded tissues in two cases within 3 years. Based on the TRS result, Sanger sequencing of BRAF and HRAS was performed in fifteen cases (including the two cases with TRS performed), with three cases excluded due to the limited amount of tissue.

Results: Histopathologically, all the cases were composite PCC/PGL-ganglioneuroma (GN). The GN components were either closely admixed or juxtaposed with the PCC/PGL components, with a highly variable percentage (10–80%). All cases stained positive for SDHB and ATRX. HRAS and BRAF mutations were identified during TRS. In the subsequent Sanger sequencing, 20.0% (3/15) harbored BRAF mutations (K601E and K601N) and 46.7% (7/15) harbored HRAS mutations (Q61R, Q61L, G13R). The mutation rates were both significantly higher than reported in ordinary PCC/PGL.

Conclusions: We demonstrated that composite PCC/PGL-GN might be a unique entity with frequent HRAS and BRAF mutations rather than genetic changes of SDH and ATRX. Our findings revealed the possible pathogenesis of composite PCC/PGL-GN and provided clues for potential treatment targets.

Introduction

Composite pheochromocytoma/paraganglioma (CP) is a rare neoplasm consisting of pheochromocytoma (PCC) or paraganglioma (PGL) combined with developmentally related neurogenic tumor (1). Its neurogenic component is various, including ganglioneuroma (GN), neuroblastoma, ganglioneuroblastoma, etc (1, 2). Currently, little is known about its pathogenesis and relationship with its pure tumor counterparts. Comstock et al. investigated the N-myc
amplification status in CP, ordinary PCC, and ordinary neuroblastoma, and their findings suggested that CP might be a histologic variant of PCC (2). Nevertheless, more cases and research are required to explore its pathogenesis.

A series of genes have been reported to be closely related to ordinary PCC and PGL, both hereditarily and somatically (3, 4). Among them, the succinate dehydrogenase (SDH) gene family is the most commonly mutated gene (5). Recently, some researchers have also identified co-occurring SDHB and ATRX chromatin remodeler (ATRX) mutations in extensive metastatic cases, and ATRX mutation has been shown to be an independent risk factor of metastasis (6). In contrast, researchers have not encountered CP with definite germline SDH mutations based on the limited data (7, 8). It remains unclear whether CP also harbors mutations of SDH and ATRX and whether their mutation status also has an impact on clinicopathological features. Therefore, one of our objectives is to screen loss-of-function mutations of SDH and ATRX in CP.

A series of other mutations have been identified in PCC/PGL, such as BRAF, CDKN2A, DNMT3A, FH, H3F3A, HRAS, MAX, NF1, RET, and VHL (9, 10). Among them, HRAS and BRAF are two well-known proto-oncogenes: HRAS is involved in the kinase receptor signaling pathway and its mutation activates its downstream effectors, which leads to cell proliferation and tumor formation (11). BRAF belongs to the RAF family of serine/threonine kinases and its mutation significantly influences the prognosis and treatment in various malignancies (12). However, previous research in ordinary PCC/PGL indicates that HRAS somatic mutation only serves as a small part of the multiple pathways of PCC/PGL (13). Regarding BRAF, the mutation is even rarer (9, 14). Interestingly, we performed targeted region sequencing (TRS) in CP and identified HRAS and BRAF mutations. Herein, we also focused on these two genes in a larger case series and identified frequent mutations in our cohort.

Materials and methods

Clinicopathological information

Eighteen CP cases were diagnosed between February 2005 and June 2020 in Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences. The institutional review board of PUMCH approved the study. Consent has been obtained from each patient after a full explanation of the purpose and nature of all procedures used. Clinicopathological information was gathered from medical records and pathological reports. Tumor sections from formalin-fixed paraffin-embedded (FFPE) samples were stained with hematoxylin–eosin (HE) and reviewed by two experienced pathologists independently. TNM staging was based on the 2017 World Health Organization Classification of Tumors of Endocrine Organs.

Immunohistochemistry (IHC)

IHC staining was performed on 4 μm thick sections with FFPE tissues using the following antibodies: ATRX (ZA-0016, ZSGB-BIO), BRAF V600E (clone VE1, Ventana), CgA (clone LK2H10, ZSGB-BIO), Ki-67 (clone MIB1, ZSGB-BIO), SDHB (clone OTI1H6, ZSGB-BIO), and S-100 (Dako).

Genomic DNA preparation

PCC/PGL or GN component was labeled under the microscope and separated by macroadissection. Intermingled parts of the tumors were used for genetic analysis of composite components. DNA extraction was performed from FFPE samples using QIAamp DNA FFPE Tissue Kit (Cat No. 56404).

TRS

Probes about 560 genes were designed on the website of Agilent. In our panel, genes related to PCC included: ATRX, BRAF, CDKN2A, DNMT3A, FH, H3F3A, HRAS, IDH1, MAX, MEN1, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TPS3, and VHL. DNA fragmentation was carried out by the hydrodynamic shearing system (Covaris, Massachusetts, USA). Extracted DNA was amplified by ligation-mediated PCR, purified, and hybridized to the probe for enrichment. Both non-captured and captured ligation-mediated PCR products were subjected to real-time PCR to estimate the magnitude of enrichment. Each captured library was then loaded on a HiSeq platform. The average cover depth was 804×. Valid sequence data were mapped to the reference human genome (UCSC hg19) by the Burrows–Wheeler Aligner software. MuTect and Strelka were used respectively to call somatic single nucleotide variations (SNV) and small insertions and deletions (InDel). The cut-offs for mutational calling were 2%.

Sanger sequencing of BRAF and HRAS

The BRAF and HRAS gene fragments were amplified by PCR. Each 20 μL PCR reaction mixture included 1× HotStarTaq buffer, 0.2 μM of each primer, 2.0 mM MgCl2, 0.2 mM of each
dNTP, 1 U HotStarTaq polymerase, and 1 μL template DNA. The sequencing primers were provided in Supplementary Table 1 (see section on supplementary materials given at the end of this article). Sequencing was performed with the DNA analyzer ABI3130XL at Genesky Biotechnologies Inc (Shanghai, China). Data were analyzed with Polyphred.

Results

Clinicopathological characteristics

Sixteen composite pheochromocytomas (Table 1, cases 1–16) and two composite paragangliomas (Table 1, cases 17 and 18) were collected. Clinicopathological features were summarized in Table 1.

The tumors were diagnosed at the mean age of 51 (range: 23 to 68). The average size of the tumor was 4.9 cm (range: 0.9 to 14 cm). Thirteen of 18 were symptomatic with chief complaints such as headache and hypertension. One patient (case 12) showed a weight loss of 3.5 kg in 6 months. The 24-h urinary catecholamines were measured in 17 patients, and 13 of them elevated. Prior to the surgery, 13 cases (cases 1–4, 6–9, 11–14, 17) were diagnosed as PCC/PGL, 3 cases (cases 5, 10, 16) were diagnosed as adrenal cortical adenoma, 1 case (case 15) was diagnosed as GN and 1 case (case 18) occurring in the urinary bladder was misdiagnosed as urothelial carcinoma. All the cases were clinically sporadic without any family histories of related diseases.

Histopathologically, the PCC or PGL component exhibited a Zellballen pattern composed of polygonal tumor cell nests separated by capillaries. The tumor cells showed nuclear polymorphism but few mitoses. The cytoplasm was granular and basophilic to amphophilic. In all the cases, the composite components exhibited a clear histoarchitecture of GN, which was characterized by large ganglion cells distributed in Schwannian stroma (Fig. 1). The mixture patterns and percentages of the two components were variable. There were both relatively intermingled and relatively separated areas in ten cases, whereas in the other eight cases they were clearly juxtaposed. The percentage of GN components varied from 10 to 80%. Immunohistochemically, the polygonal cells in PCC or PGL were positive for Syn and CgA. The ganglion cells were immunoreactive for NeuN; the Schwann cells and sustentacular cells were immunoreactive for S-100. Seven cases had a low Ki-67 index (<1%), ten cases were 1–3%, and one case was 5% (Fig. 2A, B and C).

Sixteen cases were classified as stages I–II, with cases 14 and 17 belonging to stage III due to invasion of the extra-adrenal or extra-capsule adipose tissue. Follow-up of nine patients was available. The median follow-up time was 52 months. Overall, the survival rate was excellent, with no recurrence, metastasis, or death.

Staining of SDHB and ATRX by IHC

All the cases expressed SDHB in the cytoplasm and expressed ATRX in the nuclear (Fig. 2D and E). Endothelial cells were used as a positive internal control.

TRS in cases 14 and 17

Two cases within 3 years (cases 14 and 17 in Table 1) were available for TRS with paired normal tissues. In case 14, HRAS mutation (exon 3, Q61R) was detected in PCC component, and concomitant BRAF mutation (exon 15, K601N) and HRAS mutation (exon 3, Q61R) mutations were identified in composite areas (due to the highly intermixing of GN component in case 14, only PCC component was separated). In case 17, HRAS mutation (exon 3, Q61L) was detected in PGL component, GN component, and composite component (Table 2). Normal paired tissue was negative for ATRX, BRAF, CDKN2A, DNMT3A, FH, H3F3A, HRAS, IDH1, MAX, MEN1, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TPS3, and VHL in both cases.

Mutation status of BRAF and HRAS in composite PCC/PGL-GN and comparison with literature data in ordinary PCC/PGL

Fifteen cases were available for Sanger sequencing (cases 1, 2, 5–9, 11–18 in Table 1). Cases 3, 4, and 10 were excluded due to the limited amount of tissue. The hotspot mutations of BRAF (V600E, K601N, K601E) and HRAS (Q61R, Q61L, G13R) were screened.

Three cases (20.0%) harbored BRAF mutation, and seven (46.7%) harbored HRAS mutation in CP (Figs 3 and 4). The TRS results of cases 14 and 17 were confirmed. For further clarification of the mutation status in each of the components, macroadissection was performed to separate PCC/PGL and GN in 12 of the 15 cases (Fig. 3; cases 5, 8, and 18 were not separated due to their highly intermingled growth pattern). For PCC/PGL component, five cases (5/11, 45.5%) harbored HRAS mutations and two cases (2/11, 18.2%) harbored BRAF mutations (case 1 failed for sequencing). For GN component, all the cases were BRAF wild-type (0/11), and two cases were HRAS-mutant (Fig. 3).

Notably, in cases 11 and 17, both PCC/PGL component and GN harbored HRAS mutations. However, the mutation...
Table 1  Clinicopathological features of 18 composite PCC/PGL-GN cases.

| No. | Age/gender | Symptoms                              | Location              | Tumor size (cm) | PCC/PGL (%) | GN (%) | Ki-67 (%) | TNM staging | Follow-up (months) |
|-----|------------|---------------------------------------|-----------------------|-----------------|-------------|---------|-----------|-------------|-------------------|
| 1   | 53/M       | Hypertension; tachycardia              | Adrenal               | 2.5             | 70          | 30      | <1        | T1N0M0 stage I   | NA                |
| 2   | 62/M       | Hypertension                           | Adrenal               | 14              | 90          | 10      | 1         | T2N0M0 stage II  | NA                |
| 3   | 43/M       | Tachycardia                            | Adrenal               | 4              | 20          | 80      | <1        | T1N0M0 stage I   | NA                |
| 4   | 28/F       | Hypertension; headache; tachycardia; sweating | Adrenal               | 4              | 90          | 10      | 1         | T1N0M0 stage I   | NA                |
| 5   | 53/F       | Hypertension                           | Adrenal               | 3.5             | 70          | 30      | <1        | T1N0M0 stage I   | NA                |
| 6   | 68/F       | Hypertension                           | Adrenal               | 5              | 70          | 30      | 2         | T2N0M0 stage II  | NA                |
| 7   | 36/F       | Hypertension                           | Adrenal               | 5              | 90          | 10      | <1        | T2N0M0 stage II  | 91                |
| 8   | 53/M       | Hypertension                           | Adrenal               | 6              | 85          | 15      | <1        | T2N0M0 stage II  | 71                |
| 9   | 50/F       | None                                   | Adrenal               | 4.1             | 75          | 25      | 5         | T1N0M0 stage I   | 69                |
| 10  | 49/F       | None                                   | Adrenal               | 0.9             | 60          | 40      | 2         | T1N0M0 stage I   | 65                |
| 11  | 54/F       | Hypertension                           | Adrenal               | 4.3             | 75          | 25      | 2         | T1N0M0 stage I   | NA                |
| 12  | 53/M       | Weight loss                            | Adrenal               | 4.5             | 45          | 55      | 2         | T1N0M0 stage I   | 52                |
| 13  | 57/F       | Hypertension                           | Adrenal               | 6              | 80          | 20      | 1         | T2N0M0 stage II  | NA                |
| 14  | 60/M       | None                                   | Adrenal               | 9              | 40          | 60      | 1         | T3N0M0 stage III | 31                |
| 15  | 23/M       | None                                   | Adrenal               | 5.5             | 20          | 80      | 1         | T2N0M0 stage II  | NA                |
| 16  | 60/F       | None                                   | Adrenal               | 2.7             | 55          | 45      | <1        | T1N0M0 stage I   | 13                |
| 17  | 55/M       | Sweating                               | Retroperitoneum       | 5              | 85          | 15      | <1        | T3N0M0 stage III | 17                |
| 18  | 54/F       | Hypertension                           | Urinary bladder       | 1.5             | 90          | 10      | 1         | T1N0M0 stage I   | 42                |

*Reference range: norepinephrine (16.69–40.65 μg/24 h); epinephrine (1.74–6.42 μg/24 h); dopamine (120.93–330.59 μg/24 h). Bold numbers are out of the reference range.
status between PCC/PGL and GN was not always the same. One case (case 14) harbored \textit{BRAF} mutation in PCC/PGL but not in GN. Two cases (cases 14 and 16) harbored \textit{HRAS} mutations in PCC/PGL but not in GN.

Since the \textit{BRAF} mutations identified were K601E/K601N rather than the most frequent mutation spot (V600E) which occurred in multiple neoplasms, IHC for \textit{BRAF} V600E mutation was performed. All the 18 cases were negative, which further confirmed the absence of V600E mutations in CP (Fig. 2F).

A review of the English literature indicated a much lower \textit{BRAF} and \textit{HRAS} mutation rate in ordinary PCC/PGL and no concomitant mutations were reported (4, 9, 13, 14, 15, 16, 17, 18, 19, 20, 21). For ordinary GN, it is not usually associated with genetic abnormalities, and only \textit{RET} gene has been considered to be causative in its pathogenesis (22). The mutation sites of \textit{HRAS} in our cases shared similarities with previously reported sites occurring in PCC/PGL, whereas for \textit{BRAF} gene codon 601 rather than codon 600 seemed to be a hotspot in CP (Table 3).

\section*{Discussion}

CP is a rare neoplasm that has mostly been reported as case reports with variable clinical presentations (23, 24, 25). Its pathogenesis remains to be a dilemma due to the lack of pathological and molecular studies. Regarding its pure tumor counterparts, a series of susceptible genes have been reported in ordinary PCC/PGL, some of which were associated with familial syndromes (26). Among them, \textit{SDH} is the most well-known mutated gene family, and \textit{ATRX} has also been reported to be mutated in 13% of cases (27, 28, 29). Concomitant \textit{ATRX} and \textit{SDHB} mutations might indicate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(A) CP showing PCC component (left) and GN component (right) with a clear fibrous margin (HE, ×40). (B) CP showing PGL component highly intermixed with the GN component (HE, ×40). (C) PGL component showing classical paraganglioma cells with abundant cytoplasm (HE, ×100). (D) GN component with ganglion cells distributed in Schwannian stroma (HE, ×100).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{(A) Paraganglioma cells were strongly positive for CgA (IHC, ×100). (B) Schwann cells and sustentacular cells were immunoreactive positive for S-100 (IHC, ×100). (C) CP showing a low Ki-67 index of <1% (IHC, ×100). (D) Granular cytoplasmic expression of SDHB (IHC, ×100). (E) Nuclear expression of ATRX (IHC, ×100). (F) Negative staining for \textit{BRAF} V600E mutation (IHC, ×100).}
\end{figure}
more aggressive behavior in PCC/PGL (3). In contrast, the mutation status of SDH and ATRX in CP is largely unknown, with only one case reporting ATRX as a driver mutation in metastatic CP, one case reporting the possible association between SDHB mutation and neuroblastoma susceptibility, and one case reporting a composite PGL-GN in the neck with SDHA mutation (30, 31, 32). Here, all of our composite PCC/PGL-GN cases show no loss of expression of SDHB or ATRX, which suggests the lack of SDH and ATRX mutations. A review of the English literature also reveals the rarity of SDH mutation in composite PCC/PGL-GN, which might indicate its uniqueness compared with its pure counterparts (8, 30, 32, 33).

Next-generation sequencing has rarely been performed in CP as in ordinary PCC/PGL. To further explore other possible changes in composite PCC/PGL-GN, we performed TRS and identified frequent BRAF and HRAS mutations. Based on the molecular classification of ordinary PCC/PGL, HRAS mutation has been considered to be a validated driver event, with mutations occurring exclusively in sporadic cases and restricted to codon 61 and rarely in codon 13 (13, 34). Our findings suggest that composite PCC/PGL-GN does share HRAS mutation pathway and hotspots with ordinary PCC/PGL. Notably, some key genes have significantly different mutation rates among different populations (4). A recent multi-center study has demonstrated a higher frequency of HRAS mutation in the Chinese population than in the European population (16.5% vs 9.8%) (4). The even higher mutation rate (46.7%) in our case series might be attributed to both Chinese population and the important role of HRAS mutation in the pathogenesis of composite PCC/PGL-GN. For BRAF, it has been an extremely rare mutation in neuroendocrine tumors, and only two cases have been reported in ordinary PCC/PGL (9, 17). Notably, all the affected site of BRAF in composite PCC/PGL-GN is codon 601 rather than codon 600, which indicates a possibly unique driver event. There is genetic heterogeneity within the tumor, which further explains the reason why the mutation status between the two components could be different.

Clinicopathologically, we first describe the different mixed patterns and variable percentages of the two components in detail. Notably, the WHO working group points out that the diagnosis of CP requires the complete histoarchitecture of the addition tumor type, and one of the clues is the stromal features, including bundles of spindle-shaped Schwann cells and axon-like processes. However, current definitions are still vague in terms of

| Case | PCC component (VAF) | Composite component (VAF) | PGL component (VAF) | GN component (VAF) | Composite component (VAF) |
|------|---------------------|--------------------------|---------------------|--------------------|--------------------------|
| SNV  | HRAS (exon 3, Q61R) (12.2%) | BRAF (exon 15, K601N) (8.1%); HRAS (exon 3, Q61R) (16.3%) | HRAS (exon 3, Q61L) (50.1%) | None | HRAS (exon 3, Q61L) (41.7%) |
| InDel | None | None | None | None | None |

VAF, variant allele fraction.
Table 3 Review of the literature regarding HRAS and BRAF mutations in ordinary PCC/PGL.

| Year | Mutation rate | Mutation sites | Mutation rate | Mutation sites | Ref |
|------|---------------|----------------|---------------|----------------|-----|
| 2004 | N/A           | –              | 0 (0/34)      | –              | (15) |
| 2013 | 6.9% (4/58)   | Q61R, Q61K, G13R | 0 (0/58)      | –              | (16) |
| 2014 | 5.2% (14/271) | Q61R, Q61K, G13R | N/A           | –              | (13) |
| 2015 | 7.1% (6/85)   | Q61R, G13R     | 1.2% (1/85)   | V600E          | (17) |
| 2016 | 7.1% (11/156) | Q61R, Q61K, Q61L, G13R | N/A           | –              | (18) |
| 2016 | N/A           | –              | 0 (0/110)     | –              | (19) |
| 2017 | N/A           | –              | 0 (0/64)      | –              | (14) |
| 2017 | 9.8% (17/173) | Q61R, Q61K, G13R, Q61L | 0.6% (1/173) | G469A          | (9)  |
| 2019 | 5.7% (13/227) | Q61R, Q61K, G13R, G12R | N/A           | –              | (20) |
| 2020 | 6.7% (2/30)   | Q61R, G12D     | N/A           | –              | (21) |
| 2020 | 16.5% (107/650) in Chinese; 9.8% (68/692) in European | Q61R, Q61K, G13R | N/A           | –              | (4)  |

N/A, not reported; Ref, reference.

how much of each component is required. Based on our findings that CP could be a unique neoplasm, we suggest that at least 10% of each element might be required for making the diagnosis, which might provide clues for pathologists. Importantly, we have to emphasize that 10% is only a possible suggestion. Larger cohorts and more comparisons with ordinary PCC/PGL are needed to better establish the diagnostic criteria. Currently, it is suggested that all patients diagnosed with CP require long-term follow-up since the clinical course is highly unpredictable. In general, for composite PCC/PGL-GN, the prognosis has been promising based on previous publications, with extreme rare metastasis or death (Supplementary Table 2) (1, 2, 8, 23, 24, 25, 32, 35, 36, 37, 38, 39, 40, 41, 42, 43). However, careful follow-up is still recommended, and predictors for prognosis are waiting to be explored.

In summary, to the best of our knowledge, the present study is the largest series. We reveal the frequent BRAF

Figure 4

(A) The BRAF K601E (c.1801A>G) mutation in PCC and composite components (case 2) and representative case with wild type BRAF (case 9). (B) The HRAS Q61R (c. 182A>G) mutation in PCC and composite components (case 7) and representative case with wild type HRAS (case 9).
and HRAS mutations rather than SDH or ATRX mutations in composite PCC/PGL-GN, which might indicate its unique pathogenesis and provide potential targets for further treatment, especially for cases with metastasis and malignant potential in the literature (35). We also manage to separate PCC/PGL component and GN component for the first time and explore their relationships.

Several limitations are of concern: first, due to the limited amount of normal paired tissue and the extreme rarity of this entity, the number of cases for next-generation sequencing is small and could only represent part of the pathogenesis of composite PCC/PGL-GN. For instance, a recent case reports a MAX mutation in multiple and composite neuroendocrine-neuroblastic neoplasms (33). Besides, CP with other neurogenic components is not diagnosed in our study and needs more investigation. Second, in ordinary PCC/PGL, a remarkable percentage of apparently sporadic cases are carriers of germline mutations (44, 45). This point should also be kept in mind when studying CP. However, not all our cases are available for next-generation sequencing. Therefore, we did not focus on these germline changes. Thirdly, the amount of each component, which might have an impact on clinicopathological features, is not taken into consideration. Nevertheless, our research still provides important clues for the pathogenesis of composite PCC/PGL-GN. Future studies are required for deeper investigation into this rare neoplasm.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0300.

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

Funding
This work was supported by the Foundation of Pathologic Research Centre of the China Academy of Medical Sciences (No. 2016ZX310176-3) and CAMS Innovation Fund for Medical Sciences (CIFMS) (No. 2017-I2M-1-001).

References
1. Dhanasekar K, Visakan V, Tahir F & Balasubramanian SP. Composite phaeochromocytoma—a systematic review of published literature. Langenbecks Archives of Surgery 2021 [epub]. (https://doi.org/10.1007/s00423-021-02129-5)
2. Comstock JM, Willmore-Payne C, Holden JA & Coffin CM. Composite pheochromocytoma: a clinicopathologic and molecular comparison with ordinary pheochromocytoma and neuroblastoma. American Journal of Clinical Pathology 2009 132 69–73. (https://doi.org/10.1309/AJCPN76VTGWOPOAG)
3. Alrekz R, Suarez A, Ten a I & Pacak K. Update of pheochromocytoma syndromes: genetics, biochemistry, evaluation, and imaging. Frontiers in Endocrinology 2018 9 515. (https://doi.org/10.3389/fendo.2018.00515)
4. Jiang J, Zhang J, Pang Y, Bechmann Ni, Li M, Monteagudo M, Calzina B, Gimenez-Roqueplo AP, Nolting S, Besuschtein E, et al. Sino-European differences in the genetic landscape and clinical presentation of pheochromocytoma and paraganglioma. Journal of Clinical Endocrinology and Metabolism 2020 105 dgaa502. (https://doi.org/10.1210/clinem/dgaa502)
5. Kontrorovich V, King KS & Pacak K. SDH-related pheochromocytoma and paraganglioma. Best Practice and Research: Clinical Endocrinology and Metabolism 2010 24 415–424. (https://doi.org/10.1016/j.beem.2010.04.001)
6. Job S, Draskovic I, Burnichon N, Buffet A, Cros J, Lepine C, Venisse A, Robidel E, Verkarre V, Meachti T, et al. Telomerase activation and ATRX mutations are independent risk factors for metastatic pheochromocytoma and paraganglioma. Clinical Cancer Research 2019 25 760–770. (https://doi.org/10.1158/1078-0432.CCR-18-0139)
7. Turchini J & Gill AJ. Morphologic clues to succinate dehydrogenase (SDH) deficiency in pheochromocytomas and paragangliomas. American Journal of Surgical Pathology 2020 44 422–424. (https://doi.org/10.1097/PAS.0000000000001415)
8. Gupta S, Zhang J & Erickson LA. Composite pheochromocytoma/paraganglioma-ganglioneuroma: a clinicopathologic study of eight cases with analysis of succinate dehydrogenase. Endocrine Pathology 2017 28 269–275. (https://doi.org/10.1007/s12022-017-9494-3)
9. Fishbein L, Leshchiner I, Walter V, Danilova L, Robertson AG, Johnson AR, Lichtenberg TM, Murray BA, Ghayee HK, Else T, et al. Comprehensive molecular characterization of pheochromocytoma and paraganglioma. Cancer Cell 2017 31 181–193. (https://doi.org/10.1016/j.ccell.2017.01.001)
10. Buffet A, Burnichon N, Favier J & Gimenez-Roqueplo AP. An overview of individual RAS mutations in cancer biology. Frontiers in Oncology 2019 9 1088. (https://doi.org/10.3389/fonc.2019.01088)
11. Cohen R, Cervera P, Svrcek M, Pellat A, Dreyer C, de Gramont A & Andre T. BRAF-mutated colorectal cancer: what is the optimal strategy for treatment? Current Treatment Options in Oncology 2017 18 9. (https://doi.org/10.1007/s11864-017-0453-5)
12. Oudijk L, de Kruijer BR, Rapa I, Besuschtein E, de Cubas AA, Dei Tos AP, Dinjens WN, Korpershoek E, Mancikova V, Mannelli M, et al. H-RAS mutations are restricted to sporadic pheochromocytomas lacking specific clinical or pathological features: data from a multi-institutional series. Journal of Clinical Endocrinology and Metabolism 2014 99 E1376–E1380. (https://doi.org/10.1210/jc.2013-3879)
13. Vosecka T, Vich a A, Zelinka T, Jencova P, Pacak K, Duskova J, Benes J, Guha A, Stanek L, Kohonoutova M, et al. Absence of BRAF mutation in pheochromocytoma and paraganglioma. Neoplasma 2017 64 278–282. (https://doi.org/10.14499/neop.2017.215)
14. Perren A, Schmid S, Locher T, Sarembslanl P, Bonvina C, Heitz PU & Korminnoth P. BRAF and endocrine tumors: mutations are frequent in papillary thyroid carcinomas, rare in endocrine tumors of the gastrointestinal tract and not detected in other endocrine tumors. Endocrine-Related Cancer 2004 11 855–860. (https://doi.org/10.1677/erc.1.00841)
15. Crona J, Delgado Verdugo A, Maharjan R, Stalberg P, Granberg D, Hellman P & Bjorklund P. Somatic mutations in H-RAS in sporadic pheochromocytoma and paraganglioma identified by exome sequencing. Journal of Clinical Endocrinology and Metabolism 2013 98 E1266–E1271. (https://doi.org/10.1210/jc.2012-4257)
16. Luchetti A, Walsh D, Rodger I, Clark G, Martin T, Irving R, Sanna M, Yao M, Robled M, Neumann HF, et al. Profiling of somatic mutations in pheochromocytoma and paraganglioma by targeted next
gene deletion associated with a composite paraganglioma/ganglioneuroma. Journal of Medical Genetics 2009 46 215–216. (https://doi.org/10.1136/jmg.2008.060749)

32 Delgado S, Smith SM, Mehra S & Prasad ML. Composite paraganglioma: pioneering in the head and neck. International Journal of Surgical Pathology 2019 27 282–289. (https://doi.org/10.1056/NEJMra1806696)

33 Pozza C, Sesti F, Di Dato C, Stareda E, Pofi R, Schiavi F, Bonifacio V, Isidori AM, Faggioni A, Lenzi A, et al. A novel MAX gene mutation variant in a patient with multiple and “composite” neuroendocrine–neuroblastic tumors. Frontiers in Endocrinology 2020 11 234. (https://doi.org/10.3389/fendo.2020.00234)

34 Crona J, Taieb D & Pacak K. New perspectives on pheochromocytoma and paraganglioma: toward a molecular classification. Endocrine Reviews 2017 38 489–515. (https://doi.org/10.1210/er.2017-00062)

35 Lam KY & Lo CY. Composite pheochromocytoma-paraganglioma of the adrenal gland: an uncommon entity with distinctive clinicopathologic features. Endocrine Pathology 1999 10 343–352. (https://doi.org/10.1007/BF02739777)

36 Chen CH, Boag AH, Belko DT, Siemens DR, Froese A & Isotalo PA. Composite paraganglioma-ganglioneuroma of the urinary bladder: a rare neoplasm causing hemodynamic crisis at tumour resection. Canadian Urological Association Journal 2009 3 E45–E48. (https://doi.org/10.5489/cujai.1160)

37 Usuda H & Emura I. Composite paraganglioma-ganglioneuroma of the bladder. Urology. 2005 65 596–600. (https://doi.org/10.1111/j.1440-1827.2005.03875.x)

38 Dundra P, Dudorkinova D, Povysil C, Pesl M, Babjuk M, Dvoracek J & Zelinka T. Pigmented composite paraganglioma-ganglioneuroma of the urinary bladder. Pathology, Research and Practice 2003 199 765–769. (https://doi.org/10.1016/S0344-0338-0498)

39 Menon S, Mahajan P & Desai SB. Composite adrenal medullary tumor: a rare cause of hypertension in a young male. Urology. Annals 2011 3 36–38. (https://doi.org/10.4103/0974-7796.75860)

40 Choi EK, Kim WH & Park KY. A case of a composite adrenal medullary tumor of pheochromocytoma and ganglioneuroma masquerading as acute pancreatitis. Korean Journal of Internal Medicine 2006 21 141–145. (https://doi.org/10.3904/kjim.2006.21.2.141)

41 Shankar GM, Chen L, Kim AH, Ross GL, Folkerth RD, Spiotto MT, Friedlander RM. Composite ganglioneuroma-paraganglioma of the cervical spine. Journal of Neurosurgery: Spine 2010 12 709–713. (https://doi.org/10.3171/2009.12.SPINE09482)

42 Shida Y, Iwaga T, Abe K, Hakariya T, Takehara K, Onita T & Sakai H. Composite paraganglioma of the adrenal gland: a case series. BMC Research Notes 2015 8 257. (https://doi.org/10.1186/s13104-015-1233-6)

43 Khan AN, Solomon SS & Childress RD. Composite pheochromocytoma-ganglioneuroma: a rare experiment of nature. Endocrine Practice 2010 16 291–299. (https://doi.org/10.4158/EP109025.RA)

44 Brito JP, Asín N, Bancos I, Goniñorriño MD, Zeballos-Palacios CL, Leppin AL, Undavalli C, Wang Z, Domecq JJ, Prutsakis G, et al. Testing for germline mutations in sporadic pheochromocytoma/paraganglioma: a systematic review. Clinical Endocrinology 2015 82 338–345. (https://doi.org/10.1111/cen.12530)

45 Patocs A, Lendvai NK, Rutzi H, Liko I, Sapi Z, Szuc N, Toth G, Grołmusz VK, Igaz P, Toth M, et al. Novel SDHB and TMEM127 mutations in patients with pheochromocytoma/paraganglioma syndrome. Pathology Oncology Research 2016 22 673–679. (https://doi.org/10.1007/s12253-016-0050-0)