RESEARCH

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

Jingci Chen[1,*], Yan Wu[1,*], Pengyan Wang[1,*], Huanwen Wu[1], Anli Tong[2] and **Xiaoyan Chang[1]**

[1]Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
[2]Department 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.

Key Words
- composite
- pheochromocytoma
- paraganglioma
- *BRAF*
- *HRAS*

Endocrine Connections (2021) **10**, 926–934

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*

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

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 macrodissection. 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*, *TP53*, 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 Mg^{2+}, 0.2 mM of each

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

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* (exon 15, K601N) and *HRAS* (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*, *TP53*, 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, macrodissection 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

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

Table 1 Clinicopathological features of 18 composite PCC/PGL-GN cases.

No.	Age/gender	Symptoms	24-h uterine test[a] (norepinephrine; epinephrine; dopamine)	Location	Tumor size (cm)	PCC/PGL (%)	GN (%)	Ki-67 (%)	TNM staging	Follow-up (months)
1	53/M	Hypertension; tachycardia	29.37; **6.80**; **403.78**	Adrenal	2.5	70	30	<1	T1N0M0 stage I	NA
2	62/M	Hypertension	32.5; **6.63**; 294.92	Adrenal	14	90	10	1	T2N0M0 stage II	NA
3	43/M	Tachycardia	15.69; 4.08; 174.69	Adrenal	4	20	80	<1	T1N0M0 stage I	NA
4	28/F	Hypertension; headache; tachycardia; sweating	**147.96**; **7.12**; 97.98	Adrenal	4	90	10	1	T1N0M0 stage I	NA
5	53/F	Hypertension	**176.59**; **9.26**; **474.55**	Adrenal	3.5	70	30	<1	T1N0M0 stage I	NA
6	68/F	Hypertension	**50.31**; **7.51**; 314.61	Adrenal	5	70	30	2	T2N0M0 stage II	NA
7	36/F	Hypertension	**138.86**; 3.86; **401.14**	Adrenal	5	90	10	<1	T2N0M0 stage II	91
8	53/M	Hypertension	**59.14**; 2.961; 141.96	Adrenal	6	85	15	<1	T2N0M0 stage II	71
9	50/F	None	25.92; 4.32; **976.32**	Adrenal	4.1	75	25	5	T1N0M0 stage I	69
10	49/F	None	Unknown	Adrenal	0.9	60	40	2	T1N0M0 stage I	65
11	54/F	Hypertension	**200.07**; **33.48**; 264.87	Adrenal	4.3	75	25	2	T1N0M0 stage I	NA
12	53/M	Weight loss	**107.89**; 1.91; 152.65	Adrenal	4.5	45	55	2	T1N0M0 stage I	52
13	57/F	Hypertension	38.08; 1.90; **619.92**	Adrenal	6	80	20	1	T2N0M0 stage II	NA
14	60/M	None	20.45; 4.09; **1886.12**	Adrenal	9	40	60	1	T3N0M0 stage III	31
15	23/M	None	22.25; 2.97; 254.02	Adrenal	5.5	20	80	1	T2N0M0 stage II	NA
16	60/F	None	33.96; 2.55; 189.47	Adrenal	2.7	55	45	<1	T1N0M0 stage I	13
17	55/M	Sweating	**61.05**; **9.46**; **336.98**	Retroperitoneum	5	85	15	<1	T3N0M0 stage III	17
18	54/F	Hypertension	30.89; 4.03; 214.88	Urinary bladder	1.5	90	10	1	T1N0M0 stage I	42

[a]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.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

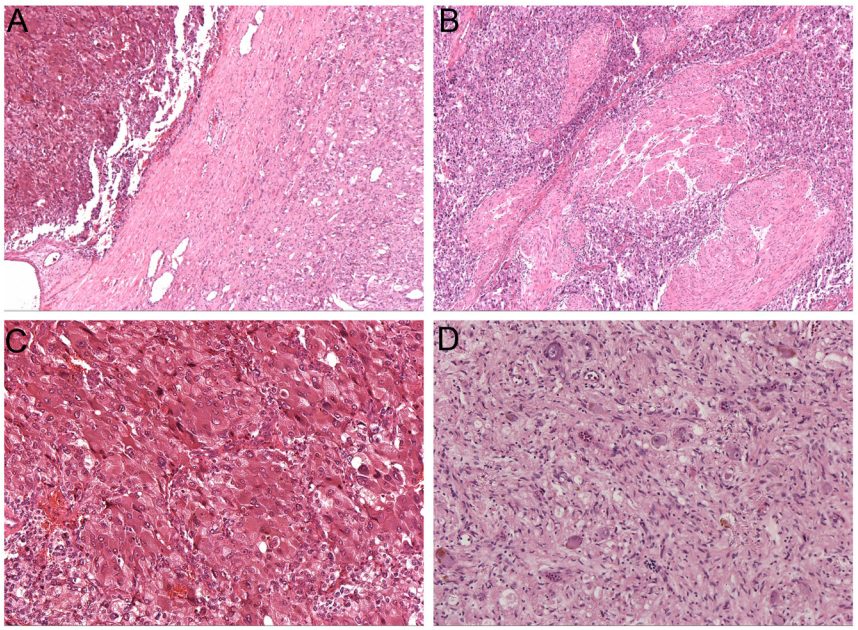

Figure 1
(A) CP showing PCC component (left) and GN component (right) with a clear fibrous margin (HE, ×40). (B) CP showing PGL component highly intermixed with the GN component (HE, ×40). (C) PGL component showing classical paraganglioma cells with abundant cytoplasm (HE, ×100). (D) GN component with ganglion cells distributed in Schwannian stroma (HE, ×100).

status between PCC/PGL and GN was not always the same. One case (case 14) harbored *BRAF* mutation in PCC/PGL but not in GN. Two cases (cases 14 and 16) harbored *HRAS* mutations in PCC/PGL but not in GN.

Since the *BRAF* mutations identified were K601E/K601N rather than the most frequent mutation spot (V600E) which occurred in multiple neoplasms, IHC for *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 *BRAF* and *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 *RET* gene has been considered to be causative in its pathogenesis (22). The mutation sites of *HRAS* in our cases shared similarities with previously reported sites occurring in PCC/PGL, whereas for *BRAF* gene codon 601 rather than codon 600 seemed to be a hotspot in CP (Table 3).

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, *SDH* is the most well-known mutated gene family, and *ATRX* has also been reported to be mutated in 13% of cases (27, 28, 29). Concomitant *ATRX* and *SDHB* mutations might indicate

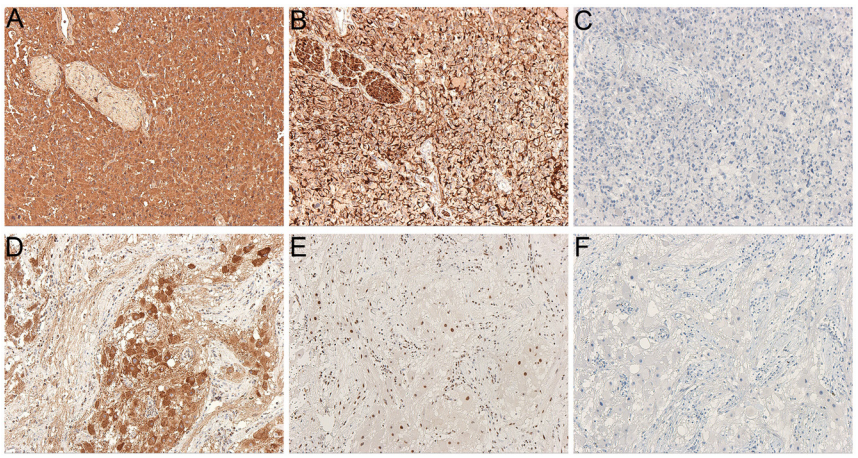

Figure 2
(A) Paraganglioma cells were strongly positive for CgA (IHC, ×100). (B) Schwann cells and sustentacular cells were immunoreactive positive for S-100 (IHC, ×100). (C) CP showing a low Ki-67 index of <1% (IHC, ×100). (D) Granular cytoplasmic expression of SDHB (IHC, ×100). (E) Nuclear expression of ATRX (IHC, ×100). (F) Negative staining for *BRAF* V600E mutation (IHC, ×100).

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

Table 2 TRS of cases 14 and 17.

	Case 14		Case 17		
	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%)	*HRAS* (exon 3, Q61L) (10.0%)	*HRAS* (exon 3, Q61L) (41.7%)
InDel	None	None	None	None	None

VAF, variant allele fraction.

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

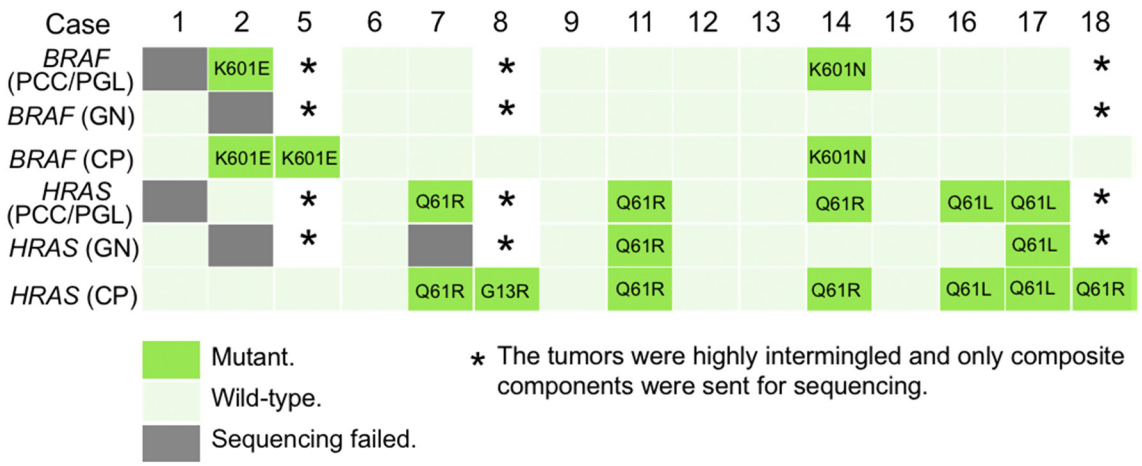

Figure 3
Sanger sequencing of 15 composite PCC/PGL-GN cases.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

Table 3 Review of the literature regarding *HRAS* and *BRAF* mutations in ordinary PCC/PGL.

Year	*HRAS*		*BRAF*		Ref
	Mutation rate	Mutation sites	Mutation rate	Mutation sites	
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*

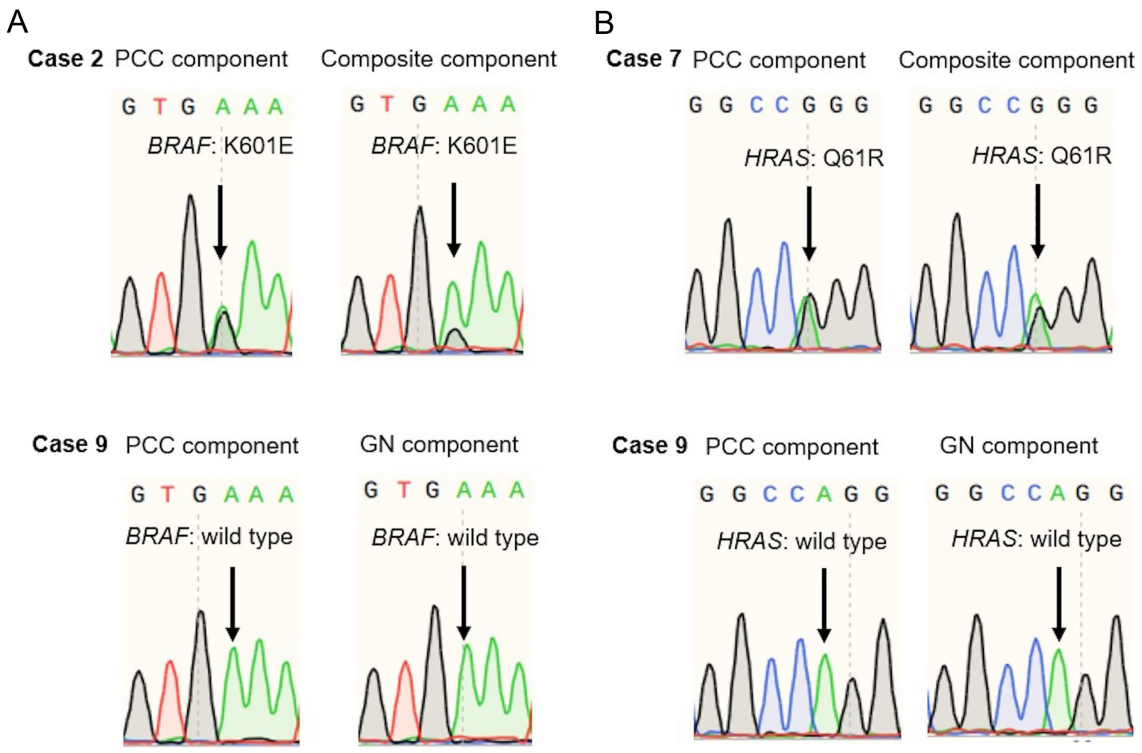

Figure 4
(A) The *BRAF* K601E (c.1801A>G) mutation in PCC and composite components (case 2) and representative case with wild type *BRAF* (case 9). (B) The *HRAS* Q61R (c. 182A>G) mutation in PCC and composite components (case 7) and representative case with wild type *HRAS* (case 9).

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-21-0300

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. Secondly, 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 phaeochromocytomas-a systematic review of published literature. *Langenbeck's 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/AJCPN76VTIGWPOAG)

3 Alrezk R, Suarez A, Tena I & Pacak K. Update of pheochromocytoma syndromes: genetics, biochemical 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 N, Li M, Monteagudo M, Calsina B, Gimenez-Roqueplo AP, Nolting S, Beuschlein F, *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 Kantorovich 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, Meatchi 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 20 years of genetic studies in pheochromocytoma and paraganglioma. *Best Practice and Research: Clinical Endocrinology and Metabolism* 2020 **34** 101416. (https://doi.org/10.1016/j.beem.2020.101416)

11 Munoz-Maldonado C, Zimmer Y & Medova M. A comparative analysis of individual RAS mutations in cancer biology. *Frontiers in Oncology* 2019 **9** 1088. (https://doi.org/10.3389/fonc.2019.01088)

12 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)

13 Oudijk L, de Krijger RR, Rapa I, Beuschlein F, 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)

14 Vosecka T, Vicha A, Zelinka T, Jencova P, Pacak K, Duskova J, Benes J, Guha A, Stanek L, Kohoutova M, *et al.* Absence of BRAF mutation in pheochromocytoma and paraganglioma. *Neoplasma* 2017 **64** 278–282. (https://doi.org/10.4149/neo_2017_215)

15 Perren A, Schmid S, Locher T, Saremaslani P, Bonvin C, Heitz PU & Komminoth 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)

16 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)

17 Luchetti A, Walsh D, Rodger F, Clark G, Martin T, Irving R, Sanna M, Yao M, Robledo M, Neumann HP, *et al.* Profiling of somatic mutations in phaeochromocytoma and paraganglioma by targeted next

generation sequencing analysis. *International Journal of Endocrinology* 2015 **2015** 138573. (https://doi.org/10.1155/2015/138573)
18 Stenman A, Welander J, Gustavsson I, Brunaud L, Backdahl M, Soderkvist P, Gimm O, Juhlin CC & Larsson C. HRAS mutation prevalence and associated expression patterns in pheochromocytoma. *Genes, Chromosomes and Cancer* 2016 **55** 452–459. (https://doi.org/10.1002/gcc.22347)
19 Paulsson JO, Svahn F, Welander J, Brunaud L, Soderkvist P, Gimm O, Stenman A & Juhlin CC. Absence of the BRAF V600E mutation in pheochromocytoma. *Journal of Endocrinological Investigation* 2016 **39** 715–716. (https://doi.org/10.1007/s40618-015-0420-6)
20 Ben Aim L, Pigny P, Castro-Vega LJ, Buffet A, Amar L, Bertherat J, Drui D, Guilhem I, Baudin E, Lussey-Lepoutre C, *et al.* Targeted next-generation sequencing detects rare genetic events in pheochromocytoma and paraganglioma. *Journal of Medical Genetics* 2019 **56** 513–520. (https://doi.org/10.1136/jmedgenet-2018-105714)
21 Tomic TT, Olausson J, Rehammar A, Deland L, Muth A, Ejeskar K, Nilsson S, Kristiansson E, Wassen ON & Abel F. MYO5B mutations in pheochromocytoma/paraganglioma promote cancer progression. *PLoS Genetics* 2020 **16** e1008803. (https://doi.org/10.1371/journal.pgen.1008803)
22 Takaya K, Yoshimasa T, Arai H, Tamura N, Miyamoto Y, Itoh H & Nakao K. Expression of the RET proto-oncogene in normal human tissues, pheochromocytomas, and other tumors of neural crest origin. *Journal of Molecular Medicine* 1996 **74** 617–621. (https://doi.org/10.1007/s001090050065)
23 Hu J, Wu J, Cai L, Jiang L, Lang Z, Qu G, Liu H, Yao W & Yu G. Retroperitoneal composite pheochromocytoma-ganglioneuroma: a case report and review of literature. *Diagnostic Pathology* 2013 **8** 63–63. (https://doi.org/10.1186/1746-1596-8-63)
24 Hirasaki S, Kanzaki H, Okuda M, Suzuki S, Fukuhara T & Hanaoka T. Composite paraganglioma-ganglioneuroma in the retroperitoneum. *World Journal of Surgical Oncology* 2009 **7** 81. (https://doi.org/10.1186/1477-7819-7-81)
25 Ohtsuki Y, Watanabe R, Okada Y, Matsuka Y, Lee GH & Furihata M. Composite paraganglioma and ganglioneuroma in the retroperitoneum: a case report. *Medical Molecular Morphology* 2012 **45** 168–172. (https://doi.org/10.1007/s00795-011-0567-y)
26 Neumann HPH, Young Jr WF & Eng C. Pheochromocytoma and paraganglioma. *New England Journal of Medicine* 2019 **381** 552–565. (https://doi.org/10.1056/NEJMra1806651)
27 Irwin T, Konnick EQ & Tretiakova MS. Malignant intrarenal/renal pelvis paraganglioma with co-occurring SDHB and ATRX mutations. *Endocrine Pathology* 2019 **30** 270–275. (https://doi.org/10.1007/s12022-019-09594-1)
28 Fishbein L, Khare S, Wubbenhorst B, DeSloover D, D'Andrea K, Merrill S, Cho NW, Greenberg RA, Else T, Montone K, *et al.* Whole-exome sequencing identifies somatic ATRX mutations in pheochromocytomas and paragangliomas. *Nature Communications* 2015 **6** 6140. (https://doi.org/10.1038/ncomms7140)
29 Mercado-Asis LB, Wolf KI, Jochmanova I & Taieb D. Pheochromocytoma: a genetic and diagnostic update. *Endocrine Practice* 2018 **24** 78–90. (https://doi.org/10.4158/EP-2017-0057)
30 Comino-Mendez I, Tejera ÁM, Curras-Freixes M, Remacha L, Gonzalvo P, Tonda R, Leton R, Blasco MA, Robledo M & Cascon A. ATRX driver mutation in a composite malignant pheochromocytoma. *Cancer Genetics* 2016 **209** 272–277. (https://doi.org/10.1016/j.cancergen.2016.04.058)
31 Armstrong R, Greenhalgh KL, Rattenberry E, Judd B, Shukla R, Losty PD & Maher ER. Succinate dehydrogenase subunit B (SDHB) gene deletion associated with a composite paraganglioma/neuroblastoma. *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/10.1177/1066896918799940)
33 Pozza C, Sesti F, Di Dato C, Sbardella E, Pofi R, Schiavi F, Bonifacio V, Isidori AM, Faggiano 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-ganglioneuroma 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, Beiko 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/cuaj.1160)
37 Usuda H & Emura I. Composite paraganglioma-ganglioneuroma of the urinary bladder. *Pathology International* 2005 **55** 596–601. (https://doi.org/10.1111/j.1440-1827.2005.01875.x)
38 Dundr 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/10.1078/0344-0338-00495)
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 & Friedlander RM. Composite ganglioneuroma-paraganglioma of the filum terminale. *Journal of Neurosurgery: Spine* 2010 **12** 709–713. (https://doi.org/10.3171/2009.12.SPINE09482)
42 Shida Y, Igawa T, Abe K, Hakariya T, Takehara K, Onita T & Sakai H. Composite pheochromocytoma 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/EP09205.RA)
44 Brito JP, Asi N, Bancos I, Gionfriddo MR, Zeballos-Palacios CL, Leppin AL, Undavalli C, Wang Z, Domecq JP, Prustsky 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, Butz H, Liko I, Sapi Z, Szucs N, Toth G, Grolmusz 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)

Received in final form 17 June 2021
Accepted 14 July 2021
Accepted Manuscript published online 14 July 2021