Whole Exome Sequencing Identifies Novel Genetic Alterations in Patients with Pheochromocytoma/Paraganglioma

Soo Hyun Seo1,*, Jung Hee Kim2,*, Man Jin Kim3, Sung Im Cho3, Su Jin Kim4, Hyein Kang4, Chan Soo Shin2, Sung Sup Park1,5, Kyu Eun Lee4,5, Moon-Woo Seong3,5

1Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam; Departments of 2Internal Medicine, 3Laboratory Medicine, 4Surgery, Seoul National University Hospital, Seoul National University College of Medicine; 5Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea

Background: Pheochromocytoma and paragangliomas (PPGL) are known as tumors with the highest level of heritability, approximately 30% of all cases. Clinical practice guidelines of PPGL recommend genetic testing for germline variants in all patients. In this study, we used whole exome sequencing to identify novel causative variants associated with PPGL to improve the detection of rare genetic variants in our cohort.

Methods: Thirty-six tested negative for pathogenic variants in previous Sanger sequencing or targeted gene panel testing for PPGL underwent whole exome sequencing. Whole exome sequencing was performed using DNA samples enriched using TruSeq Custom Enrichment Kit and sequenced with MiSeq (Illumina Inc.). Sequencing alignment and variant calling were performed using SAMtools.

Results: Among previously mutation undetected 36 patients, two likely pathogenic variants and 13 variants of uncertain significance (VUS) were detected in 32 pheochromocytoma-related genes. SDHA c.778G>A (p.Gly260Arg) was detected in a patient with head and neck paraganglioma, and KIF1B c.2787-2A>C in a patient with a bladder paraganglioma. Additionally, a likely pathogenic variant in BRCA2, VUS in TP53, and VUS in NFU1 were detected.

Conclusion: Exome sequencing further identified genetic alterations by 5.6% in previously mutation undetected patients in PPGL. Implementation of targeted gene sequencing consisted of extended genes of PPGL in routine clinical screening can support the level of comprehensive patient assessment.

Keywords: Pheochromocytoma; Paraganglioma; Whole exome sequencing; Germ-line mutation; Molecular diagnostic techniques
INTRODUCTION

Pheochromocytoma and paragangliomas (PPGL) are the most heritable tumors, with around 30% of cases caused by pathogenic variants. More than 15 germline and 30 somatic variants of causative genes have been associated with the disease, demonstrating a high degree of heterogeneity [1-7].

Molecular PPGL subtypes can be classified into three groups according to the Cancer Genome Atlas [2]. One major cluster is the pseudohypoxic group, which includes SDHx (SDHA, SDHB, SDHC, SDHD), SDHAF2, FH, MDH2, IDH1, VHL, EPAS1, and PHD1/2, with somatic and germline variants. Another cluster is the kinase signaling group, consisting of germline or somatic variants in RET, NF1, HRAS, MAX, and TMEM127. The third cluster is Wnt signaling group, which includes newly recognized somatic variants in CSDE1 as well as somatic gene fusions affecting MAML3.

Among those genes, RET, NF1, and VHL are involved in three distinct clinical syndromes associated with PPGL: multiple endocrine neoplasia type 2 ( MEN2 ) syndrome caused by RET, neurofibromatosis type I caused by NF1, and von Hippel-Lindau disease caused by VHL. Aside from those three syndromes, germline variants in the succinate dehydrogenase (SDH) genes are the most common cause of PPGL, occurring in up to 25% of all PPGL patients [8]. Clinical practice guidelines of PPGL recommend testing for germline variants in all patients with a family history of PPGL [9,10]. Clinical characteristics of PPGL can be classified according to genetic clusters, which can lead to different follow-up tests and treatments guided by the underlying molecular cause. However, some patients may not express causative genes included in the molecular subtypes, thus remaining unclassified.

In this study, whole exome sequencing (WES) was used to screen for novel causative variants associated with PPGL to improve the detection rate of rare genetic variants in our cohort. Additional screening for variants in other genes related to cancerous disease or mitochondrial function was also performed.

METHODS

Subjects

Among patients diagnosed with PPGLs at the Seoul National University Hospital, 36 were recruited due to high risk of genetic diseases: metastasis (n=9), bilateral diseases (n=2), paraganglioma (n=16), aged under 35 years (n=9). Among them, 20 patients were negative for SDHB, SDHD, VHL, and RET genes using Sanger sequencing and multiplex ligation-dependent probe amplification before March 2014. In March 2014, the targeted next-generation sequencing panel for PPGL was developed and used to test the additional 16 patients. All 16 patients were negative for MAX, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, and VHL. This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. 2004-012-1115). Informed consent from all patients was obtained.

PPGL was diagnosed by elevated levels of catecholamine and/or histological confirmation after surgery. We conducted serum fractionated metanephrine or 24-hour urine catecholamine/fractionated metanephrine. Hormone type was classified as epinephrine, norepinephrine, or nonfunctioning [11]. Elevated metanephrine with or without high normetanephrine levels was designated as epinephrine type. Elevated normetanephrine levels without high metanephrine levels were considered as norepinephrine type. Nonfunctioning type indicated a normal range of fractionated metanephrine levels.

Thoraco-abdominal computed tomography (CT) or magnetic resonance imaging was performed for anatomical localization. 123I-metaiodobenzylguanidine (MiBG), positron emission tomography/CT with 68Ga-labeled DOTANOC or 18F-labeled fluorodeoxyglucose (FDG) was conducted to detect multifocal lesions or metastasis. Metastasis was defined as the presence of PPGL tumors in non-chromaffin organs at diagnosis or during follow-up [12].

Molecular genetic testing

DNA was extracted from whole blood samples obtained from 36 patients. WES was performed using DNA samples enriched using TruSeq Custom Enrichment Kit and sequenced with MiSeq (Illumina Inc., San Diego, CA, USA). Sequencing alignment and variant calling were performed using SAMtools. Copy number variation (CNV) analysis for the genes included in the panel was not performed.

Variant filtering and interpretation of clinical significance

Exonic variants with nonsynonymous variants and intronic variants within 10 bp from the exonic region were included. Allele frequencies in normal controls (gnomeAD) and in silico prediction results were considered (SIFT, PolyPhen2, and Mutation-Taster). The highest minor allele frequency (MAF) in the patient population was taken into consideration, and variants that had MAF >0.1% were filtered out. Classification of each retained variant was performed according to the American College of
Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) 2015 guidelines [13]. For previously reported variants, segregation and functional test results were reviewed. Variants were screened for the 32 pheochromocytoma-related genes (ATRX, BRAF, CDKN2A, DLST, DNMT3A, EGLN1, EGLN2, EPAS1, FGFR1, FH, GOT2, H3F3A, HRAS, IDH1, IDH2, IDH3B, KIF1B, KMT2D, MAX, MDH2, MERTK, MET, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SLC25A11, TMEM127, and VHL). Genes related to other types of cancerous disease or encoding for mitochondria-localized proteins were screened for additional variants.

RESULTS

Patients’ characteristics
Among the 36 patients included in this study, 19 were female and 17 were male. The mean age at the time of diagnosis was 40.2 (range, 12 to 85) (Table 1). Seventeen patients were diagnosed with pheochromocytoma, four bilateral and two multifocal, while 19 patients were diagnosed with paraganglioma. None of the patients had a family history of pheochromocytoma or paraganglioma. Nine patients presented metastatic lesions, and three patients showed relapse during the follow-up period.

Identification of germline variants in pheochromocytoma-related genes
Average coverage depth in target regions of the whole exome panel was 80.7X; 99.1% of the bases had coverage of ≥20X, which was the minimal level of acceptable coverage considered. Among 36 patients, 14 patients were found to carry at least one variant of interest (VOI) in 32 pheochromocytoma-related genes. A total of 15 VOIs were detected, two were classified as likely pathogenic variants and 13 as variants of uncertain significance (VUS) (Table 2). SDHA c.778G>A (p.Gly260Arg) was detected in a patient negative for SDHB, SDHD, VHL, and RET genes. This was a previously reported variant in paraganglioma [14-16], known to be a loss-of-function variant according to functional studies [15]. KIF1B c.2787-2A>C, a likely pathogenic variant that had not been previously reported, was detected in a patient with a bladder paraganglioma. Other variants detected in pheochromocytoma-related genes lacked strong supporting evidence for pathogenic classification. SDHC c.478G>A (p.Val160Met) has not been reported previously, but other missense variants near this amino acid residue such as p. Leu158Pro and p. Leu161Val had been detected in PPGL patients [17-19]. FH c.418G>C (p.Val140Gly) involving the same amino acid residue has been reported in leiomyomatosis and renal cell cancer patients [20]. In addition, a novel nonsense variant, c.914G>A (p.Trp305*) in DNMT3A, showed variant allele frequency of 18% in exome sequencing data, and subsequent validation by Sanger sequencing showed a small alternate peak in the region (Supplemental Fig. S1).

Identification of germline variants in other genes
Also, we screened for germline variants in other cancer-related genes or mitochondria-related genes. One likely pathogenic variant in BRCA2 and one VUS in TP53 were detected in cancer-related genes (Table 2). A BRCA2 splice-site variant, c.8488-1G>A, was detected in a 25-year-old male patient with early-onset paraganglioma. He had no personal cancer history, nor a family history of cancer related to BRCA2. A patient with VUS in TP53, c.566C>T (p.Ala189Val), had previously been diagnosed with breast cancer, endometrial polyp and also had a brother who had been diagnosed with choriocarcinoma. Additionally, we found a missense VUS c.473G>A in NFU1, which is a causative gene of multiple mitochondrial dysfunctions syndrome 1 (MMD1).

DISCUSSION

Among the 36 patients found to be negative for routine clinical gene testing, only two were found to be positive for likely pathogenic variants (2/36=5.6%). SDHA c.778G>A (p.Gly260Arg) was shown to be a loss-of-function variant in functional studies in a yeast strain lacking Sdh1 [15]. Pathogenic germline SDHA variants were previously identified in 7.6% of patients with PGL, with diagnosis occurring at a significantly younger age in patients carrying the SDHA variants [21]. The patient carrying the likely pathogenic SDHA variant in this study was diagnosed with head and neck paraganglioma at the age of 20 and was the second youngest patient of our study cohort. Missense variants in the KIF1B gene had been previously detected in samples of pheochromocytoma [22,23], along with a splice site variant [24]. Yet, no previous reports of paraganglioma with a pathogenic KIF1B variant have been published. Our patient carrying a KIF1B c.2787-2A>C had a bladder paraganglioma, which may be the first paraganglioma to be reported carrying a KIF1B variant. The overall positive rate of pathogenic variants in the whole cohort of the apparently sporadic PPGL in our institution was 21.7% (35 among 161 PPGL patients). The most commonly mutated gene was RET (31.4%), followed by VHL (25.7%),
Table 1. Clinical Characteristics of the Patients Included in This Study

| ID | Sex | Age at Dx | uPCC | Bilateral | HNPGL | uPCC (others) | Tumor size (max) | Meta | Multifocal | Hormone type | Recurrence | FHx of PPGL | FHx of other cancer | Variant of interest |
|----|-----|-----------|------|-----------|--------|---------------|------------------|------|------------|--------------|------------|-------------|---------------------|------------------|
| 1  | F   | 46        | N    | Y         | N      | N             | 8.5              | Y    | N          | E/M          | Y          | N           | NA                  |                  |
| 2  | M   | 48        | Y    | N         | N      | N             | NA               | Y    | N          | E/M          | N          | N           | NA                  |                  |
| 3  | F   | 39        | N    | Y         | N      | Y             | 6.7              | Y    | Y          | E/M          | N          | N           | NA                  |                  |
| 4  | M   | 86        | N    | N         | N      | Y             | 11.8             | N    | N          | E/M          | N          | N           | NA                  | NM_000143.3(FH):c.260G>A p.(Arg87His) |
| 5  | F   | 54        | N    | N         | N      | Y             | 5.5              | N    | N          | E/M          | N          | N           | NA                  | NM_002168.3(IDH2):c.424A>C p(Ile142Leu) NM_001933.4(DLST):c.973C>T p(Arg325Trp) |
| 6  | M   | 52        | Y    | N         | N      | N             | NA               | Y    | N          | NE/NM        | Y          | NA          | NA                  |                  |
| 7  | F   | 60        | Y    | N         | N      | N             | NA               | Y    | Y          | NE/NM        | N          | N           | NA                  | NM_003001.3(SDHC):c.478G>A p(Val160Met) |
| 8  | M   | 60        | N    | N         | N      | Y             | 6.5              | N    | N          | NE/NM        | N          | N           | NA                  |                  |
| 9  | F   | 33        | N    | N         | N      | Y             | 9.9              | N    | N          | NE/NM        | N          | N           | NA                  |                  |
| 10 | M   | 36        | N    | N         | N      | Y             | 8                | N    | N          | E/M          | N          | N           | NA                  | NM_022552.4(DNMT3A):c.914G>A p(Trp305*) |
| 11 | M   | 38        | N    | N         | Y      | N             | 2.7              | Y    | N          | NA           | N          | N           | NA                  |                  |
| 12 | M   | 24        | Y    | N         | N      | N             | 5                | N    | N          | NE/NM        | N          | N           | NA                  |                  |
| 13 | F   | 27        | N    | Y         | N      | Y             | 2.5              | N    | Y          | E/M          | N          | N           | NA                  | NM_001430.4(EPAS1):c.1565A>G p(Asn522Ser) |
| 14 | F   | 27        | N    | N         | N      | Y             | 3.4              | N    | N          | NE/NM        | N          | N           | NA                  | NM_000077.4(CDKN2A):c.236C>T p(Thr789Le) |
| 15 | M   | 47        | N    | N         | N      | Y             | 5.9              | N    | N          | E/M          | N          | N           | NA                  | NM_003482.3(KMT2D):c.15707A>G p(Asn5236Ser) |
| 16 | F   | 20        | N    | N         | Y      | N             | 3.2              | N    | N          | NA           | N          | N           | NA                  | NM_004168.3(SDHA):c.778G>A p(Gly260Arg) |
| 17 | M   | 68        | N    | N         | N      | Y             | 5.5              | N    | N          | E/M          | N          | N           | NA                  | NM_002168.3(IDH2):c.247G>A p(Asp83Asn) |
| 18 | M   | 67        | N    | N         | N      | Y             | 2                | N    | N          | E/M          | N          | N           | NA                  | NM_053046.3(EGLN2):c.773C>G p(Ala258Gly) |
| 19 | M   | 50        | Y    | N         | N      | N             | 9.3              | N    | N          | E/M          | N          | N           | NA                  |                  |
| 20 | F   | 45        | N    | N         | N      | Y             | 3.5              | N    | N          | NE/NM        | N          | N           | NA                  |                  |

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Table 1. Continued

| ID  | Sex | Age at Dx | uPCC | Bilateral | HNPGL | PGL (others) | Tumor size (max) | Meta | Multifocal | Hormone type | Recurrence | FHx of PPGL | FHx of other cancer | Variant of interest |
|-----|-----|-----------|------|-----------|--------|--------------|------------------|------|------------|--------------|------------|-------------|----------------------|---------------------|
| 21  | F   | 55        | N    | N         | N      | N            | Y                | Y    | N          | E/M          | N          | N          | NA                   | NM_000143.3(FH):c.418G>C p.(Val140Leu) |
| 22  | F   | 56        | Y    | N         | N      | N            | 3.5              | N    | N          | E/M          | N          | N          | NA                   | NM_015700.3(NFU1):c.473G>A p.(Arg158Gln) |
| 23  | F   | 48        | N    | N         | N      | Y            | 3                | N    | N          | NA           | N          | N          | NA                   | NM_015074.3(KIF1B):c.2787-2A>C p.? |
| 24  | F   | 45        | N    | N         | N      | Y            | 4.8              | N    | N          | E/M          | N          | N          | NA                   | NM_003482.3(KMT2D):c.4987G>A p.(Glu1663Lys) |
| 25  | M   | 36        | N    | N         | N      | Y            | 4.8              | N    | N          | NA           | N          | N          | NA                   | NM_003482.3(KMT2D):c.4942G>A p.(Asp1648Asn) |
| 26  | M   | 29        | Y    | N         | N      | N            | 3.8              | N    | N          | NE/NM        | N          | N          | NA                   | NM_000546.5(TP53):c.566C>T p.(Ala189Val) |
| 27  | M   | 23        | Y    | N         | N      | N            | 2.4              | N    | N          | E/M          | N          | N          | NA                   | NM_003482.3(KMT2D):c.4942G>A p.(Asp1648Asn) |
| 28  | F   | 22        | Y    | N         | N      | N            | 6                | N    | N          | E/M          | N          | N          | NA                   | NM_003482.3(KMT2D):c.4942G>A p.(Asp1648Asn) |
| 29  | F   | 35        | N    | Y         | N      | N            | 5.5              | N    | N          | E/M          | N          | N          | NA                   | NM_000546.5(TP53):c.566C>T p.(Ala189Val) |
| 30  | F   | 23        | Y    | N         | N      | N            | 3.5              | N    | N          | NE/NM        | N          | N          | NA                   | NM_000546.5(TP53):c.566C>T p.(Ala189Val) |
| 31  | F   | 43        | N    | N         | N      | Y            | 4.5              | N    | N          | NE/NM        | Y          | N          | Rt. breast cancer, endometrial polyp Brother with choriocarcinoma, metastasis to lung, kidney, brain | NM_000059.3(BRCA2):c.8488-1G>A p.? |
| 32  | F   | 51        | Y    | N         | N      | N            | 8.0              | Y    | N          | NE/NM        | N          | N          | Sister: breast cancer Brother: thyroid cancer | None |
| 33  | M   | 27        | N    | N         | N      | Y            | 11.5             | Y    | N          | E/M          | N          | N          | None | None |
| 34  | F   | 42        | Y    | N         | N      | N            | 1.5              | N    | N          | E/M          | N          | N          | None | None |
| 35  | M   | 12        | Y    | N         | N      | N            | 2.5              | N    | N          | NE/NM        | N          | N          | None | None |
| 36  | M   | 25        | N    | N         | N      | Y            | 4.7              | N    | N          | NE/NM        | N          | N          | None | None |

Dx, diagnosis; uPCC, unilateral pheochromocytoma; HNPGL, head and neck paraganglioma; PGL, paraganglioma; Meta, metastasis; FHx, family history; PPGL, pheochromocytoma and paragangliomas; N, not present; Y, present; E/M, epinephrine/metanephrine; NA, not available; NE/NM, norepinephrine/normetanephrine.
SDHB (17.1%) and SDHD (14.3%) (unpublished data). Positive rate for SDHA was 2.9%, though the result seems underestimated since SDHA has been included in the panel recently. Our data showed targeted gene panel with extended genes related to PPGL would benefit by increasing the positive rate of pathogenic variants.

Among the VUSs, a novel nonsense variant, c.914G>A (p.Trp305*) was detected in DNMT3A. This variant may be a likely hematopoietic somatic mosaic variant unrelated to the paraganglioma. Germline variants of DNMT3A previously reported in paraganglioma had been gain-of-function missense variants [25], while most of the likely hematopoietic somatic mosaic variants detected in multiple cancers were loss-of-function variants [26].

In other cancer-related genes, BRCA2 c.8488-1G>A, detected in a 25-year-old male patient with early-onset paraganglioma. The patient had no personal history or family history of BRCA2 related cancer. Germline BRCA1/2 variants, most commonly associated genes in familial breast and ovarian cancer, are also known to be associated with other cancers such as prostate, colon, gastric, pancreatic cancer. BRCA1/2 variants are not regarded as genetic causes for adrenal tumors, but there had been a previously reported case of pheochromocytoma who carried BRCA2 variants [27]. A 40-year-old Ashkenazi woman was diagnosed with pheochromocytoma, and later diagnosed with infiltrating ductal carcinoma at 61 years of age. The patient carrying a BRCA2 splicing variant in our study had been diagnosed with paraganglioma at the age of 25, and yet he had not been diagnosed with additional cancer until now. However, at 15 months of age, the patient underwent a Fontan operation [28]. Thus, hypoxic condition may be the second hit for development for paraganglioma. Although the causative role of this variant for the diagnosis of pheochromocytoma cannot be proven, the BRCA2 germline variants may be associated with an increased

| ID  | Gene   | Transcript | Base change | AA change | In silico prediction (SIFT/Polyphen/Mutation Taster) | gnomAD MAX frequency | ACMG-AMP classification |
|-----|--------|------------|-------------|-----------|-------------------------------------------------|----------------------|--------------------------|
| 23  | KIF1B  | NM_015074  | c.2787-2A>C | p.?       | −/−/D                                           | −                    | LP                       |
| 16  | SDHA   | NM_004168  | c.778G>A    | p.Gly260Arg| D/D/D                                           | EAS 0.006%           | VUS                      |
| 14  | CDKN2A | NM_000077  | c.236C>T    | p.Thr79Ile | D/D/D                                           | AFR 0.012%           | VUS                      |
| 5a  | DLST   | NM_001933  | c.973C>T    | p.Arg325Trp| D/D/D                                           | AFR 0.0062%          | VUS                      |
| 10  | DNMT3A | NM_175629  | c.914G>A    | p.Trp305* | −/−/D                                           | AFR 0.0062%          | VUS                      |
| 18  | EGLN2  | NM_053046  | c.773C>G    | p.Ala258Gly| T/B/D                                           | EAS 0.011%           | VUS                      |
| 13  | EPAS1  | NM_001430  | c.1565A>G   | p.Asn522Ser| T/B/N                                           | EAS 0.033%           | VUS                      |
| 4   | FH     | NM_000143  | c.260G>A    | p.Arg87His | D/D/D                                           | EAS 0.0054%          | VUS                      |
| 17  | IDH2   | NM_002168  | c.247G>A    | p.Asp83Asn | D/D/D                                           | −                    | VUS                      |
| 5a  | IDH2   | NM_002168  | c.424A>C    | p.Ile142Leu| D/P/D                                           | −                    | VUS                      |
| 28  | KMT2D  | NM_003482  | c.492G>A    | p.Asp164Asn| D/P/D                                           | EAS 0.015%           | VUS                      |
| 25  | KMT2D  | NM_003482  | c.498G>A    | p.Glu1663Lys| D/P/D                                         | NFE 0.001%           | VUS                      |
| 15  | KMT2D  | NM_003482  | c.15707A>G  | p.Asn5236Ser| T/P/D                                           | EAS 0.006%           | VUS                      |
| 7   | SDHC   | NM_003001  | c.478G>A    | p.Val160Met| T/P/D                                           | −                    | VUS                      |
| 36  | BRCA2  | NM_000059  | c.8488-1G>A | −/−/D     | −                                               | Pathogenic for breast/ovarian cancer |
| 21a | NFU1   | NM_015700  | c.473G>A    | p.Arg158Gln| D/D/D                                           | EAS 0.0054%          | VUS                      |
| 31  | TP53   | NM_000546  | c.566C>T    | p.Ala189Val| D/D/D                                           | EAS 0.027%           | VUS                      |

AA, amino acid; ACMG-AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology; D, deleterious/damaging/disease causing; LP, likely pathogenic; EAS, East Asian; VUS, variant of uncertain significance; AFR, African; T, tolerated; B, benign; N, polymorphism; P, possibly damaging; NFE, non-Finnish European.

aHighest minor allele frequency among different populations (gnomAD); bTwo variants detected in the same patient (ID5, ID 21); cA likely hematopoietic somatic mosaic variant.
risk for adrenal tumors. Another missense VUS, c.473G>A (p.Arg158Gln) in NFU1, was found in an individual who also carried a missense variant c.418G>C (p.Val140Leu) in FH. NFU1 is an essential iron–sulfur (Fe/S) protein implicated in multiple metabolic pathways and energy production, and acts as a maturation factor of respiratory complex II (SDH) [29]. Though this gene is known to be associated with MMDS1, which is inherited in an autosomal recessive pattern, a variant that affects the function of the protein may be involved with compromised SDH function [30,31]. Association of the disease and the BRCA2 variant, as well as VUS detected in other genes, should be assessed in further studies.

This study has several limitations. Though VUS reclassification is considered important as the genetic testings are becoming more available [32], familial screening was not performed in any of the patients. Segregation data would have provided more evidence that can lead to the reclassification of numerous VUS detected. Also, gross defects such as CNV were excluded, only analyzing single nucleotide variants or small insertion/deletions. Moreover, somatic variants in the tissue were not analyzed though it would have explained the additional driver alteration of the disease other than the germline portion. Further evaluation regarding family testing, CNV analysis, and sequencing of the tissue samples would improve the overall detection rate of the causative genetic variants.

In conclusion, we analyzed the WES data of PPGL patients with no causative genetic variant detected in routine clinical gene testing. Likely pathogenic variants were detected in two patients, which led to a 5.6% increase in molecularly confirmed PPGL patients. While implementation of WES for detection of germline variants in PPGL patients has not yet been widely adopted in clinical laboratories, implementation of targeted gene sequencing consisted of extended genes of PPGL in routine clinical screening can support the level of comprehensive patient assessment.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conception or design: S.H.S., J.H.K., M.W.S. Acquisition, analysis, or interpretation of data: S.H.S., J.H.K., M.J.K., S.I.C., S.J.K., H.K., C.S.S., S.S.P., K.E.L., M.W.S. Drafting the work or revising: S.H.S., J.H.K., K.E.L., M.W.S. Final approval of the manuscript: K.E.L., M.W.S.

ORCID

Soo Hyun Seo https://orcid.org/0000-0002-6899-4967
Jung Hee Kim https://orcid.org/0000-0003-1932-0234
Kyu Eun Lee https://orcid.org/0000-0002-2354-3599
Moon-Woo Seong https://orcid.org/0000-0003-2954-3677

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