INTRODUCTION

Head and neck paraganglioma (PGL) are a group of neurogenic tumors originating from neural crest cells in the ectoderm. The incidence of the disease ranges from one in 30 thousand to one in 100 thousand, which accounts for 0.6% of head and neck tumors, with 15% of the tumors displaying malignancy.1 The tumor usually has no endocrine function and is most commonly found in the carotid body, accounting for about 60% of head and neck PGL.2 It can also occur in the jugular foramen, the tympanum, or the vagus nerve.3 Currently, it is believed that the disease is related to genetic abnormalities and environmental factors. In 2000, Baysal et al. first discovered the mutation of the SDHD gene in patients with PGL.4 After that, mutations of the SDHC, SDHB, SDHA, SDHAF2, VHL, RET, NF1, TMEM127, and MAX genes were found.5 Paraganglioma in the head and neck can be familial or sporadic occurrences. Familial occurrences are usually characterized by autosomal dominant inheritance, 80% of which are multiple PGL. Multiple PGL occur as sporadic occurrences in only 10% to 20% of patients.5

Because of the complex anatomical structure of the head and neck, tumors are often adjacent to large vessels and important nerves, leading to a greater risk of surgery for PGL of the head and neck and a higher probability of serious postoperative complications. Therefore, early diagnosis and treatment of PGL of the head and neck are particularly important. A study showed that even patients with sporadic PGL had a 30% chance of carrying a related gene mutation. Accordingly, related gene mutations should be detected in every patient with PGL, and follow-up of the carriers of these pathogenic gene mutations should be conducted regularly to reduce the risk of surgery and improve prognosis of the patients.5 However, in clinical practice, considering the high cost and time-consuming nature of gene detection, this often is only performed in patients with an obvious family history, systemic syndrome or metastasis, multiple or bilateral tumors, and younger age. In this study, we detected the genes associated with multiple PGL in the head and neck and screened for gene mutations. In addition, we focused on finding new mutations and identifying families

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previously not suspected of being at risk, and we discuss the pathogenesis of multiple PGL.

MATERIALS AND METHODS

Patients

Of the 70 patients with head and neck PGL admitted to the Department of Otologyngology, Head and Neck Surgery in Beijing Tongren Hospital (Capital Medical University, Beijing, People’s Republic of China) between January 2013 and February 2017, this study examined 23 of the patients who were diagnosed with multiple PGL. The disease was found in patients at 1 to 15 years of follow-up.

Diagnosis of PGL was by clinical characteristics and family history, urine catecholamine determination, otolaryngology head and neck surgery-related specialist examination (e.g., laryngoscope and electric otoscope), and neck computed tomography/magnetic resonance imaging (CT/MRI) angiography. Malignant PGL is accompanied by distant metastasis, and the diagnosis depends on postoperative pathology.

Collection of Peripheral Blood/Fresh Tissue Specimens and DNA Extraction

This study was approved by the hospital ethics committee. The content and significance of the study were discussed with every patient, who then signed informed consent forms to participate in the research. Clinical information and specimens (blood/fresh tissue) were collected. DNA was extracted from the paraffin-embedded tumor tissue of some patients from the pathology department, and the genes related to PGL were detected.

Experimental Method

DNA was obtained from blood or from fresh tissue, and the exons of SDHD/SDHB/SDHC/SDHA/SDHAF2/RET/VHL and other genes were analyzed. The solutions were vortexed at room temperature for 5 to 10 seconds under low-speed centrifugation. After mixing with a prepared enrichment system, the solutions were placed in the preset polymerase chain reaction (PCR) instrument. The temperature was reduced to 65°C for 2 minutes, and 26 μL of enrichment buffer solution was added with a Transferpette pipette. Once the enriched samples had been cleaned and eluted, the PCR system was configured and placed on the PCR instrument. After purification, the samples were stored below −20°C. The target genes in the stored samples were captured using a liquid capture kit. An Illumina HiSeq 2500 (San Diego, CA) new generation sequencing instrument was used to carry out high-throughput sequencing, with an average depth of greater than 200×. The sequencing results were compared with the standard sequence published in the Human Gene Mutation Database.

RESULTS

Clinical Phenotypic Results

Of the 23 patients with multiple PGL in the head and neck, there were 17 cases of bilateral carotid body tumors (CBTs), one case of bilateral CBT with glomus tympanicum tumor, one case of bilateral CBT with glomus jugulare–tympanic PGL, one case of unilateral CBT with jugular foramen tumor, one case of bilateral CBT complicated by retroperitoneal PGL, and two cases of unilateral CBT with pheochromocytoma (PCC). In all 23 patients, there were tumors in the carotid body, with the second predilection site at the jugular foramen and the tympanic cavity, and two cases had PGL extending outside the head and neck region. There were nine men and 14 women, with age ranging from 25 to 69 years. There were 13 cases of familial occurrence, with eight male patients and five female patients and an average age of 41 years. There were 10 cases of sporadic occurrence, with three male patients and seven female patients and an average age of 39 years. Complications included the unintentional discovery of bilateral neck tumors (13 cases, 52.4%), hospitalization after resection of the tumor on one side in another hospital (3 cases, 14.3%), discovery of cervical tumors when the patient was in the Department of Endocrinology for hypertension (3 cases, 14.3%), hoarseness after CBT resection in another hospital (2 cases, 9.5%), pulsatile tinnitus (1 case, 4.8%), and repeated ear spills with ipsilateral feeling of numbness of tongue (1 case, 4.8%).

Collection of Specimens and Results of Gene Detection

For the family proband and corresponding family members, fresh tissue and blood-matching tests were carried out. Four families were identified from the probands (Fig. 1). Of the 10 patients with sporadic manifestations, six blood samples were collected from patients with head and neck PGL; and five tissue samples (including blood samples from four patients) were collected. Six cases with DNA from blood samples (all from women) and five cases with DNA from tissue samples (one case from a male patient and four cases from female patients) were evaluated by gene sequencing.

In the 10 patients with sporadic manifestations, five cases of SDHD germline mutation and one case of RET somatic mutation were detected. One case was not detected successfully due to the poor quality of paraffin wax, and three patients did not agree to gene detection. Two of the new mutations were the c.387_393del7 mutation of the SDHD gene and the c.3247T>G mutation of the RET gene (Table I).

Thirty-one members of family 1 (F1) underwent genetic testing. Twelve cases of germ cell mutation were found, all of which were c.112C>T mutations of the SDHD gene (Table II). Eight cases showed bilateral CBTs, with one malignant case (F1:III-14). For three members of family 2 (F2), three mutations were detected. The proband contained a c.C242T mutation of the SDHD gene (Table II). The uncle and cousin of the patient had the same mutation site. Of the three cases that developed, one showed bilateral CBTs, whereas two cases had unilateral CBTs. The c.112C>T mutation of the SDHD gene was detected in the proband and cousin in family 3 (F3) (Table II). Two cases of unilateral CBT and PCC were diagnosed in the family. The proband and family members of family 4 (F4) did not agree to gene detection. The incidence in the four families is illustrated in Table II. Mutations in SDHA, SDHAF2, SDHC, SDHB, and VHL genes were not found in this study. The
c.112C>T mutation of the SDHD gene was the most common mutation.

**DISCUSSION**

Four genetic pedigrees, comprising three cases of familial PGL syndrome and one case of familial PCC/PGL syndrome, were examined. Due to financial reasons and patients’ will, we only sequenced those patients with multiple tumors and their relatives who agreed to gene detection. All the probands had multifocal PGL. The gene mutations in F1, F2, and F3 were SDHD germline mutations (3 of 3), and the mutant amino acids were R38X (2 of 3) and P81L (1 of 3). F4 did not agree to gene detection. No mutations in SDHA, SDHAF2, SDHC, SDHB, and VHL genes were found in familiar occurrences. This may be related to family size and differences in race. In this study, 12 cases of SDHD gene mutation of germ cells were found in F1, and tumors had developed in eight of these cases. Each case had bilateral CBTs, including one case of malignant transformation of liver and lung metastasis, albeit with a relatively low rate of malignant transformation. Studies have shown that, in the familial PGL of SDHD gene mutations, only 5% of the tumors were associated with malignancy and metastasis, whereas the ratio of SDHB gene mutation to malignant tumor with metastasis was up to 13% to 23%. In our study, F1, F2, and F4 families displayed patterns consistent with autosomal dominant inheritance. There were no patients with PGL in the first generation or second generation of F3, whereas the third-generation members showed two cases of PGL, which may be related to the delayed and occult nature of this tumor.

In addition to a distinct family history, some patients with hereditary head and neck PGL had sporadic manifestations. In this study, 10 patients with sporadic occurrences were evaluated by genetic sequencing. Five of the patients who displayed SDHD germline mutations were the probands, and they initially presented sporadic manifestations and potentially lineal consanguinity illness. The cause of PGL is often the result of the environment or gene mutation; therefore, there are also cases that manifested only as sporadic occurrences. In patients with sporadic manifestations, five cases of SDHD-related mutations were found after gene detection, with a new mutation site c.387_393del7 in the SDHD gene. The mutation is a frameshift mutation, and its clinical phenotype is bilateral CBTs and retroperitoneal PGL.
frameshift mutation is the insertion of a gene locus into a normal DNA molecule or the deletion of a number of bases that are not divisible by three, causing the three-codon reading frame to shift after this point and displacing a series of gene-coding sequences. Therefore, the clinical phenotype caused by the frameshift mutation could be more serious than the missense mutation.

In 2013, Bacca et al.7 reported the relationship between gene mutation and clinical phenotype of head and neck PGL. They found that a frameshift mutation could cause tumor recurrence, and the local tumor had strongly invasive characteristics. The other four sporadic SDHD gene mutations were a c.112C>T nonsense mutation (two cases), a c.1A>G nonsense mutation, and a c.188_198del deletion mutation. The tumor was resected completely during surgery. No recurrence was found postoperatively.

Head and neck PGL is often accompanied by mutations in the SDH-related gene, and mutation of the RET gene is rare. This is the first study to report a patient with multiple PGL in the head and neck also carrying the RET somatic mutation. The patient’s complaint was left-pulsating tinnitus for 14 years and aggravated accompanying headache for 3 years with no family history. A bilateral neck tumor and left tympanic cavity tumor were found through head and neck CT/MRI. Preoperative evaluation and staged surgery were performed before the operation. Postoperative pathology was a bilateral CBT and left glomus jugulare tumor. Postoperative follow-up

### TABLE I.
Detection of Gene Mutations in Patients With Sporadic Manifestations.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Germline Mutation</th>
<th>Somatic Mutation</th>
<th>Age, Years</th>
<th>Sex</th>
<th>Disease</th>
<th>Benign or Malignant</th>
<th>Recrudescence</th>
<th>Codon</th>
<th>Mutation Site</th>
<th>Change of Amino Acid</th>
<th>Mutation Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SDHD</td>
<td>–</td>
<td>44</td>
<td>F</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>2</td>
<td>c.112C&gt;T</td>
<td>p.R38X</td>
<td>Nonsense mutation</td>
</tr>
<tr>
<td>2</td>
<td>SDHD</td>
<td>–</td>
<td>33</td>
<td>F</td>
<td>CBT + jugular spheroid tumor</td>
<td>Benign</td>
<td>–</td>
<td>3</td>
<td>c.188_198del</td>
<td>p.S63fs</td>
<td>Deletion mutation</td>
</tr>
<tr>
<td>3</td>
<td>Poor paraffin quality</td>
<td>–</td>
<td>42</td>
<td>M</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>SDHD</td>
<td>–</td>
<td>40</td>
<td>F</td>
<td>Bilateral CBT + jugular orifice–typanic paraganglioma</td>
<td>Benign</td>
<td>–</td>
<td>1</td>
<td>c.1A&gt;G</td>
<td>p.M1V</td>
<td>Nonsense mutation</td>
</tr>
<tr>
<td>5</td>
<td>SDHD</td>
<td>–</td>
<td>29</td>
<td>F</td>
<td>Bilateral CBT + retroperitoneal paraganglioma</td>
<td>Benign</td>
<td>Yes</td>
<td>4</td>
<td>c.387_393del7*</td>
<td>p.?</td>
<td>Frameshift mutation</td>
</tr>
<tr>
<td>6</td>
<td>Do not agree to test</td>
<td>Do not agree to test</td>
<td>38</td>
<td>M</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Do not agree to test</td>
<td>Do not agree to test</td>
<td>30</td>
<td>M</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>SDHD</td>
<td>–</td>
<td>44</td>
<td>F</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>2</td>
<td>c.112C&gt;T</td>
<td>p.R38X</td>
<td>Nonsense mutation</td>
</tr>
<tr>
<td>10</td>
<td>Do not agree to test</td>
<td>Do not agree to test</td>
<td>43</td>
<td>F</td>
<td>Bilateral CBT</td>
<td>Benign</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* A new type of mutation.
CBT = carotid body tumor; F = female; M = male.

### TABLE II.
Overview of SDHD Gene Mutations in Four Families.

<table>
<thead>
<tr>
<th>Family</th>
<th>Diagnosis</th>
<th>Gene Mutation</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino Acid</th>
<th>Mutation Type</th>
<th>Number of CBT Patients (diagnostic age range, years)</th>
<th>Number of PCC Patients (diagnostic age range, years)</th>
<th>Number of Mutant Gene Carriers (age range, years)</th>
<th>Number of Patients With Distant Metastasis (age range, years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>PGL</td>
<td>SDHD</td>
<td>2</td>
<td>c.112C&gt;T</td>
<td>P.R38X</td>
<td>Nonsense mutation</td>
<td>8(29–69)</td>
<td>–</td>
<td>4(3–50)</td>
<td>1(31)</td>
</tr>
<tr>
<td>F2</td>
<td>PGL</td>
<td>SDHD</td>
<td>3</td>
<td>c.C242T</td>
<td>p.P81L</td>
<td>Missense mutation</td>
<td>3(25–50)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F3</td>
<td>PGL/PCC</td>
<td>SDHD</td>
<td>2</td>
<td>c.112C&gt;T</td>
<td>P.R38X</td>
<td>Nonsense mutation</td>
<td>2(28–34)</td>
<td>2(27–33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F4</td>
<td>PGL</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3 (32–45)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

CBT = carotid body tumor; PCC = pheochromocytoma; PGL = paraganglioma; SDHD = succinic dehydrogenase.
showed that the patient recovered well, and no neurological complications were found. Fresh tumor tissue and a 2 mL blood sample with ethylenediaminetetraacetic acid as anticoagulant were collected for gene mutation detection. Analysis of the samples showed a RET gene mutation in exon 20, nucleotide changes to c.3247A>G, and peptide change in amino acid to p.T1083A. No related mutations were detected in the blood, and no SDHx gene mutation was found. A search through the genetic database showed the RET mutation to be of a novel type. The protein expressed by the RET gene is a transmembrane receptor of a tyrosine kinase family, which regulates the proliferation, differentiation, and apoptosis of cells. The study showed that 5% to 10% of patients with PCC carried RET gene mutations, and the mutation was rarely expressed in the head and neck PGL. Given the homology of PCC and PGL, it is possible that the RET gene may be the pathogenetic gene of multiple PGL in the head and neck in this case. In addition, studies have shown that 98% of multiple endocrine neoplasia 2A, 95% of multiple endocrine neoplasia 2B, and 88% of familial medullary thyroid carcinoma had RET gene germ cell mutations. Therefore, medullary thyroid carcinoma may be more likely to occur in RET gene mutation carriers. According to the medullary thyroid carcinoma guidelines of the American Thyroid Association, patients should be followed up closely; receive thyroid ultrasonography every 6 months or annually; have their serum calcitonin level monitored; and if necessary, have prophylactic total thyroidectomy performed.

In the study of familial PGL, four gene carriers (with ages of 3, 4, 7, and 50 years) were found in family 1. The European-American Paraganglioma Study Group recommends that, for hereditary PGL, gene detection in close relatives should start from 13 years of age. Renard et al. suggested that gene screening should start at the age of 10 years, with the youngest known occurrence at the age of 5 years. The age of the gene carriers found in family 1 is relatively young; therefore, the age of gene screening for first-generation relatives of the proband can be advanced. There are different opinions on the penetrance of SDHx-related genes. Neumann et al. found that the penetrance of SDHB and SDHD mutation carriers increased with increasing age. The older they were, the higher the penetrance. Peczowska et al. carried out an investigation of 25 carriers in 16 families with the SDHD c.33C>A mutation. The results showed that the penetrance rate was 50% at 30 years old, with complete penetrance of mutation carriers by the age of 54. For familiar patients, carriers should be closely followed up and treated early. In particular, the first and second generations of relatives in the family should receive gene sequencing. However, because gene detection is time-consuming and costly, many clinical scholars have proposed a progressive gene detection based on clinical features. If malignant PGL is diagnosed clinically, SDHB detection should be performed first. If negative, SDHD detection should be performed. For patients younger than 35 years of age with an obvious family history or sporadic benign PGL, SDHx gene detection should be performed preferentially. If negative, then SDHB and SDHC related tests should be performed. In order to speed up testing and reduce the cost, immunohistochemical analysis of SDHB protein expression in tumor tissue has been used recently as a specific and sensitive method in SDHx gene correlation analysis. In addition, high-performance liquid chromatography is also used by clinicians due to its high efficiency and low cost. Moreover, all of the SDHD mutations found in this study were of paternal imprint. To improve the efficiency, some scholars have suggested that only the next generation of paternal gene SDHD mutations should be tested via gene detection.

**CONCLUSION**

This study found that the SDHD gene mutation rate was higher in patients and family members with multiple PGL in the head and neck, which suggests that the SDHD gene plays an important role in the pathogenesis of multiple PGL in the head and neck. The SDHD gene should be studied for mutations in patients with multiple PGL, with a close follow-up of family members with the gene mutation for early detection of tumors. Two new mutations, the c.387_393del7 mutation of the SDHD gene and the c.3247A>G mutation of the RET gene, were found in patients with sporadic manifestations; and new mutation sites are provided for genetic screening of multiple PGL in the head and neck.

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