Carotid Body Paraganglioma and SDHD Mutation in a Greek Family

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Abstract. Background: Carotid body (CB) is a highly specialized paraganglion originating from the neural crest ectoderm. CB paraganglion can be caused either by a genetic predisposition (hereditary paraganglia) or by chronic hypoxic stimulation. Germline mutations in any of the following genes: SDHD, SDHC, SDHB, PGL2 or other unknown genes, can cause paragangliomas (PGLs). Materials and Methods: We studied a Greek family in which the two daughters had carotid body paraganglioma, whereas both parents did not. RNA extraction, reverse transcriptase polymerase chain reaction and direct DNA sequencing were performed, in order to identify SDHD mutations in all four exons. Results: Our results revealed the existence of the missense mutation Y114C, in exon-4 of the SDHD gene, in the unaffected father and both affected sisters. Conclusion: DNA testing was performed, for the first time in Greece, on patients with carotid body tumor. This marks a new geographical location, in the literature, for this mutation.

Carotid body (CB) is a highly specialized paraganglion, derived from both mesodermal elements of the third branchial arch and neural elements originating from the neural crest ectoderm. CB is a small and highly vascularized ovoid or irregularly-shaped organ located at the medial aspect of the carotid artery bifurcation, within the adventitia. In adults, CB has an average dimension of 1.7 x 2.2 x 3.3 mm. Microscopically, CB has multiple lobules composed of two major cell types: chief cells and sustentacular cells, the first of which arrange in nests called zell-ballen (1, 2). CB functions as a chemoreceptor sensitive to changes in arterial P_{O2}, P_{CO2}, and pH, which induces reflex changes in vasomotor action and respiration (3, 4).

CB, other head and neck paraganglia, adrenal medulla and other extra adrenal paraganglia altogether comprise a dispersed multifocal neuroendocrine system with similar histology and embryology. The tumors arising from any of the components of this neuroendocrine system are called paragangliomas (PGLs) (1, 5).

PGLs are highly vascularized, painless, hormonally-silent, slow-growing tumors. The annual incidence of all paraganglia is ~1 in 300,000. CB and the adrenal medulla are the most common hereditary paraganglioma tumor locations (6, 7). CB tumors can be etiologically classified on the presence or absence of additional tumors (8). CB paragangliomas can be caused either by a genetic predisposition (PG) or by chronic hypoxic stimulation (e.g. individuals dwelling at high altitudes, certain medical conditions such as cyanotic heart or chronic lung disease). In 30% of published cases, PGLs are inherited, while chronic hypoxia is a risk factor for sporadic PGLs (9).

PGLs can be caused by germline mutations in any of the following genes: SDHD, SDHC, SDHB, PGL2 or other unknown genes (10). The SDHD was the first gene to be identified with hereditary paraganglioma (PGL-1) at 11q23 by positional cloning (9, 11). The transmission of SDHD mutations clearly displays a parent of origin. The disease phenotype occurs in an age-dependent autosomal dominant fashion after paternal transmission, whereas after maternal transmission no disease phenotype is seen, suggesting the operation of genomic imprinting at the PGL-1 locus (12).

SDHD spans over 19 Kb and consists of 4 exons of 52, 117, 145 and 163 bp, respectively. It encodes the small subunit of cytochrome-b in succinate–ubiquinone oxidoreductase (mitochondrial complex II). Complex II is an important heterotetrameric compound, crucial for both the tricarboxylic acid cycle and the aerobic respiratory chains of mitochondria (11). SDHD also functions as a tumor suppressor gene. The main role of such genes is the inhibition of cell proliferation. The characterization of tumor suppressor genes is important both for the understanding of processes of tumor-genesis and for practical use in the diagnostics, prognostics and therapy of tumors (13, 11).

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Materials and Methods

Patients. Clinical examination in a 42-year-old woman revealed two visible bulges, one in each side of the neck, which were palpable and movable only at the horizontal axis. Computerized tomography (CT) in the neck showed a tumor in the parapharyngeal region. Angiography revealed a characteristically highly vascularized ovoid tumor blush at the carotid bifurcation, with wide separation of the internal and external carotid arteries. After surgical removal of both tumors, histological examination revealed PGLs. In 1995, the sister of the above patient underwent surgical removal of a left CB tumor, histological examination of which revealed PGLs. CT of the abdominal region and 24-h-urine metanephrin analysis excluded the existence of pheochromocytoma in both of them. Blood samples were collected from the two sisters and their parents (while tissue sample was collected only from the first woman).

RNA extraction-isolation and reverse transcriptase polymerase chain reaction. DNA was extracted from whole blood, using a standard salting out method. Total RNA was extracted from the CB tumor tissue using Trisol reagent (Invitrogen, Cat.No. 15596-026), according to the procedure described by Chomczynski and Sacchi. Reverse transcriptase reaction was carried out using: Depc–ddH2O (Sigma, D05758), d-NTP's (10 Mm) (Invitrogen, Cat.No. 10297-014), SuperScript II RNAse H (Invitrogen, Cat.No. 18064-014) and Random Primers (0.3 µg/µl) (Invitrogen, Cat.No. 48190-011). Min Oil (Sigma, M-5904) was added to minimize evaporation and cross contamination. The reaction was incubated at 42 °C for 50 minutes and inactivated at 70 °C for 20 minutes.

Polymerase chain reaction was performed for both whole blood DNA and RT product, using: Depc–ddH2O (Sigma, D05758), d-NTPs (10 Mm) (Invitrogen, Cat.No. 10297-014), Taq. Polymerase (5 U/µl) (Invitrogen, Cat.No. 10342-020), Primers (10 Mm) of the four exons of the SDHD gene (Invitrogen) and Min Oil (Sigma, M-5904) added to minimize evaporation and cross contamination. The reaction was incubated at 42 °C for 50 minutes and inactivated at 70 °C for 20 minutes.

Polymerease chain reaction was performed for both whole blood DNA and RT product, using: Depc–ddH2O (Sigma, D05758), d-NTPs (10 Mm) (Invitrogen, Cat.No. 10297-014), Taq. Polymerase (5 U/µl) (Invitrogen, Cat.No. 10342-020), Primers (10 Mm) of the four exons of the SDHD gene (Invitrogen) and Min Oil (Sigma, M-5904) (Figure 1). The primers were designed from the genomic sequence of the SDHD gene region (Gene bank accession number AB026906) (Table I). The amplified products were then checked on 1.8% ethidium bromide-stained agarose gel (Quiagquick Nucleotide Removal Kit; Catalogue no. 28304; Qiagen, Hilden, Germany).

Table I. Primers that were used.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Size (bp)</th>
<th>Accession number</th>
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<tbody>
<tr>
<td>EXON-1</td>
<td>281</td>
<td>ABO26906</td>
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</table>
| F: GTT CAC CCA GCA TTT CTT
| R: TGC TGT GAT TTC GGT ATT TTC |                  |
| EXON-2  | 375       | ABO26906        |
| F: ATG TTA TCC ATT ATT TAT TGT T
| R: TCT GCC CAA AGG TGT AAA CTA |                  |
| EXON-3  | 316       | ABO26906        |
| F: CAC TGC CTT TCA GTT TGG GTT AC
| R: GGG CAT TTC AAT CAA CTT TTC CTC CC |                  |
| EXON-4  | 389       | ABO26906        |
| F: TCC CCT AAA GAA GCA AAC AGT GAC
| R: GAG CTT AAT GGC ATG ACA AAG CAG |                  |

Figure 1. The expression of all four SDHD exons detected in tissue and blood samples, as analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) method, described in detail in the Materials and Methods section.

Table I. Primers that were used.
The mitochondrial enzyme complex II (SDH) is comprised of SDHA, B, C and D and it is important for the tricarboxylic acid cycle and the aerobic respiratory chain in mitochondria. The SDHD gene, on chromosome 11q23, is the first and most common gene to be mutated in a patient with PGLs. The SDHD gene encodes the small subunit of cytochrome-b in the succinate-ubiquinone oxidoreductase complex. Thus, SDHD is probably critical for oxygen sensing and its loss may lead to chronic hypoxic stimulation of cellular proliferation leading to tumor growth (11). SDHD mutations have been identified in all four exons, without the observation of phenotype - genotype correlation. Some of them have been identified in several families (9, 16 - 19).

In the family being studied, the mutation Y114C in exon-4 of the SDHD gene was detected in the unaffected father and both affected sisters. The disease is inherited in an autosomal dominant fashion and subject not only to age-dependent penetrance, but also imprinting. Thus, affected individuals inherit the disease from their father, and expression is not observed in the offspring of affected mothers (12). It has been shown that offspring who inherit a gene mutation from their mother do not manifest the disease. However, male offspring may have affected children (11).

The mutation found, Y114C in exon-4, is a missense mutation, which changes the protein sequence from the normal tyrosine to cysteine, a non-conservative amino acid substitution that could dramatically alter the protein conformation. The same mutation was found in a German family for the first time and, to date, 58 cases have been described in the literature with the same mutation (20).

Moreover, the carotid body (CB) functions as an oxygen-sensor and stimulates the cardiopulmonary system in acute hypoxia (21). The increased incidence of PGLs in humans and other mammals living at very high altitudes (4000m above sea level) (22) establishes chronic hypoxia as a risk factor for sporadic PGLs. On the basis of this observation and the phenotypic similarity between sporadic PGLs and hereditary paraganglioma (PGL–1), it has been hypothesized that mutations in SDHD disrupt oxygen sensing in the CB and lead to tumor formation, effectively mimicking the pathogenesis of sporadic tumors at high altitudes (11). It was also suggested that the increased prevalence of SDHD mutations in the Netherlands, attributable to multiple founder mutations, can be explained, in part, by the low altitudes in that country, which presumably reduce gene penetrance and relax the natural selection (23). The family being studied lives in a small village in Southern Greece at an altitude of 630 m above sea level, which hardly explains the existence of PGLs.

It has been shown that the R22X SDHD mutation leads to overproduction of angiogenic factors, such as VEGF and EPAS1, which probably contribute to the increased angiogenesis of these tumors (24). Furthermore, it has been suggested that the hypoxia-inducible factor-1 (HIF-1) transcription factor may play an important role in the cellular response to hypoxia in CB (11) and could represent
one such imprinting candidate. The gene for HIF-1 has been mapped to 14q21-q24 (25). Although this is not a region currently known to be subject to imprinting, this hypothesis warrants further investigation. Although hypotheses and studies have been made in order to explain the existence of PGLs and their transcription, the role of genomic imprinting and the role of the environment in the penetrance of SDHD mutations are not well documented. The discovery of a genomic mutation in a PGL patient is important for the genetic counseling of his or her family, including predictive DNA testing mutations in all relatives and PGL screening at a presymptomatic stage. Indeed, early PGL detection reduces the incidence of morbidity and mortality (26).

References