A thyroid nodule revealing a paraganglioma in a patient with a new germline mutation in the *succinate dehydrogenase B* gene

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**Abstract**

A 32-year-old asymptomatic female was diagnosed with an isolated thyroid nodule of 2.5 cm diameter. Fine needle aspiration suggested a medullary thyroid carcinoma. Consequently, a total thyroidectomy was performed. The nodule stained positive for chromogranin A, neurone-specific enolase and synaptophysin, but not for calcitonin. Finally, pathological analysis showed a thyroid paraganglioma. Although the tumour appeared to be sporadic in a patient with no personal or familial history of paraganglioma and/or pheochromocytoma, we have identified a new mutation (392delC) of the *succinate dehydrogenase-B* (*SDHB*) gene in the genomic DNA extracted from the leukocytes of the patient. That mutation induced a shift in the reading frame of the gene creating a premature stop codon (P131fsX135) which was predicted to result in a truncated SDHB protein of 135 amino acids.

This report highlights the difficulties of this unexpected diagnosis of hereditary thyroid paraganglioma. It also discusses the clinical involvements in terms of familial screening and the necessary follow-up of the patient.

**Introduction**

Paragangliomas are usually benign tumours of the autonomous nervous system that are composed of cells derived from the primitive neural crest (1). They can be found in different locations from the middle ear and the skull base to the pelvic floor. In the head and the neck area, paragangliomas are located in close association with the parasympathetic nervous system along the cranial nerves and the arterial vasculature, but they may also be found in unusual sites such as the orbit, the paranasal sinuses or the thyroid (1 – 3). Thyroid paragangliomas are thought to arise from the inferior laryngeal paraganglia (2) which are sometimes situated within the thyroid capsule. They are extremely rare tumours which can be misinterpreted as medullary thyroid carcinomas (MTCs) and/or paragangliomas (4). Thyroid paragangliomas are thought to arise from the inferior laryngeal paraganglia (2) which are sometimes situated within the thyroid capsule. They are extremely rare tumours which can be misinterpreted as medullary thyroid carcinomas (MTCs) and/or paragangliomas (4). The symptoms are pulsatile tinnitus, hearing loss, deafness for head and neck locations or cranial nerves or skull base. Paragangliomas are inherited in around 30% of cases. Usually, hereditary paragangliomas are multiple, recurrent and sometimes malignant. Three genes, the *succinate dehydrogenase-B*, *SDHC* and *SDHD*, which encode three protein subunits of the cytochrome b of complex II in the mitochondrial respiratory chain, have been implicated in the genetics of hereditary paragangliomas (5 – 7). Germline mutations of *SDHD* gene are mostly found in patients with head and neck hereditary paragangliomas (8), whereas *SDHB* germline mutations have also been identified in patients with familial paragangliomas (5, 8), familial pheochromocytomas (5) and apparently sporadic paragangliomas (8) and/or pheochromocytomas (5). Only two mutations have been reported in the *SDHC* gene in a family with hereditary paragangliomas as well as in one case of apparently sporadic pheochromocytoma (6, 8, 9). Herein, we report a case of a thyroid paraganglioma appearing as a classic solitary thyroid nodule which was first misdiagnosed as an MTC and in which we have found a new germline mutation of the *SDHB* gene.
Patient and methods

Patient

The patient was a 32-year-old female who had an asymptomatic, single thyroid mass of about 2.5 cm diameter. Routine laboratory data showed normal plasma thyrotrophin (1.29 mIU/l), as well as undetectable plasma calcitonin (<5 ng/l). Anti-peroxidase and anti-thyroglobulin antibodies were negative in her blood. The patient’s family history revealed only Hodgkin’s disease in her dead father. Her personal medical history was negative for hypertension, thyroid or other endocrine disorders, neck irradiation, respiratory diseases, cyanotic heart diseases and other causes of chronic hypoxia. Ultrasound examination showed a 2.5 cm hypervascularized solid nodule of the upper pole of the left thyroid lobe. Fine needle aspiration of the nodule was performed and an MTC was then suspected. The patient underwent total thyroidectomy with resection of the cricoid cartilage and bilateral node dissection. The pathologist’s description first suggested an MTC with amyloid stroma and unexpected immunohistochemical staining. Surprisingly, the tumour cells were strongly positive for synaptophysin, neuron-specific enolase, serotonin and chromogranin A, but they were negative for calcitonin. Further pathological evaluation diagnosed a thyroid paraganglioma. The results were negative in the search for other paraganglioma locations by octreotide scintigraphy and for catecholamine secretion by measurement of urinary metanephrines. Six years after the surgical operation, the patient is still alive. In addition, she feels well under substitutive treatment with L-thyroxine and without any evidence of recurrent disease.

Methods

Three haematoxylin and eosin-stained (HES) blocks of tissue were sent by the pathologist for review. Immunohistochemical stains were performed against the following antigens: thyroglobulin, calcitonin, chromogranin A, neuron-specific enolase, synaptophysin, serotonin, S-100 protein and carcinoembryonic antigen. Written informed consent was obtained for the DNA analysis. Peripheral DNA was obtained from a venous blood sample. Four exons of the SDHD gene, eight exons of the SDHB gene and six exons of the SDHC gene were amplified and directly sequenced as previously described (10).

Results

Pathological findings (Fig. 1a and b)

The tumour was mainly composed of large polyhedral cells and sometimes fusiform with poorly eosinophilic cytoplasm. Some nuclei were hyperchromatic or with irregular outlines. Cells were disposed in nests or sometimes in trabeculae separated by a thin conjunctivovascular area. The proliferation was obviously endocrinoid, the trabeculae were separated by capillaries with well-recognized endothelium. In many areas, cells were round with a clear finely granular cytoplasm. Within the periphery, there were small-sized cells with elongated nuclei (sustentacular cells). The proliferation was lobulated by fibro-vascular trabeculae, surrounded by a fibrous condensation which contained vessels with a thick wall. It also separated the tumour from the cellulo-adipose tissue and the normal adjacent thyroid tissue. Fibrous condensation was infiltrated in some areas and mitoses were found in the tumour.

Immunohistochemical findings (Fig. 1c, d and e)

The tumour was strongly and entirely positive for neuron-specific enolase, chromogranin A, synaptophysin and locally positive for serotonin. The sustentacular...
cells were positive for the S-100 protein. The tumour was negative for thyroglobulin, calcitonin and carcinoembryonic antigen.

**Mutation analysis (Fig. 2)**

We found no mutation in the SDHD and the SDHC genes but we identified a heterozygous germline mutation in exon 4 of the SDHB gene. Direct sequencing showed the deletion of cytosine at the 392 nucleotide position (392delC). That mutation led to a shift in the reading frame of the gene creating a premature stop codon (P131fsX135) which was predicted to result in a truncated SDHB protein of 135 amino acids.

**Discussion**

Intrathyroidal paragangliomas are rare tumours affecting women between 40 and 50 years of age. Most of the time they appear as an asymptomatic thyroid nodule (4). The diagnosis of intrathyroidal paraganglioma may be extremely difficult on a purely morphological basis, and immunohistochemistry is essential to show the difference from other types of tumours (11). Those tumours are essentially MTC, Hurthle cell neoplasm, metastatic renal cell carcinoma, hyalinizing trabecular adenoma, atypical follicular adenoma and metastatic carcinoid tumours of the thyroid (4). In the present case, the presence of nests of large polygonal cells separated by variable amounts of amyloid and richly vascularized fibrous stroma as well as the presence of mitoses and nuclear abnormalities first led to the diagnosis of MTC. However, the lack of staining for calcitonin and for carcinoembryonic antigen as well as the identification of S-100 protein-positive sustentacular cells later excluded the diagnosis of MTC. Calcitonin immunostaining is always present in MTC, and its intensity is used as a prognosis marker: poorly stained tumours are less differentiated and more aggressive (12). However, diagnosis may be very difficult. Hence, a battery of epithelial, neural and hormonal immunohistochemical markers are mandatory in the differential diagnosis between paraganglioma and MTC (4).

At least four genetic loci have been implied in the pathogenesis of hereditary paragangliomas. Three genes of the loci, paraganglioma1 (PGL1) on chromosome 11q23 (13), PGL3 on chromosome 1q21 (14) and PGL4 on chromosome 1p36 (5) have been identified. They encoded for the mitochondrial complex II (succinate dehydrogenase, succinate:ubiquinone oxidoreductase) subunits SDHD (7), SDHC (6) and SDHB (5) respectively. The PGL2 gene located at the 11q13 chromosome position (15) still has to be confirmed and identified. The succinate dehydrogenase participates in the electron transport chain and in the Krebs tricarboxylic acid cycle in the mitochondria (16–19). It is formed by two catalytic subunits (SDHA and SDHB) which are anchored to the membrane by two hydrophobic integral membrane proteins (SDHC and SDHD). It was previously demonstrated that inactivation of SDHD or SDHB genes induced complete loss of succinate dehydrogenase activity and activation of the angiogenic pathway (10, 20). Several mechanisms such as oxygen sensing and/or the apoptosis hypothesis (21) are currently being discussed to explain the tumorigenesis triggered by the loss of succinate dehydrogenase activity. The SDHD mutations are usually reported in hereditary paragangliomas located in the head and neck (most often developed in the carotid glomus). They are also found in familial and/or apparently

**Figure 2** Electrophoretogram corresponding to a control normal SDHB sequence (upper electrophoretogram) and to the patient sequence (lower electrophoretogram) showing the deletion of cytosine 392 and the introduction of a stop codon (TGA) at amino acid position 135.
sporadic pheochromocytomas (7, 18, 22, 23). The SDHB mutations are preferentially observed in familial and apparently sporadic pheochromocytomas. Table 1 shows the mutations reported in the SDHB gene. In the present patient we found a heterozygous deletion of cytosine at nucleotide 392 in exon 4 of the SDHB gene resulting in a false-sense codon (proline changed to histidine) in the 131 amino acid position, and in a termination codon at 135. This cytosine was previously reported to be changed in guanine inducing a missense mutation (P131R) in the SDHB gene in one Polish family with head and neck hereditary paragangliomas (23). Familial paragangliomas caused by SDHD mutations demonstrate maternal genomic imprinting effects and the disease phenotype is manifested only after paternal transmission (24). The phe-notypic expression of SDHB and SDHC mutations do not reveal parent-of-origin effects which would be clinical evidence for genetic imprinting of these genes (5, 6). Other differences between SDHD and SDHB mutations have been noted. There is no evidence for a founder effect in the different mutations of SDHB (8), contrasting with SDHD gene mutations which show a founder effect in Dutch families (25). The SDHD gene is included within the region of loss of heterozygosity exclusively targeting the maternal allele on chromosome 11q22-23 which has been observed at distant flanking markers in paragangliomas (20, 22, 24 – 27). Gimenez-Roqueplo et al. (28) have recently shown that germline mutations of the SDHB gene associated with a loss of heterozygosity at the 1p36 chromosome were strongly associated with extra-adrenal pheochromocytomas and conferred a high risk of recurrence or malignancy. Although our patient’s paraganglioma was apparently sporadic, the presence of a germline mutation in her genome demonstrated an inherited disease (29 –34). Her parents could not be studied but there was no clinical evidence of the disease, suggesting a de novo mutation. Whether our patient is the cas simplex remains hypothetical. In any case, there are two important reasons for involvement. On a familial basis, a presymptomatic genetic testing should be proposed for her first-degree related parents and all individuals determined to be at risk of the familial disease should be engaged in regular follow-ups for the possibility of paragangliomas and/or pheochromocytomas. On a clinical basis, this patient has a high risk of recurrence and should be regularly screened.

Finally, attention should be drawn to apparent MTCs which do not show calcitonin expression. The diagnosis of paraganglioma with an unusual location in the head and neck should lead to the analysis of the SDHB gene.

Table 1 Principal causal mutations of the SDHB gene.

<table>
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<th>Exon</th>
<th>Nucleotide change*</th>
<th>Amino acid change**</th>
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*Nucleotide number on the cDNA sequence; **amino acid number; ***F-PGL, familial paraganglioma; S-Pheo, apparently sporadic functional paraganglioma (catecholamine-secreting paraganglioma or pheochromocytoma); F-Pheo, familial pheochromocytoma; S-PGL, apparently sporadic paraganglioma.
Acknowledgements

This study was supported by Groupement d’Intérêt Scientifique-Institut des Maladies Rares and the Cortico Medullo Tumeurs Endocriines network. A-P G-R holds a Contrat Individuel d’Objectif from INSERM.

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Received 14 June 2004
Accepted 20 July 2004