CASE REPORT

A novel TMEM127 mutation in a patient with familial bilateral pheochromocytoma

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Abstract

Objective: In this report, we describe a new patient with unexplained familial bilateral pheochromocytoma. Following the recent description of TMEM127 as a new pheochromocytoma susceptibility gene, the aim of this study was to test the hypothesis of a causative TMEM127 gene mutation in this patient.

Design: Pheochromocytoma susceptibility genes were analyzed in germline DNA and losses of heterozygosity (LOH) assessed by BAC array comparative genomic hybridization in tumor DNA. SDHB expression and S6 kinase (S6K) phosphorylation were analyzed by immunohistochemistry. Genome-wide expression microarray studies were performed, and vascular density was quantified after CD34 immunohistochemistry.

Results: A first germline variant was identified in the SDHB gene (c.158G>A; p.Gly53Glu). However, a positive SDHB immunostaining in the tumor indicated that this SDHB variant was a non-functional polymorphism. A novel TMEM127 germline mutation (c.140C>A, p.Ala47Asp) associated with a 2q11 LOH was found. Transcriptome and immunohistochemical analyses showed that TMEM127-related pheochromocytoma clustered with NF1-related and RET-related tumors in a large series of pheochromocytomas and paragangliomas, exhibited a reduced TMEM127 mRNA expression and displayed a low vascularization. The phosphorylation of S6K observed in this tumor was suggestive of an activation of the MTOR pathway.

Conclusions: Pathological and genomic data demonstrated that a TMEM127 gene mutation not previously described was causative of a new case of familial bilateral pheochromocytoma. This report highlights the importance of supplementary analyses on tumor tissue to provide an accurate pheochromocytoma/paraganglioma genetic testing result to affected patients.

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Introduction

Pheochromocytomas and paragangliomas are rare catecholamine-secreting tumors arising from the adrenal medulla (pheochromocytoma proper) or from extra-adrenal chromaffin tissues. Although usually sporadic, pheochromocytomas and paragangliomas can occur in the context of inherited cancer syndromes in ~ 30% of cases (1). These hereditary diseases include multiple endocrine neoplasia type 2, von Hippel–Lindau disease, neurofibromatosis type 1, and hereditary paragangliomas caused respectively by germline mutations in the RET, VHL, NF1, SDHB, SDHC, SDHD, SDHA (SDHx), and SDHAF2 genes (2–4). Some familial clusters of yet unexplained pheochromocytomas and/or paragangliomas remained, suggesting the existence of new genes predisposing to these tumors (5). Recently, Qin et al. (6) reported heterozygous germline mutations in TMEM127 gene in seven patients affected by pheochromocytoma. They demonstrated that TMEM127 is a new tumor suppressor gene involved in hereditary pheochromocytoma/paraganglioma syndrome, according to a two-hit model of inactivation (germline mutation associated with loss of wild-type allele).
Moreover, microarray-based expression profiling analyses showed that TMEM127-mutated tumors present a transcription signature comparable to that of RET- and NF1-mutated pheochromocytomas (characterized by enrichment for kinase receptor signaling pathways), differentiating them from SDHx- and VHL-related tumors, which are characterized by activation of the hypoxic pathway.

Here, we report a patient with familial bilateral pheochromocytoma, harboring a non-functional missense variant in the SDHB gene and a causative germline mutation in the TMEM127 gene. This report illustrates the importance of clinical, pathological, and genomics data to interpret pheochromocytoma/paraganglioma genetic testing, and confirms that the TMEM127 gene is a new tumor suppressor gene involved in pheochromocytoma predisposition.

Subjects and methods

Case history

The patient is a woman who reported hyperadrenergic symptoms (sweating, headache, and tachycardia) since she was 20 years old and who suffered from hypertension and diabetes during both her pregnancies. At the age of 44 years, she became permanently hypertensive. She had asthenia and presented unexplained weight loss, symptoms that led to consult a specialized physician. Her family history was remarkable. Her mother had bilateral adrenal surgery for hypertension and diabetes during both her pregnancies. She was 20 years old and who suffered from pancreatitis. Her brother died during childhood after general anesthesia.

Clinical investigations revealed high concentrations of urinary fractionated metanephrines (193.9 μmol/24 h, normal range <3.7, with a predominant elevation of normetanephrines). Computed tomography scan disclosed two heterogeneous adrenal masses (40×26 mm on the left side and 150×50 mm on the right side) suggestive of bilateral pheochromocytoma. Whole body metaiodobenzylguanidine scintigraphy was performed using the following antibodies: anti-SDHB (HP A002868, Sigma–Aldrich), chemical analyses were performed using the following primers available on request.

DNA samples

Patient signed a written informed consent for germline and somatic DNA analyses as well as for the collection of tumor samples at the time of surgery. Tumor samples were immediately frozen in liquid nitrogen to be included in the COMETE network. Ethical approval for the study was provided by the institutional review board (CPP Paris-Cochin, January 2007). A Caucasian reference population was used to obtain 170 control DNAs. Germline DNAs were extracted from leukocytes according to standard protocols. Tumor DNAs and RNAs were extracted using AllPrep DNA/RNA Mini Kit (Qiagen).

Genetic testing

Mutation analysis for RET, VHL, SDHB, SDHC, and SDHD genes was performed by direct sequencing. VHL, SDHB, SDHC, and SDHD genes were also analyzed for the presence of large deletions by Multiplex Ligation-dependent Probe Amplification (MLPA) method as described previously (2, 7). The four exons and the intron–exon boundaries of TMEM127 gene in the germline and tumor DNAs were directly sequenced (primers available on request).

Microarray and BAC array comparative genomic hybridization

Microarray and BAC array comparative genomic hybridization (CGH) analyses were performed as previously described (3, 8).

Immunohistochemistry

Paraffin blocks were cut and 6 μm-thick sections were mounted on Superfrost plus slides. Immunohistochemical analyses were performed using the following antibodies: anti-SDHB (HPA002868, Sigma–Aldrich), anti-SDHA (HPA002867, Sigma–Aldrich), anti-NF1 (HPA009935, Sigma–Aldrich).

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anti-CD34 (Clone QBEND 10, Immunotech, Marseille, France 1/100) for the quantification of vascular density (8), anti-S6 kinase (S6K) (sc-8418, Santa Cruz Biotechnologies), and anti p-S6K (sc-7984-R, Santa Cruz Biotechnologies, Santa Cruz, CA, USA).

Results

Identification of a non-functional SDHB variant

In 2008, genetic testing was proposed to the patient, accordingly to international recommendations for patients with paraganglioma/pheochromocytoma (9, 10). The search for germline mutation in VHL, RET, SDHC, and SDHD genes was negative, but a heterozygous missense variant was identified in the SDHB gene (c.158G>A; p.Gly53Glu; Fig. 1B). This variant was previously reported as a possible polymorphism in the NCBI’s Entrez system (dbSNP reference: rs34916635) and in the TCA cycle gene mutation database (11). However, it appeared homozygous after SDHB direct sequencing from tumor DNA (Fig. 1B) suggesting a loss of heterozygosity (LOH) at the SDHB locus (1p36), which was further confirmed by BAC array CGH (Fig. 2B). To assess the functionality of this variant, we therefore performed SDHB immunohistochemistry on the tumor tissue (Fig. 1C) (12). Such analysis of SDHB expression revealed a clear cytoplasmic SDHB staining in the patient’s tumor, comparable to that observed in non-SDHx pheochromocytomas, clearly confirming that the c.158G>A SDHB variant is a non-functional polymorphism.

Identification of a TMEM127 mutation associated with LOH

In 2010, TMEM127 germline mutations were reported in seven patients affected by pheochromocytoma (6). We thus analyzed the TMEM127 gene by direct sequencing in our patient and identified a germline heterozygous missense mutation (c.140C>A, p.Ala47Asp) in leukocyte DNA that appeared homozygous in tumor DNA (Fig. 2A). This variant was not found in 340 control chromosomes. In the patient’s tumor, LOH at the TMEM127 locus (2q11) was confirmed by BAC array CGH experiments (Fig. 2B) performed in a series of 202 pheochromocytomas/paragangliomas (comprising 75 inherited tumors and 127 sporadic tumors) collected by the COMETE network. It is worth noting that in this tumor, no LOH was found at the SDHA (5p15) or SDHAF2 (11q13) loci, thus excluding the involvement of these recently identified extra-adrenal paraganglioma susceptibility genes for our patient (Fig. 2B). In the whole series of 202 tumors, a 2q11 LOH was observed in nine additional tumors, including one SDHB-related paraganglioma (c.200+1G>A) and eight tumors with an apparently sporadic presentation. These nine additional candidates were thus submitted to TMEM127 sequencing. Only one synonymous variant (c.621G>A; p.Ala207Ala (db SNP reference: rs3852673)) was identified in three other patients (heterozygosity frequency = 31%). Among the healthy control population, a new missense variant (c.121A>G; p.Ile41Val) was identified. This variant has not yet been annotated and should be considered as a rare polymorphism (frequency < 1%).

Microarray and immunohistochemical analyses of the patient’s tumor

The transcriptome of the patient’s tumor was analyzed by genome-wide expression microarray in the context of a large study that included 188 tumors of the COMETE cohort (3, 8). The unsupervised clustering performed with genes’ expression involved in energy metabolism (combination of oxidative phosphorylation and glycolytic pathways) or in hypoxia pathway classified the

Figure 2 (A) Sequence chromatograms of TMEM127 gene obtained in the patient’s leukocyte and tumor DNAs compared to a control DNA. Direct sequencing of the patient’s leukocyte DNA shows the TMEM127 heterozygous mutation c.140C>A and the predominance of the mutant allele in the tumor tissue indicating LOH at the TMEM127 locus. (B) BAC array CGH analysis reveals 1p, 2q, 11p, and 21q losses and gain of 1q and 20pq in TMEM127 tumor. Green and red lines indicate chromosomal losses and gains respectively. The arrow points to the TMEM127 locus at 2q11.
TMEM127-related tumor in the subgroup of RET- and NF1-related pheochromocytomas (data not shown), further confirming the absence of any functional SDHx gene mutations in this tumor. The evaluation of angiogenesis by CD34 immunohistochemistry showed that the vascular density in the TMEM127-related tumor (13 ± 2 blood vessels/0.65 mm²) was comparable to that previously reported in 4 RET/NF1 tumors (mean = 9 ± 2 blood vessels/0.65 mm²) and significantly lower than in 15 SDHx/VHL-related tumors (mean = 28 ± 3 blood vessels/0.65 mm²) (Fig. 3A) (8). Analysis of TMEM127 mRNA levels based on microarray expression data was performed in the patient’s pheochromocytoma and compared to tumors with LOH at 2q11 locus (n = 8) or without LOH at 2q11 (n = 179). We observed an overall 35% reduction in TMEM127 expression levels in tumors harboring a 2q11 LOH as compared to tumors with no LOH at the TMEM127 locus. There was a significant association between TMEM127 level and the presence of the 2q11 LOH (P = 4.10^{-5}, T-test). In the patient’s tumor, a 61% reduction in TMEM127 mRNA was observed (Fig. 3B).

Finally, Qin et al. (6) reported that TMEM127-mutated tumors displayed increased phosphorylation of MTOR target S6K, as compared with normal adrenal or tumors without TMEM127 mutation. We thus performed immunohistochemistry to evaluate the expression of S6K and the presence of its phosphorylated form in the patient’s tumor and confirmed a putative activation of the MTOR pathway in this tissue (Fig. 3C).

**Discussion**

In this report, we describe a patient affected by familial bilateral pheochromocytoma, harboring a non-functional variant in the SDHB gene (p.Gly53Glu) and a new TMEM127 gene mutation (p.Ala47Asp) associated with 2q11 LOH. The presence of these variants could not be confirmed in the patient’s tumor mother who was also affected by bilateral pheochromocytoma, as she died several years ago. However, genetic and expression data indicate that the TMEM127 germline mutation identified in the patient is causative of her familial predisposition to pheochromocytoma and could be used with confidence for the genetic counseling of her first relatives.

This report underlines the importance of complementary tests to provide validated genetic results. Herein, we demonstrate that SDHB immunohistochemistry was positive in this case and confirms that such a pathological analysis is a powerful means to orientate the genetic testing among the nine genes that are now to be considered (13) and to validate the functionality of SDHx unknown variants (12).

Most of our data are in accordance with the recent publication on TMEM127 gene by Qin et al. (6). The family history and the bilateral and benign nature of the pheochromocytoma are in keeping with the clinical characteristics of the first seven patients carrying a TMEM127 germline mutation. This report confirms that TMEM127 gene is a tumor suppressor gene following the two-hit Knudson model with LOH in the tumor DNA. Using the transcriptome analysis of the large series of pheochromocytomas and paragangliomas collected by the COMETE network, we were also able to confirm the cluster association between the TMEM127-related pheochromocytoma and NF1- and RET-related tumors. By the same token, the evaluation of angiogenesis assessed by CD34 immunohistochemistry revealed, in the TMEM127-related pheochromocytoma, a low vascular density as observed in the group of RET/NF1-related tumors. Analysis of TMEM127 transcript levels indicated a 61% reduction for the tumor associating germline TMEM127 mutation and LOH at TMEM127 locus compared with wild-type TMEM127 tumors. This down-regulation was slightly lower than that previously described (78%), but is also in favor of the instability of the mutant transcript. Interestingly, in patients with no TMEM127 mutation but with 2q11 LOH, TMEM127 mRNA levels were also decreased by 35%, thus showing that 2q11 LOH itself had a dosage effect. Such a correlation has already been reported for many other genes, such as 19p13 LOH and GNG7 expression in esophageal cancer (14) or 3p14 LOH and FHIT expression in breast cancer (15).

Finally, as previously reported, we observed a putative activation of the mTOR pathway characterized by the phosphorylation of S6K in tumor cells. Whether analysis of these immunohistochemical criteria could
be used as a predictive tool for the presence of a TMEM127 mutation will have to be evaluated in large cohorts.

Qin et al. (6) reported that 3% of apparently sporadic pheochromocytomas were caused by TMEM127 gene mutations. In the COMETE series of 127 sporadic pheochromocytomas and paragangliomas, we limited the TMEM127 gene sequencing to the eight patients harboring a 2q11 LOH (6%) and found no mutation. The exact prevalence of TMEM127-related pheochromocytomyomas will need to be defined by the TMEM127 genotyping of further large series. Anyhow, the present knowledge suggests that TMEM127 genotyping should now be added to VHL and RET analyses for patients with bilateral pheochromocytoma and/or a family history and with a positive tumor SDHB immunostaining.

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.

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