Familiar Papillary Thyroid Carcinoma in a Large Brazilian Family Is Not Associated with Succinate Dehydrogenase Defects

Elen Dias Accordi^a Paraskevi Xekouki^c Bruna Azevedo^a
Rodrigo Bertollo de Alexandre^a Carla Frasson^{a,b} Siliane Marie Gantzel^a
Georgios Z. Papadakis^d Anna Angelousi^c Constantine A. Stratakis^c
Vanessa Santos Sotomaior^a Fabio R. Faucz^{a,c}

^aGroup for Advanced Molecular Investigation (NIMA), Graduate Program in Health Sciences (PPGCS), School of Medicine (EM), Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, and ^bÁlvaro Center for Analysis and Clinical Research – Diagnósticos da América (DASA), Cascavel, Brazil; ^cSection on Endocrinology and Genetics, Program on Developmental Endocrinology and Genetics (PDEGEN) and Pediatric Endocrinology Inter-Institute Training Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), and ^dDepartment of Radiology and Imaging Sciences, Clinical Center, National Institutes of Health (NIH), Bethesda, Md., USA

Key Words

Succinate dehydrogenase · Mutations · Papillary thyroid cancer · Genetics · Endocrine tumors

Abstract

Background: Thyroid cancer is the most common endocrine gland malignancy. Advances in understanding the genetic basis for thyroid cancer revealed the potential involvement of several genes in the formation of thyroid tumors. Mutations in the gene coding for succinate dehydrogenase subunit B (*SDHB*) have been implicated in papillary thyroid cancer (PTC). Succinate dehydrogenase (SDH) is a heterotetrameric protein composed of four subunits, *SDHA*, *SDHB*, *SDHC*, and *SDHD*, and participates in both the electron transport chain and the tricarboxylic acid cycle. The aim of the study was to evaluate the association between variants in the *SDHA*, *SDHB*, *SDHC*, and *SDHD* genes and familial PTC in a large Brazilian family. **Method:** Four patients with PTC, 1 pa-

tient with PTC and gastrointestinal stromal tumor (GIST), 1 patient with GIST, and their relatives – several of them with different thyroid problems – from a large Brazilian family were screened for genetic variations of *SDHx* genes with the use of polymerase chain reaction-single-stranded conformational polymorphism and direct sequencing. **Results:** Only one rare variation in *SDHA* was found in some of the family members, but not segregating with the disease. No other genetic variants of these genes were detected in the family members that presented with PTC and/or GIST. **Conclusion:** Familiar PTC and a GIST were not associated with *SDHx* mutations; additional genetic defects, yet unknown, may be responsible for the development of tumor.

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E.D.A. and P.X. contributed equally to this article.

Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for most cancers of the endocrine system. It has a frequency that is higher in women (approximately 3:1 ratio), which means an increased incidence rate by gender. Some investigators have suggested that the higher occurrence rate in women may be due to hormonal, reproductive, dietary, and molecular factors, the last of which are unknown [1]. In Brazil, the estimated incidence rate of thyroid cancer evaluated by the Brazilian National Cancer Institute (INCA) [2] was 7.91 cases per 100,000 females. In males, however, due to the low incidence, no calculation was performed. In the United States, 62,980 new cases were expected in 2014, with 47,790 of them in females [3].

There are two types of endocrine thyroid cells from which thyroid cancers are derived: follicular thyroid cells and parafollicular C cells [4]. Medullary thyroid cancers that are parafollicular thyroid C cells in origin have been observed in about 5% of all thyroid cancers [1]. Follicular thyroid cell-derived tumors include papillary thyroid cancer (PTC), follicular thyroid cancer, and anaplastic thyroid cancer [5–9]. PTC and follicular thyroid cancer are collectively classified as well-differentiated thyroid cancers and account for around 95% of thyroid malignancies. Although the majority of papillary and follicular thyroid carcinomas are sporadic, familial tumors may account for 5–10% of thyroid carcinoma cases [1, 10].

Recent progress in the understanding of the molecular pathogenesis of thyroid cancer has stemmed from the identification of molecular alterations, including the genetic and epigenetic alterations of signaling pathways, such as the RAS-RAF-MEK-MAPK-ERK pathway (MAPK pathway) and the PI3K-AKT pathway. The identification of these alterations in different signaling pathways has led to the development of more effective treatment strategies that target these particular signaling perturbations [8, 11].

Mutations in genes coding for the different subunits of succinate dehydrogenase (*SDHx*) have also been associated with the development of thyroid cancer [12, 13]. SDH or complex II is a heterotetrameric protein composed of two hydrophilic catalytic subunits (SDHA and SDHB) and two hydrophobic subunits (SDHC and SDHD) anchored to the mitochondrial membrane [14, 15]. SDH is situated at the intersection of the tricarboxylic acid cycle and mitochondrial oxidative phosphorylation. Hence, SDH is at the center of the two essential en-

ergy-producing processes of the cell, and its dysfunction due to genetic alterations may lead to tumor formation through a complex mechanism involving hypoxia-signaling abnormalities [16–18].

Mutations or loss of heterozygosity of the genes *SDHA*, *SDHB*, *SDHC*, and *SDHD* have been described in paragangliomas, pheochromocytomas [19–22], renal cell carcinoma [19, 23, 24], gastrointestinal carcinoma [19], familial pheochromocytoma and paraganglioma [14, 24–27], Cowden's syndrome [22, 23], medullar [1, 19] and papillary thyroid carcinoma [14, 19, 24], and more recently pituitary adenomas [28–30].

In the course of this study, we investigated the *SDHA*, *SDHB*, *SDHC* and *SDHD* genes in a large Brazilian family, several members of whom presented with PTC.

Materials and Methods

DNA Collection

The sampling of epithelial buccal cells was performed as previously described [31]. After the collection 10 µl of proteinase K (20 mg/ml) were added to the solution, being left overnight at 65°C, DNA was purified by adding ammonium acetate 10 M, precipitated with isopropanol and resuspended with 50 µl of Tris 10 mM (pH 7.6) and EDTA 1 mM.

Polymerase Chain Reaction-Single-Stranded Conformational Polymorphism

To examine the association of the SDH genes with familial thyroid papillary carcinoma, the coding regions of *SDHA* (NM_004168 – 15 exons), *SDHB* (NM_003000 – 8 exons), *SDHC* (NM_003001 – 6 exons) and *SDHD* (NM_003002 – 4 exons) were analyzed.

Polymerase chain reaction (PCR)-single-stranded conformational polymorphism (SSCP) and direct sequencing were used for the study. For the PCR-SSCP, 25 µl of final reaction volume were used. The analysis of all samples was performed as previously described [31] using the primers and conditions described in online supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000444522).

Direct Sequencing

To characterize the SSCP common pattern, DNA samples from 2 healthy individuals, 35 and 50 years of age, were used as technical controls. None of them had any reports of developing cancer in their families to date of collection. Two samples of each exon were sequenced in the case no different pattern was found by SSCP and all samples were sequenced to confirm the mutation segregation pattern every time a mutation was found. The PCR product was amplified using BigDye® Terminator V3.1 (Life Technologies, Grand Island, N.Y., USA) purified using the ZR DNA Sequencing Clean-Up Kit™ (Zymo Research, Irvine, Calif., USA) and analyzed by classical bidirectional Sanger sequencing.

Fig. 1. Pedigree of the family.

Results

Clinical Findings

Patients

A large family (fig. 1) was studied. The first and second generations of the family are from Northern Brazil (State of Ceará) and the third, fourth, and fifth generations reside in Southern Brazil (State of Paraná). In summary, the family group is characterized by 4 individuals with PTC, 1 individual with PTC and gastrointestinal stromal tumor (GIST), 1 individual with GIST, 2 individuals with hypothyroidism, 1 individual with Hashimoto's thyroiditis, 1 individual with Hashimoto's thyroiditis and multinodular goiter, 4 individuals with uni- or multinodular goiter, and 5 individuals with normal thyroid glands. The remaining 13 individuals of the family were not available for clinical evaluation (fig. 1). Written informed consent was obtained from all participants and the Institutional Review Boards of the participating institutions approved the study.

Case II-9. A 62-year-old female presented with multinodular goiter. Thyroid ultrasound (US) showed an enlarged multinodular thyroid gland (thyroid volume was 76.89 cm^3 , normal range for females: $10\text{--}12 \pm 2 \text{ cm}^3$), with a predominant nodule measuring 2.0 cm in its largest diameter. Thyroid function tests showed that thyrotropin (TSH) and free thyroxine hormone levels (fT_4) were within the normal range: $0.5 \text{ } \mu\text{UI/ml}$ (normal range: $0.35\text{--}5.00 \text{ } \mu\text{UI/ml}$) and 0.9 ng/dl (normal range: $0.7\text{--}1.8 \text{ ng/dl}$), re-

spectively. US-guided fine-needle aspiration (FNAC) of the largest nodule revealed a PTC. Total thyroidectomy (with lymph node ablation due to an enlarged nodule) was performed and histological analysis confirmed the presence of PTC. Unfortunately, the information regarding histological subtype, multifocality, and involvement of the lymph nodes that were removed was not available since this case was operated more than 20 years ago and not all the medical records were available.

Case II-10: A 65-year-old female presented with a 4-cm mass and tenderness in the upper left abdominal quadrant. An upper-gastrointestinal barium study and gastric endoscopic examination revealed a submucosal tumor, without obvious ulceration, in the upper part of the stomach. Endoscopic ultrasonogram showed an approximately 4-cm hypoechoic lesion with a hyperechoic area. An endoscopic biopsy was performed and confirmed GIST.

Case III-9. A 45-year-old male presented with a solitary solid thyroid nodule, immobile in palpation, measuring $1.5 \times 0.8 \text{ cm}$. US-guided FNAC revealed a PTC. Total thyroidectomy was performed and the histological analysis confirmed the diagnosis of PTC. Information regarding histological subtype was not available as in case III-9.

Case III-11. A 44-year-old female presented with multinodular goiter (thyroid gland volume: 65.3 cm^3), with a predominant nodule measuring 3.8 cm in its largest diameter. Thyroid function tests were within the normal range (TSH: $1.9 \text{ } \mu\text{UI/ml}$, fT_4 : 1.1 ng/dl). US-guided FNAC re-

Table 1. Reported *SDHx* mutations in patients diagnosed with thyroid carcinomas

Type of thyroid cancer	Histology subtype	SDH gene	Mutation	Nature of cancer	Reference
Papillary	Not available	<i>SDHB</i>	c.328T>C	Familial PGL	Neumann et al. [13]
Papillary	Not available	<i>SDHD</i>	c.14G>A	Familial PGL	Neumann et al. [13]
Papillary	Not available	<i>SDHB</i>	exon 1 del	Familial PGL	Solis et al. [34]
Papillary	Not available	<i>SDHC</i>	c.397C>T	Familial PGL	Zbuk et al. [35]
Papillary	Not available	<i>SDHC</i>	c.183G>A	Sporadic	Garrel et al. [36]
Follicular	–	<i>SDHC</i>	c.346C>T	Cowden-like syndrome	Ni et al. [24]
Papillary	Not available	<i>SDHB</i>	c.69C>G	Cowden-like syndrome	Ni et al. [24]
Papillary	Not available	<i>SDHB</i>	c.359A>G	Cowden-like syndrome	Ni et al. [24]
Papillary	Not available	<i>SDHB</i>	c.794G>A	Cowden-like syndrome	Ni et al. [24]

PGL = Paraganglioma.

vealed a PTC in a hyperplastic goiter area. Total thyroidectomy was performed and the histological analysis confirmed the presence of conventional PTC. The patient was also diagnosed with intestinal polyps 16 years later. Endoscopic biopsy of one of the polyps concluded it to be a GIST.

Case III-16. A 51-year-old female was diagnosed with multinodular goiter. US showed a normal thyroid volume (14.8 cm³) and bilateral hypoechoic nodules with a predominant nodule measuring 1.6 cm in its largest diameter. US-guided FNAC of this nodule revealed a PTC. Thyroid function tests were normal (TSH: 3.22 µUI/ml). A total thyroidectomy was performed and histological analysis confirmed the presence of a conventional PTC in the predominant nodule.

Case III-23. A 29-year-old female was diagnosed with multinodular goiter. US-guided FNAC of one of the nodules revealed a PTC. Thyroid function tests were within the normal range (TSH: 4.47 µUI/ml; fT₄: 1.5 ng/dl). A total thyroidectomy was performed and histological analysis confirmed the diagnosis of a conventional PTC.

Genetic Analysis

No novel or previously reported genetic variants in the genes *SDHB*, *SDHC*, and *SDHD* were detected. One missense mutation on *SDHA* (NM_004168 – OMIM: 600857) c.1919A>G (p.E640G/rs372480044) was found, but it did not segregate with the disease since it was found in 2 affected members (III-11, who had PTC and GIST, and III-23) and in 3 unaffected individuals (III-21, IV-31, and IV-33). This mutation is rare (not found in the 1000 Genomes Project), although it is predicted to be nonpathogenic by in silico prediction using the PolyPhen2 analysis tool (<http://genetics.bwh.harvard.edu/pph/>). All available family members and all patients were screened (com-

pletely sequenced) and no disease segregating mutations were found for the genes *BRAF*, *JUNB*, *CDKN1B*, or *PTEN* (data not shown).

Discussion

SDHx variants can be associated with PTC [13]. In addition, approximately 12% of the wild-type GIST without personal or family history of paraganglioma have germline mutations in *SDHB* or *SDHC* [32]. We described a large Brazilian family that presents with PTC and/or GISTs, but no *SDHx* mutations were detected except for a rare genetic variant, which was shown to be nonpathogenic by in silico analysis. Although the *SDH*-related tumor spectrum has been recently expanded, there are only a few reports of nonpheochromocytoma/paraganglioma tumors in *SDHx*-mutated patients [14, 31–36]. In particular, 1–5% of *SDHB/SDHD* mutation carriers with paragangliomas/pheochromocytomas were found to present renal cells or PTC [14]. The association of germline mutations of the succinate dehydrogenase subunit genes and thyroid cancer are reported in table 1. Neumann et al. [13] was the first to report the possible association of *SDHx* mutations with thyroid cancer. Other studies [14, 24] have shown that germline variants of the *SDHB*, *SDHC*, or *SDHD* genes may occur in a subset of *PTEN* mutation-negative Cowden and Cowden-like syndrome individuals. These individuals presented more frequently with breast, thyroid, and renal cancers beyond those conferred by germline *PTEN* mutations [14]. In the absence of *PTEN* alteration, Cowden or Cowden-like syndrome-related *SDHx* variants show increased phosphorylation of AKT and/or MAPK [14].

The proposed molecular mechanism of carcinogenesis in cases presenting with *SDHx* variants was based on the disruption of the complex II leading to mitochondrial metabolite imbalance, HIF1 α accumulation, and ROS generation [23, 24]. SDH loss causes succinate accumulation and activation of pseudohypoxia signaling via overexpression of HIF proteins. Activation of insulin-like growth factor 1-signaling is also typical of these tumors. SDH-deficient GISTs are a unique group of GISTs with an energy metabolism defect as the key oncogenic mechanism [37].

Our family included 4 cases with PTC, 1 case with coexistence of PTC and GIST, and 1 case with GIST alone. The coexistence of bilateral pheochromocytomas, abdominal paraganglioma, papillary thyroid carcinoma, and parathyroid adenoma has already been described in the literature [38–40]. GIST and thyroid cancer coexisted in 2.5% of the cases [38]. In our cases, sporadic coincidence of the described tumors is highly unlikely considering that 5 out of 13 family members presented with PTC. Recently, the Slit-Robo Rho GTPase activating protein 1 gene (*SRGAP1*) was identified as a candidate gene in PTC susceptibility in families with nonmedullary thyroid cancer using linkage analysis and association studies [41]. The authors speculate that 2 missense variants in *SRGAP1* (p.Q149H and p.R617C) could be loss-of-function-type changes affecting CDC42 activity; the latter has a well-defined function in cell proliferation, survival, and migration. Although *SRGAP1* is a low-penetrant susceptibility gene in PTC, screening for genetic variations of this gene is warranted [41].

No genetic alterations were identified in another study with sporadic PTC which analyzed the association between *SDHB* and *SDHC* genes, but very significant reductions in the *SDHC* expression were. By analyzing the expression of the *SDHx* gene in differentiated carcinoma

samples, a classic PTC histology was overrepresented among patients with low overall *SDHx* expression compared with those with other histologic types, especially the follicular variant form of PTC [42].

In this article we report a large pedigree with familial PTC associated also with other thyroid disorders and/or GISTs in which we were not able to identify genetic variations in the genes *SDHA*, *SDHB*, *SDHC*, and *SDHD* (mutations in the genes *BRAF*, *JUNB*, *CDKN1B*, and *PTEN* had already been excluded). Although the family is large and presents a variety of thyroid diseases showing great significance for research, it is interesting to note that 25% of consanguineous individuals were unavailable for genetic analysis and relevant information, which was a limitation of our study. These data suggest the likely presence of a different molecular pathway that should be investigated. Further genetic analysis, including next-generation sequencing studies is needed to identify new genes and understand the carcinogenic process leading to thyroid carcinoma formation.

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Disclosure Statement

The authors have nothing to disclose and they report no competing interests.

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