ARTICLE

Carney triad can be (rarely) associated with germline succinate dehydrogenase defects

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Carney triad, the association of paragangliomas/pheochromocytomas, gastrointestinal stromal tumors and pulmonary chondromas, is a sporadic condition that is significantly more frequent in females; its genetic etiology remains unknown. Carney triad is distinct from the dyad of paragangliomas/pheochromocytomas and gastrointestinal stromal tumors, known as Carney–Stratakis syndrome, which is inherited in an autosomal-dominant manner and is almost always caused by succinate dehydrogenase subunit mutations. In the present study, we investigated the largest cohort of Carney triad patients that is available internationally: 63 unrelated patients. Six patients (9.5%) were found to have germline variants in the *SDHA*, *SDHB* or *SDHC* genes. All six patients, except one, had multifocal gastrointestinal stromal tumors, chondromas and/or paragangliomas. A patient with Carney triad and *SDHC* variant had a ganglioneuroma. One of the patients with Carney triad and *SDHB* mutation had a nephew with the same sequence defect, who developed a neuroblastoma. Other relatives, carriers of the identified *SDHA*, *SDHB* or *SDHC* mutations, have not developed any of the components of Carney triad or Carney–Stratakis syndrome. None of the other 57 Carney triad patients had any genomic defects of *SDHA*, *SDHB* or *SDHC* genes. We conclude that, in rare occasions, Carney triad can be allelic to Carney–Stratakis syndrome. Although for the vast majority of patients with Carney triad the causative defect(s) remain(s) unknown, testing for *SDHA*, *SDHB* or *SDHC* variations should be offered, as carriers may develop isolated paragangliomas/pheochromocytomas and occasionally other tumors.

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INTRODUCTION

Carney triad (CTr) describes the association of paragangliomas (PGL)/pheochromocytomas (PHEO) with gastrointestinal stromal tumors (GISTs) and pulmonary chondromas (CHOs).^{1,2} CTr is a non-hereditary, sporadic condition, primarily seen in young women.³ Most CTr patients present with GIST and pulmonary CHO; PGL/PHEO is rarely the presenting tumor but most often found at screening, in addition to other lesions, such as adrenocortical tumors.⁴ A marked decrease in the enzymatic activity of succinate dehydrogenase (SDH) has been detected in tumors associated with CTr, but in the first cohort of 37 CTr patients studied by Matyakhina *et al.*⁵ no mutations of the genes coding for the four SDH subunits (*SDHA*, *SDHB*, *SDHC* and *SDHD*, respectively, collectively known as the ‘*SDHx*’ genes) were found.

Instead, the investigation led to the description of a related disease, the dyad of PGL/PHEO and GIST, also known as Carney–Stratakis syndrome (CSS), which affects males and females equally, is almost always inherited as an autosomal-dominant trait, and is never associated with CHO.⁶ Soon thereafter, *SDHx* sequencing defects

were found to be the cause of CSS.^{6,7} A large number of patients presenting with isolated GIST were also found to have germline *SDHx* defects but not necessarily CSS,⁸ whereas *SDHB*, *SDHC* and *SDHD* mutations may also cause the hereditary-isolated PGL/PHEO syndromes PGL4, PGL3 and PGL1, respectively.⁹ A similar condition, PGL2, is caused by mutations in a gene that is associated with the maintenance of proper SDH activity, *SDHA*2 (*SDH5*).¹⁰ *SDHA* defects have been found in both CSS and isolated GIST, but rarely in isolated PGL/PHEO.¹¹

To date, the genetic defect(s) causing CTr remain(s) unknown.¹² CTr-associated PGL/PHEO, GIST and CHO fail to express the *SDHB* protein, similar to tumors caused by *SDHB* and other *SDHx* variations^{8,12} providing a rapid diagnostic tool for their identification.¹³ Recently, *SDHC* locus-specific methylation was found to be present in CTr tumors,¹⁴ a defect that was also later described in GISTs that lack *SDHB* immunoreactivity but have no variations in the *SDHx* genes.¹⁵ This was a distinct defect, separate from the overall increased methylation of the genome seen in lesions that bear *SDHx* defects.^{16,17} Thus, *SDHB* deficiency by immunohistochemistry (IHC)

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and *SDHC* gene-specific methylation provide the most recent means of identification of a CTr-associated tumor, in the absence of a known causative genetic defect.

In the present study, we investigated the largest available, international cohort of 63 patients with CTr for germline variations and/or deletions in the *SDHx* genes; we included here the previously studied 37 patients and resequenced them all, and, in addition, other genomic investigations were performed. Six patients were found to have germline sequence variants in *SDHA*, *SDHB* or *SDHC*; one patient who was previously classified as CSS was found to have CTr, as a CHO was confirmed by biopsy. All six patients, except one, had multifocal gastric GIST and pulmonary CHOs at diagnosis, whereas one had only CHO; two developed PGL, confirmed by the biopsy. One of the patients with CTr and an *SDHB* variant had a nephew with the same sequence defect and a neuroblastoma (NB), confirming a previously reported association of NB with PGL/PHEO. The findings have significant implications for our understanding of CSS and CTr and for genetic counseling of both groups of patients.

MATERIALS AND METHODS

Clinical studies

A total of 63 unrelated patients were included in the study. Patients were seen mostly (but not exclusively) at the National Institutes of Health (NIH) Clinical Center from 1995 to the present time for evaluation and treatment of various tumors including PHEOs/PGLs. Diagnosis of all lesions was confirmed after surgical removal of the tumors and appropriate histology. The Institutional Review Boards of the participating institutions approved the studies: all patients provided written consents (and older children assented); those who were found to carry *SDHx* defects were counseled accordingly.¹⁸

IHC was performed in fixed specimens obtained from surgically excised tumors of the studied patients, as published previously.^{8,13} The diagnoses of PGL, PHEO, GIST or CHO were made by established criteria using the appropriate antibodies, respectively.^{4,8,13}

Genetic studies

DNA was obtained from peripheral blood lymphocytes (PBLs) or tumors, and extracted according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). For germline DNA testing, deletion analysis and direct bidirectional sequencing were used to analyze all coding regions and the flanking exon/intron junctions of the *SDHA* (OMIM: 600857, NM_004168.3), *SDHB* (OMIM: 185470, NM_003000.2), *SDHC* (OMIM: 602413, NM_003001.3), *SDHD* (OMIM: 602690, NM_003002.3) and *SDHAF2* (OMIM: 613019, NM_017841.2) genes, by standard methods as described previously.^{8,13} All detected variations were submitted to ClinVar, a public database, and can be found in <http://www.ncbi.nlm.nih.gov/clinvar/submitters/505422/> under the accession IDs SCV000222637–SCV000222642. The possible functional impact of an amino-acid change was predicted by two different *in silico* prediction tools: (a) the PolyPhen (Polymorphism Phenotyping) program (<http://genetics.bwh.harvard.edu/pph2/>), which calculates the position-specific independent count score that represents the probability that a substitution is damaging; values nearer to 1 are more confidently predicted to be deleterious; (b) the Sorting Intolerant From Tolerant (SIFT) (<http://sift.jcvi.org>) calculates normalized probabilities for all possible substitutions from the alignment. Positions with normalized probabilities <0.05 are predicted to be deleterious; those ≥0.05 are predicted to be tolerated. Prediction of splicing *in silico* was performed using the Berkeley Drosophila Genome Project (BDGP) (<http://www.fruitfly.org/seqtools/splice.html>) and the SplicePort analysis tool (<http://www.spliceport.org>). Patients with known variations were excluded. All the variations detected were compared with the TCA Cycle Gene Mutation Database.¹⁹

Tumor DNA studies were as published previously.^{5,13} In five patients with CTr, we also obtained comparative genomic hybridization (CGH) of PBL DNA, with methods that we have recently published.²⁰ There were no consistent or

informative changes beyond what has already been published⁵ and these data are not shown.

Relatives of the patients were screened for these genes only in the presence of a sequence variant in the studied gene or if there was suggestive clinical history; patients with PHEO only were also screened for all known PHEO-causing genes (data not shown). All GISTs have also been sequenced at least for exons 9, 11, 13 and 19 of the *KIT* and exons 12 and 18 of the *PDGFRA* gene and found to be negative.

Statistical comparisons between the total cohort of patients with CTr and those with *SDHx* variants were performed by *t*-test analysis for parametric data or χ^2 analysis for non-parametric comparisons.

RESULTS

The patient age range at diagnosis was 8–50 years (median, 23 years); 44 patients were 21 years old or younger. A total of 92% of the patients were female. GIST was located in the gastric area in all patients. Six patients (9.5%) had germline variants in *SDHA*, *SDHB* or *SDHC* (Table 1). The median age in these six patients was 21 years, whereas three were females ($P < 0.05$ compared with the total cohort).

Multiple synchronous GISTs were detected in 3/5 patients. The median diameter of the dominant tumor was 3.5 cm (2–15 cm). Histopathologic examination of GIST in all five patients showed reactivity for cKIT, whereas four had *SDHB* loss by IHC. Epithelioid histology was found in the GIST of three patients, whereas two had spindle histology in their GIST. The median mitotic rate per 50 hpf was 7.5 (5–14).

We were able to identify inheritance of PGL only in one family; there was no evidence of GIST (CTR-NIH593.01; Figure 1). The proband had incomplete CTr, with PGL and CHO (Figure 2). His mother and sister were carriers of the same *SDHB* defect, diagnosed with PGL/PHEO, and his nephew, also a carrier, was diagnosed with NB at the age of 3 years. A female patient with CTr and an *SDHC* mutation also had a ganglioneuroma, a tumor similar to NB.

Although PGLs or PHEOs were found in the six probands with CTr and *SDHx* variants, none reported symptoms related with functional PGL/PHEO (palpitations, high blood pressure, excessive sweating). Measurement of plasma and 24-h urine catecholamines in five of the studied five patients revealed no abnormalities (in one patient this measurement was never done). Thus far, none of the carriers, relatives of the CTr patients, have developed any tumors other than PGL/PHEO.

Three of the sequence defects were missense substitutions that led to amino-acid changes, which are highly conserved across species. One splice site variant was identified in *SDHC* at position +1 of intron 5 (c.405+1G>A). This appears to be a pathogenic mutation as has been reported previously in patients with PGL4 and in those with CSS, as well in those with sporadic *SDH*-deficient GIST; it is known to cause deletion of exon 5 and, therefore, results in a frameshift and premature termination of the protein. Two variants led to premature stop codons and are presumed pathogenic (Table 1). None of the variants was novel: all have been previously reported as affecting protein expression or function. None of the other 57 CTr patients had mutations, deletions or other genomic defects affecting the *SDHx* genes.

DISCUSSION

The present study of an expanded cohort of patients with CTr confirmed the data by Matyakhina *et al.*⁵ the vast majority of patients with CTr have no identifiable *SDHx* coding sequence defects. Nevertheless, the study also showed that, rarely, *SDHA*, *SDHB* and *SDHC* defects can be found in selected patients with CTr. Were there any differences in these patients with CTr compared with the total NIH cohort, including the ones studied by Matyakhina *et al.*⁵ First, the

Table 1 Clinical and molecular genetics of patients with Carney Triad and SDHx variants

Age ^a	Gender	Clinical findings					Molecular genetics								
		GIST	location	PGL	CHO	Other tumors	Tumor in other family carriers	Carney Triad ^b	Inheritance	Gene	Nucleotide/amino-acid change	Exon	Type	SDHB IHC	SDHA IHC
CTR-NIH808.01	21	M	G	No	Yes	No	No	I	No	SDHA	c.91C>T, p.(R31*)	2	NS	NEG	NEG
CTR-NIH72.02	23	F	G	No	Yes	No	No	I	No	SDHA	c.295C>T, p.(H99Y)	3	MS	NEG	NEG
CTR-NIH593.01	28	M	No	No	Yes	No	PGL CHO NB	I	Yes	SDHB	c.380T>G, p.(I127S)	4	MS	NA	NA
CTR-NIH602.03	20	F	G	No	Yes	Ganglioneuroma	No	I	No	SDHC	c.6del, p.(A3Rfs*4)	1	FS	NEG	POS
CTR-NIHITA	20	M	G	Yes	Yes	No	No	C	No	SDHC	c.224G>A, p.(G75D)	4	MS	NEG	NA
CTR-NIH12.03	13	F	G	No	Yes	No	No	I	No	SDHC	c.405+1G>A ^c , splicing	Intron 5/6	SS	NEG	POS

Abbreviations: GIST = gastrointestinal stromal tumor; PGL = paraganglioma; CHO = chondroma; NB = neuroblastoma; SS = splice site; FS = frameshift; MS = missense; NS = nonsense; M = male; F = female; G = gastric; POS = positive; NEG = negative; NA = not available.

^aAge at diagnosis.

^bCarney triad status (I = incomplete; C = complete).

^cWith reference sequence NG_012767.1, it is g.47466G>A; reference sequences: SDHA – NM_004168.3; SDHB – NM_003000.2; SDHC – NM_003001.3; SDHD – NM_003002.3.

similarities: they were equally young when they presented (at the age of 21 years *versus* 23 years for the whole cohort), their tumors were not more or less aggressive, and they are all alive at the time of this writing. However, unlike the rest of the CTr patients who are overwhelmingly female, those with *SDHx* defects were disproportionately male (50% *versus* 8%, $P<0.05$) following the pattern of other diseases associated with *SDHx* mutations that typically have no gender predilection.^{9,18,21} The other interesting finding was the association with other neural crest tumors that have not previously been associated with CTr: ganglioneuroma in one patient with *SDHC* variation and NB in the nephew of another, in association with the same *SDHB* defect; in this family, no other patient had CTr: the obligate carriers had PGL or PHEO. In other words, in accordance with all known CTr patients in whom the disease appears sporadically, even in these patients where *SDHx* mutations were present, CTr was never inherited in its full form.

A germline heterozygous missense *SDHB* variant (*SDHB*, c.600G>T, p.(W200C)) in a patient with PHEO and a calcified lung nodule has been reported, but there was never biopsy of the lesion to confirm the diagnosis of CHO.²² The combination of NB, PGL and renal cell carcinoma was first described by Fairchild *et al.*²³ Most recently, the case of a patient who was found to have at the same time malignant NB, PHEO and renal cell carcinoma was described; sequence analysis revealed an *SDHB* mutation.²⁴ In another patient with NB and a PGL, a germline *SDHB* gene deletion was found.²⁵ All in all, our two patients with *SDHx* variants and family occurrence of NB or co-occurrence in the same patient of ganglioneuroma confirm the possibility of an association of SDH deficiency with these neural crest tumors. It is noteworthy that none of the other CTr patients (that had normal *SDHx* coding sequence) had a similar tumor, whereas 2/6 (33.3%) CTr patients with *SDHx* defects had such an association; one could speculate that this finding could reflect a higher predisposition of CTr patients with *SDHx* germline variations to such neural crest lesions. Interestingly, too, only one of the *SDHx* variants in the present study affected splicing of the corresponding gene; it is possible that this may represent a difference with CSS, in which a higher number of splicing *SDHx* mutations has been identified.

What do the above tell us about the possible genetic defect(s) leading to CTr? CGH in tumors had previously shown copy number gains or losses in 1q, 1p and less frequently in the 14q and 22q chromosomal loci.⁵ Although the *SDHB* gene is located on chromosome 1p36.1–p35 and *SDHC* on 1q21–q23.3, deletions or coding sequence mutations had not been found. Now, the expanded CTr cohort confirms the involvement of the *SDHx* genes at the germline level in a small number of CTr cases.

The first time that SDH deficiency was implicated in the pathogenesis of CTr was when we found lack of immunoreactivity for SDHB in CTr tumors that had normal *SDHx* sequence.^{8,13} The recent identification of *SDHC*-specific methylation¹⁴ established further the connection between SDH deficiency and CTr. However, one could only speculate on what may be the defect in the vast majority of CTr cases with normal *SDHx* sequence: it could be defects in a molecule involved in SDH function, from a noncoding RNA to a protein that has not been studied yet, or it could even be aberrant genomic architecture in the vicinity of the *SDHx* genes that we have not detected using classic CGH or other whole-genome studies. It remains a mystery why the full spectrum of CTr is not inherited, even in the *SDHx*-mutated cases reported in the present study. This finding and the fact that the *SDHx* defects that we describe here have been reported previously in patients that did not have CTr suggest that perhaps multiple hits – beyond the known tissue-specific loss of the

Figure 1 Families with CTr and SDH subunit gene mutations.

Figure 2 Pulmonary chondroma of the patient with CTr and a germline *SDHB* mutation. The lesion was composed of hypocellular cartilage undergoing central osseous and fatty metaplasia. It was surrounded by a thick fibrous capsule (hematoxylin and eosin staining, $\times 40$).

normal *SDHx* allele – is needed to develop CTr; these additional hits may not be in the SDH enzyme but in other genes or genetic loci.

We conclude that CTr may, rarely, be associated with *SDHx* mutations that affect protein function. Although this finding does not change the fact that the vast majority of CTr patients get the disease from a yet elusive genetic defect, it does add significant information that needs to be considered in the genetic counseling of patients with CTr and their family members.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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