# Toward an improved definition of the genetic and tumor spectrum associated with *SDH* germ-line mutations

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The tricarboxylic acid, or Krebs, cycle is central to the cellular metabolism of sugars, lipids, and amino acids; it fuels the mitochondrial respiratory chain for energy generation. In the past decade, mutations in the Krebs-cycle enzymes succinate dehydrogenase, fumarate hydratase, and isocitrate dehydrogenase have been documented to be causally involved in carcinogenesis. This review is focused on the relationship between *SDH* mutations and the carcinogenic phenotype. The succinate dehydrogenase complex catalyzes the oxidation of succinate to fumarate; mutations in its subunits *SDHA*, *SDHB*, *SDHC*, and *SDHD*, and in the assembly factor *SDHAF2*, result in syndromes with distinct tumor types, including pheochromocytoma/paraganglioma, gastrointestinal stromal tumor, and, less often, renal-cell carcinoma

and pituitary adenoma. In this study we collected all previously reported *SDH* mutations with the aim of defining their nature and tumor spectrum. In addition, genotype–phenotype correlations as well as mechanisms of biallelic inactivation were analyzed in the *SDH*-deficient setting. Finally, we performed bioinformatics analysis using SIFT, Polyphen2, and Mutation Assessor to predict the functional impact of nonsynonymous mutations. The prediction of the latter was further compared with available SDHA and/or SDHB immunohistochemistry data.

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The tricarboxylic acid (TCA) cycle enzyme succinate dehydrogenase (SDH) is a heterotetramer protein complex consisting of four subunits encoded by nuclear genes. These include SDHA and SDHB, which form the catalytic domain, and SDHC and SDHD, which anchor the complex to the inner mitochondrial membrane.[1] The assembly factors, SDHAF1 and SDHAF2, ensure both structural and functional integrity of the complex.[2,3] SDH, also called mitochondrial complex II, is the only enzyme involved in both the electron transport chain and the TCA cycle, where it catalyzes the oxidation of succinate to fumarate.[1] The TCA cycle is central to the metabolism of sugars, lipids, and amino acids and is a major source of adenosine triphosphate in cells. In addition, the cycle also seems to be involved in tumorigenesis; enzymes of the TCA cycle are involved in the pathogenesis of several tumor types. *SDH* mutations have been involved in the etiopathogeny of pheochromocytomas (PCCs), paragangliomas (PGLs), gastrointestinal stromal tumors (GISTs), renal-cell carcinomas (RCCs), and pituitary adenomas (PAs).[1,2,4–9] In addition, mutations in fumarate hydratase (*FH*), another member of the TCA cycle and which catalyzes the hydration of fumarate to malate, predispose to tumor formation, including RCCs, cutaneous and uterine leiomyomas, and PCCs/PGLs.[10,11] Finally, isocitrate dehydrogenase (*IDH*), which catalyzes the oxidative decarboxylation of isocitrate, is frequently mutated in specific types of cartilaginous tumors,

hematological malignancies, and gliomas.[12–14] The currently known mechanisms underlying tumorigenesis linked to defects in the TCA cycle are well reviewed.[15,16] Defects in the *SDH*, *FH*, and *IDH* genes inhibit prolyl hydroxylases, leading to decreased hydroxylation of hypoxia-inducible factor-$\alpha$. This results in activation of the hypoxia pathway, which supports tumor formation by activating angiogenesis, glucose metabolism, cell motility, and cell survival. Furthermore, defects in these enzymes lead to epigenetic alterations through an accumulation of oncometabolites inhibiting $\alpha$-ketoglutarate-dependent dioxygenases, which are involved in DNA and histone demethylation. In addition to *SDH*-associated tumorigenesis, constitutional complex II deficiencies caused by *SDHA*, *SDHB*, *SDHD*, and *SDHAF1* mutations may also lead to Leigh syndrome, infantile leukodystrophies, and cardiomyopathy.[3,17–19]

In the current review, our aim is to report all currently known *SDH* mutations and define their nature and spectrum in *SDH*-related tumors, including PCCs/PGLs, GISTs, RCCs, and PAs, as well as in other unusual tumors arising in *SDH* mutation carriers. We performed bioinformatics analysis using SIFT, Polyphen2, and Mutation Assessor and compared the results with those of SDHA/SDHB immunohistochemistry (IHC) to predict the functional impact of nonsynonymous mutations. Finally, we explored and report here the nature of the second hit in all tumors arising in the SDH deficiency setting.

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## MATERIALS AND METHODS

The search for *SDH* mutations was performed using the Leiden Open Variation Database (http://www.lovd.nl/3.0/home) and publications indexed in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) from 2000, the year of the first published mutation in a paraganglioma, to April 2014.[1,2,4–6] The following search terms were used: *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2*, in combination with the tumor type names *pheochromocytomas*, *paragangliomas* (sympathetic and parasympathetic), *gastrointestinal stromal tumors*, *renal-cell carcinomas*, and *pituitary adenomas*.[20]

With the aim of predicting the functional impact of the *SDH* mutations on the corresponding proteins, a bioinformatics analysis was performed using the ANNOVAR software.[21] First, the variants found in the literature were checked in the Leiden Open Variation Database, and the genomic positions were obtained using Mutalyzer.[20,22] Then the variants were annotated using ANNOVAR with the following databases: hg19, RefSeq with HGVS as the nomenclature type, 1000 Genomes 2012APR, NonFlagged dbSNP138, CLINVAR20140211, and COSMIC68.[23–25] 1000 Genomes 2012APR and NonFlagged dbSNP138, describing only single-nucleotide polymorphisms with a frequency lower than 1%, were used to determine whether the changes were reported as single-nucleotide polymorphisms.[25,26] CLINVAR20142011 describes the associations between DNA variations and human health, whereas COSMIC68 encompasses somatic mutations in cancers.[27,28] Finally, the scores of SIFT, Polyphen-2, and Mutation Assessor were calculated by means of the ANNOVAR software to assess the potential functionally damaging impact of the amino acid changes.[29–31] The Consensus Deleteriousness software, which combines information derived from SIFT, Polyphen-2, and Mutation Assessor, was used to predict the functionality of nonsynonymous mutations.[32] In addition, we calculated the Combined Annotation Dependent Depletion score (http://cadd.gs.washington.edu/score), which is another predictor for the pathogenicity of an amino acid change and includes the previous prediction models.[33]

## RESULTS

### Pheochromocytomas and paragangliomas

In total, 445 different germ-line mutations in the *SDH* genes were reported in the literature. Of these, 403 mutations have been described in PCCs/PGLs: 211 (52%) in *SDHB*, 141 (35%) in *SDHD*, 42 (10%) in *SDHC*, 7 (2%) in *SDHA*, and 2 (1%) in *SDHAF2* (**Figure 1**). Missense mutations occurred most frequently in all genes, with the exception of *SDHD*, in which more frameshift mutations were observed (34%) (**Figure 1**). In total, 141 missense mutations (35%) and 104 frameshift mutations (26%) were observed in PCCs/PGLs (**Figure 1**). One somatic missense mutation has been identified in *SDHB* (c.299C>T, p.S100F). Seventy-eight mutations (19%) were described in malignant tumors: 59 (76%) in *SDHB*, 15 (19%) in *SDHD*, and 4 (5%) in *SDHC* (**Figure 2**). In these genes, no hotspot mutations were related to malignant behavior.

### Gastrointestinal stromal tumors, renal-cell carcinomas, and pituitary adenomas

Fifty-five germ-line mutations were identified in the GISTs, of which 26 (47%) occurred in *SDHA*, 14 (25%) in *SDHB*, 9 (16%) in *SDHC*, and 6 (11%) in *SDHD* (**Figure 1**). Missense mutations occurred most frequently in each gene except *SDHD*, in which frameshift mutations were the most common (34%; **Figure 1**). Thirty mutations were found in RCCs: 25 (83%) in *SDHB*, 3 (10%) in *SDHC*, and 2 (7%) in *SDHD* (**Figure 1**). Most mutations were missense mutations (47%). In addition, eight *SDH* mutations were reported in PAs: three (38%) occurred in *SDHD*, two each (25%) in *SDHB* and *SDHC*, and one (12%) in *SDHA* (**Figure 1**). Five of the PA-related mutations (62%) were missense. Three somatic mutations were observed in *SDHA*: one in a GIST (c.113A>T, p.D38V) and two in one PA (c.725_736del and c.989_990insTA).

### Mutations occurring in two or more tumor types

Fifty-three germ-line mutations have been described in more than one tumor type: 29 (55%) occurred in *SDHB*, 10 (19%) in *SDHC*, 10 (19%) in *SDHD*, and 4 (7%) in *SDHA* (**Figure 3a**). Twenty-three (44%) were missense mutations. In total, 19 mutations (36%) were observed in PCCs/PGLs and RCCs, 16 (30%) caused PCCs/PGLs and GISTs, and 6 (11%) were found in PCCs/PGLs and PAs. In addition, five mutations (9%) were reported in PCCs/PGLs, GISTs, and RCCs. Furthermore, five mutations (9%) were identified in at least one causally related tumor type (PCC/PGL, GIST, RCC, and PA) and one unusual tumor type (thyroid carcinoma, uterine myoma, breast cancer, and neuroblastomas), and two mutations (4%) have been identified in the four causally related tumors (**Figure 3a**). Seventeen different mutations—of which seven were missense—that caused multiple causally related tumors (PCCs/PGLs, GISTs, RCCs, and PAs) in single patients were identified: seven (41%) occurred in *SDHB*, seven (41%) in *SDHD*, and three (18%) in *SDHC* (**Figure 3b**).

### Mutations occurring exclusively in GISTs, RCCs, or PAs

Of 445 different germ-line mutations reported in the *SDH* genes, 39 (9%) were exclusively found in GISTs, RCCs, or PAs and not in PCCs/PGLs: 24 (61%) occurred in *SDHA*, 8 (21%) in *SDHB*, 5 (13%) in *SDHC*, and 2 (5%) in *SDHD* (**Figure 4a**). In addition, 33 mutations (85%) were observed in GISTs, 5 (13%) in RCCs, and 1 (2%) in PAs (**Figure 4a**). In total, 44% of the mutations reported exclusively in GISTs, RCCs, and PAs were missense (**Figure 4a**).

### Mutations associated with tumors occurring rarely in the context of SDH deficiency

Twenty-three different germ-line *SDH* mutations were identified in patients with other tumor types, including thyroid carcinomas, neuroblastomas, and breast cancer, in addition to their PCC/PGL. Of these 23 mutations, 9 (39%) occurred in *SDHB*, 8 (35%) in *SDHD*, 4 (17%) in *SDHC*, and 2 (9%) in *SDHA* (**Figure 4b**). In total, 35% were missense mutations (**Figure 4b**).
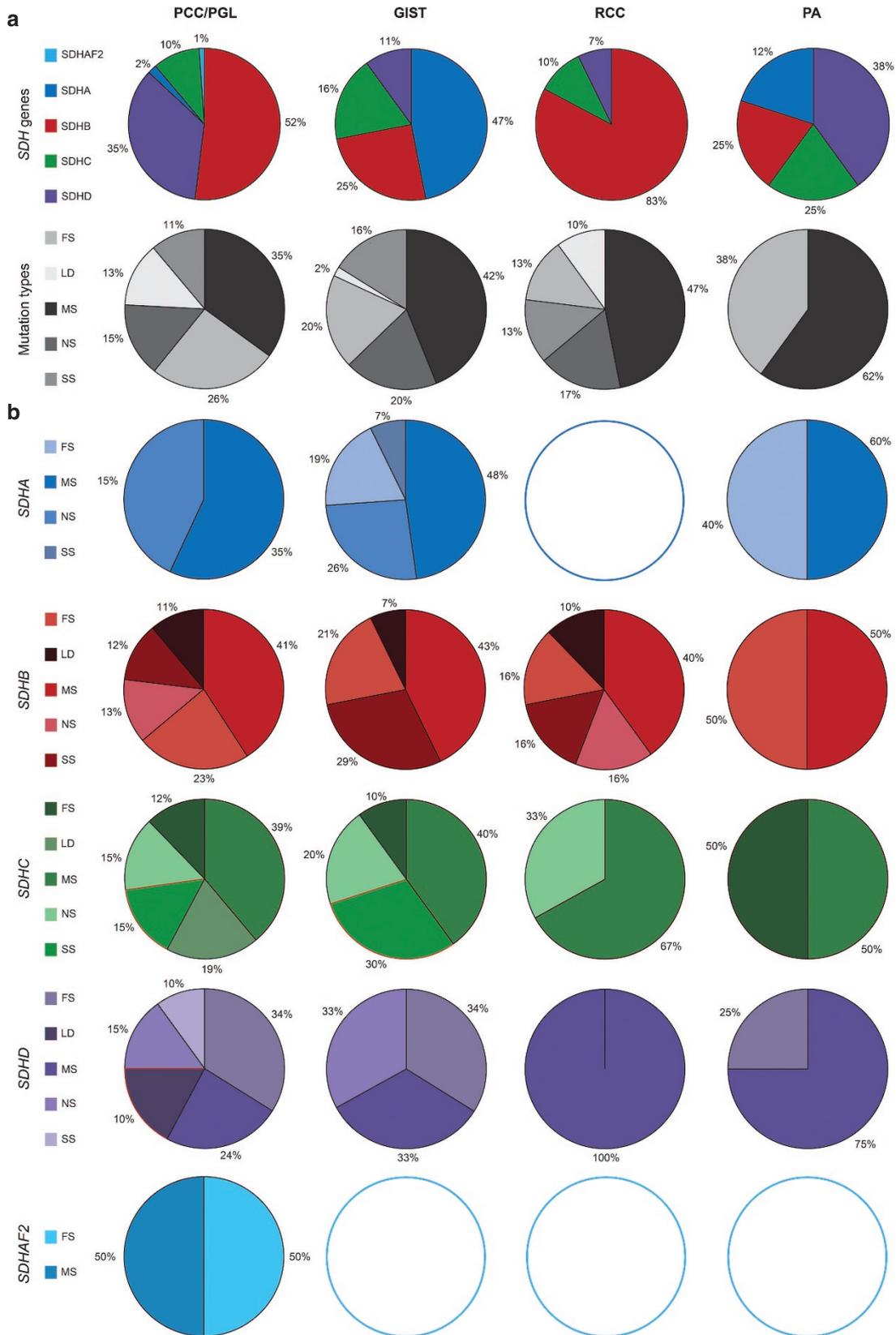
**Figure 1 Spectrum and distribution of *SDH* germ-line mutations as reported in *SDH*-deficient tumors including pheochromocytomas (PCCs)/paragangliomas (PGLs), gastrointestinal stromal tumors (GISTs), renal-cell carcinomas (RCCs), and pituitary adenomas (PAs).** (**a**) Percentages of mutations in each *SDH* gene and of the different types of mutations: frameshift deletion/duplication/insertion (FS), large deletion (LD), missense (MS), nonsense (NS), and splice site (SS). (**b**) Percentages of the different mutation types for each *SDH* gene.
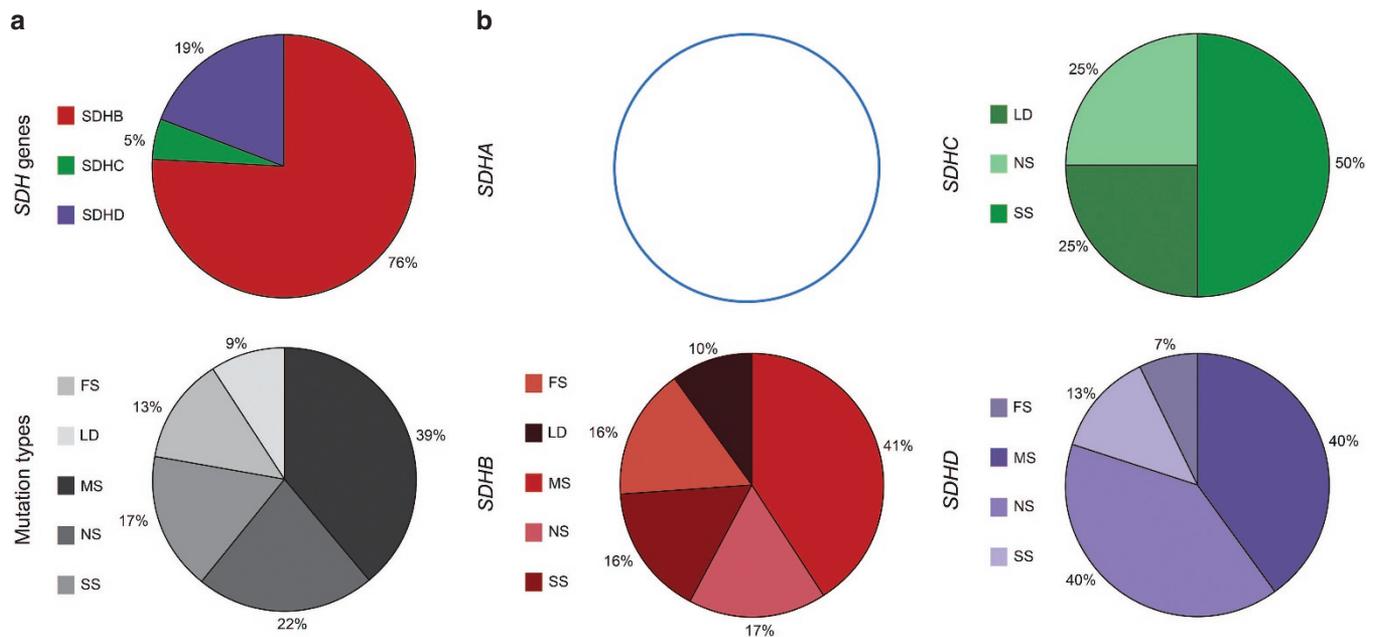
**Figure 2 Spectrum and distribution of *SDH* germ-line mutations as reported in malignant pheochromocytoma (PCC)/paragangliomas (PGL).** (**a**) Percentages of mutations in each *SDH* gene and of the different types of mutations reported in malignant PCCs/PGLs. (**b**) Percentages of different mutation types identified in malignant tumors for each *SDH* gene.

Establishing the causal link between these mutations and these tumors was not possible because IHC data were not reported. However, one study reported a patient suffering from a PGL and a testicular seminoma who had an *SDHD* mutation (c.129G>A, p.W43X) and showed loss of the wild-type (WT) allele in the tumor (**Supplementary Table S2** online).

**In silico analysis of the functional impact of *SDH* mutations**
An in silico analysis was performed to predict the functional impact of each *SDH* nonsynonymous mutation. Three scores were assessed: SIFT, Polyphen2, and Mutation Assessor. In addition, the mutations were associated with the immunohistochemical results of the SDHA and SDHB staining because this technique has been proven to reliably predict the presence of an *SDH* mutation: A negative *SDHB* staining is predictive of the presence of a mutation in any *SDH* subunit, with a sensitivity of 100% and a specificity of 84%, whereas a negative SDHA staining predicts an *SDHA* mutation with a sensitivity of 100% and a specificity of 97%.[34,35] The IHC results were available for 145 previously reported mutations. In the vast majority (134, or 92%), the IHC pattern was consistent with the prediction obtained by bioinformatics (**Supplementary Table S1** online). A total of 129 mutations were predicted to be damaging to the protein and showed consistent negative SDHA and/or SDHB staining (**Supplementary Table S1** online). The remaining five mutations were predicted to be nondeleterious and showed positive SDHA and/or SDHB staining (**Supplementary Table S1** online). Nevertheless, some exceptions were reported. Two *SDHA* mutations reported in GISTs (c.113A>T, p.Asp38Val and c.1969G>A, p.Val657Ile) were predicted to be nondamaging but showed negative SDHA and SDHB staining (**Table 1**).

Moreover, nine mutations predicted to be deleterious had positive SDHA and/or SDHB IHC results, although they were expected to have negative results (**Table 1**). For the mutations showing nonconsistent IHC results, we calculated the Combined Annotation Dependent Depletion.[33] A Cscore of 10 indicates that the variant is part of the 10% most damaging substitutions in the human genome. Considering as damaging a variant with a Cscore ≥15 is therefore suggested (http://cadd.gs.washington.edu/info). The Consensus Deleteriousness and the Combined Annotation Dependent Depletion scores reach the same prediction for each mutation.

**Second hit**
The nature of the second hit has been reported for 85 *SDH* mutations (**Supplementary Table S2** online). Inactivation of the WT allele by loss of heterozygosity (LOH) has been observed 62 times (73%) and by somatic mutations in 12 cases (14%). In addition, eight mutations (9%) co-occurred with retention of heterozygosity, and epigenetic inactivation of the second allele was reported once. A combination of multiple second hits has been found for two *SDHA* mutations: c.91C>T, p.Arg31* and c.1873C>T, p.His625Tyr (**Supplementary Table S2** online).

## DISCUSSION
In this review, we present an overview of all currently reported *SDH* mutations and analyze potential genotype–phenotype correlations with regard to the development of neoplasia. In addition, we performed bioinformatics analysis on the functional impact of the reported *SDH* mutations and compared the results with available SDHA and SDHB IHC results. Furthermore, we explored the nature of the second hit observed
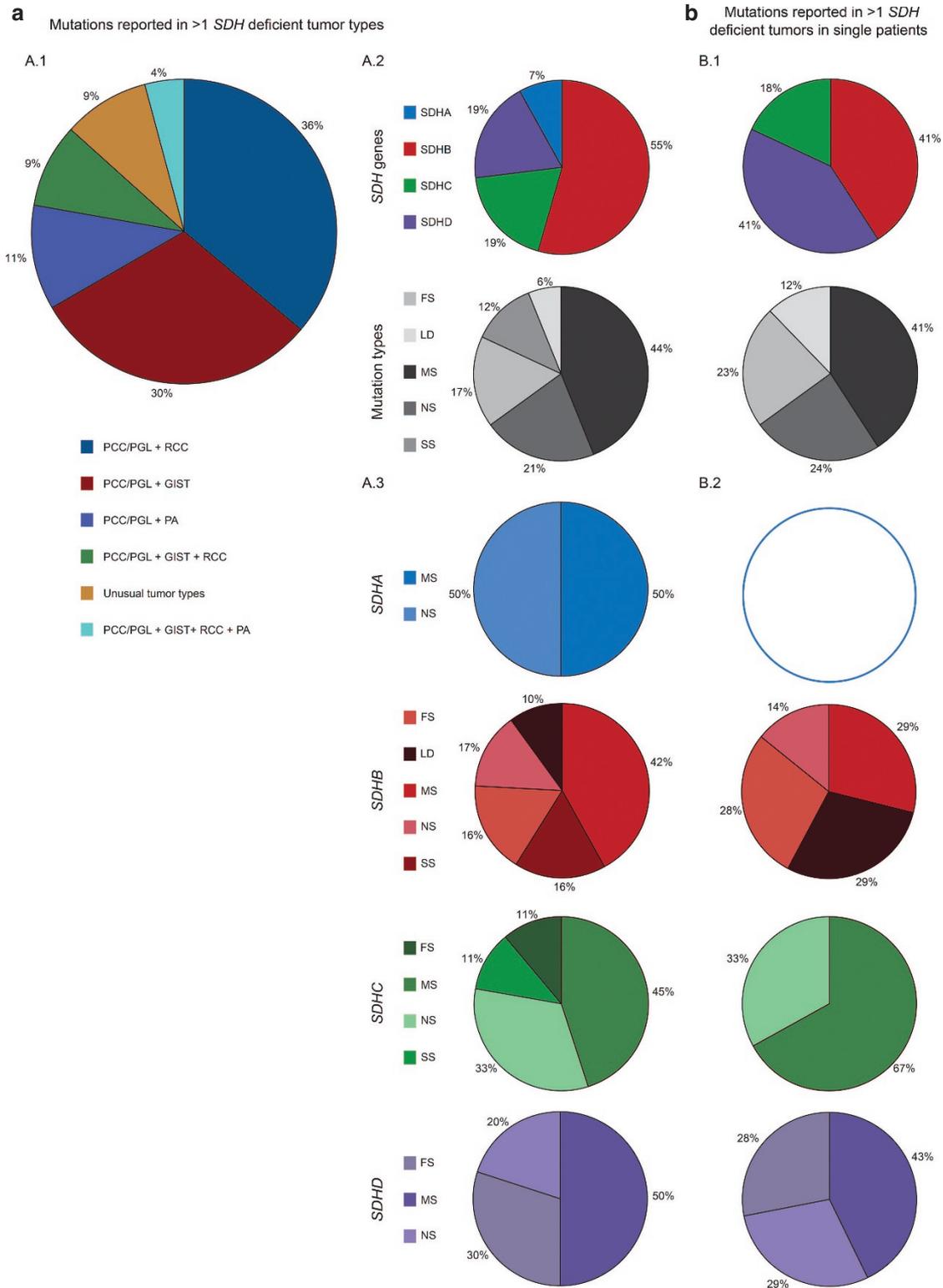
**Figure 3 Spectrum and distribution of *SDH* germ-line mutations reported in more than one *SDH*-deficient tumor type.** (**a**) Mutations causally related to more than one *SDH*-deficient tumor type: percentages of mutations observed in the different tumor types (A.1); percentages of mutations in each *SDH* gene and of the different types of mutations (A.2); and percentages of the different mutation types for each *SDH* gene (A.3). (**b**) Mutations harbored by single patients suffering from more than one *SDH*-deficient tumor type: percentages of mutations in each *SDH* gene and of the different types of mutations (B.1) and percentages of the different mutation types for each *SDH* gene (B.2).
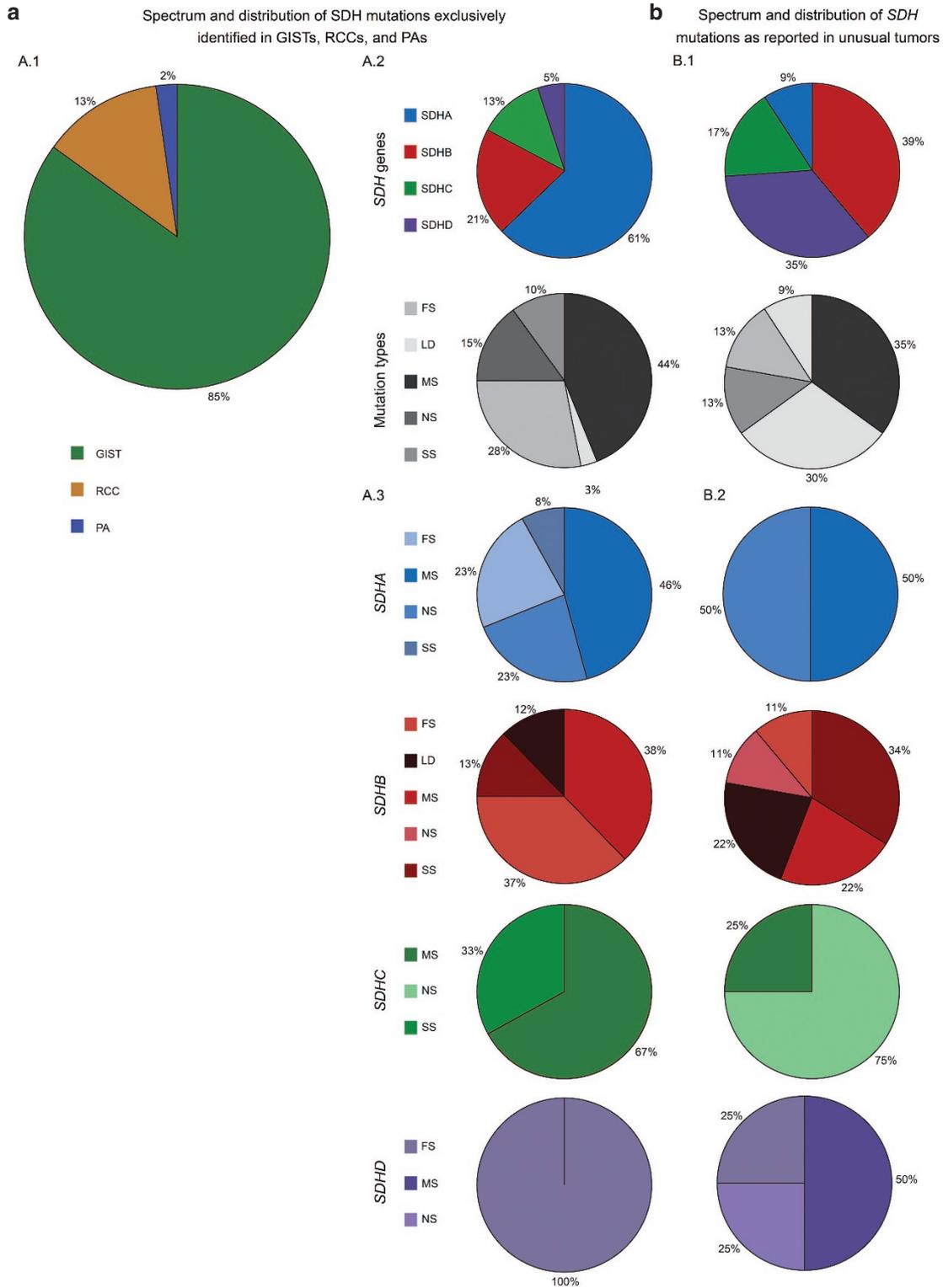
**Figure 4  Spectrum and distribution of *SDH* germ-line mutations exclusively identified in gastrointestinal stromal tumors (GISTs), renal-cell carcinomas (RCCs), or pituitary adenomas (PAs) and as reported in tumors occurring rarely in the context of *SDH* deficiency.** (**a**) Spectrum and distribution of *SDH* mutations exclusively identified in GISTs, RCCs, and PAs: percentages of mutations as reported only in GISTs, RCCs, and PAs in each of those tumor types (A.1); percentages of the mutations reported in the related tumor types except pheochromocytomas (PCCs)/paragangliomas (PGLs) in each *SDH* gene and of the different types of mutations (A.2); and percentages of the different mutation types identified in the related tumor types except PCCs/PGLs for each *SDH* gene (A.3). (**b**) Spectrum and distribution of *SDH* mutations as reported in tumors occurring rarely in the context of SDH deficiency: percentages of mutations in each *SDH* gene and of the different types of mutations reported in different tumors including thyroid carcinomas and neuroblastomas (B.1), and percentages of different mutation types identified in these different tumors for each *SDH* gene (B.2).

in tumors arising in both germ-line and somatic *SDH* mutation carriers.

## Genotype–phenotype correlation analysis

Two genotype–phenotype correlations were highlighted. The first was the high frequency of *SDHB* mutations in malignant PCCs/PGLs: 76% of the mutations identified in malignant cases occurred in *SDHB* (**Figure 1a**). The association between *SDHB* mutations and malignancy has been reported many times, but only a few studies provide mechanistic insights.[36–40] Taking the results of those studies together, the association between malignancy and *SDHB* mutations might be due to a hypermethylator phenotype that was observed in *SDHB*-mutated PCCs/PGLs in particular.[37] This hypermethylation would lead to a high epigenetic silencing of genes that play a role in differentiation and in the epithelial-to-mesenchymal transition.[36,37] By contrast, *SDHA* mutations seem to show no association with malignancy. This could be because of the small numbers of *SDHA*-mutated cases known to date; only seven different *SDHA* mutations have been identified in PCCs/PGLs, and there is a lack of follow-up of patients with apparently benign disease (**Figure 1a**).

The rarity of *SDHA*-associated PCC/PGL might be related to the low frequency of 5p15 loss seen in PCC and PGL, which is the chromosomal location of *SDHA*. By contrast, PCC and PGL show high frequencies of loss of 1p36 and 11q23, the chromosomal areas where *SDHB* and *SDHD* are located, respectively.[6]

The second strong genotype–phenotype correlation concerns the association of GIST with *SDHA*; 47% of the mutations reported in this tumor type occurred in *SDHA* (**Figure 1a**). It was recently shown that *SDHA* mutations occur in ~30% of *SDH*-deficient GISTs. These GISTs occur in patients at an older age and have a lower female-to-male ratio than other *SDH*-deficient malignancies. No differences in histology between *SDHA* WT and mutant GISTs have been discerned.[41] Most *SDH*-deficient GISTs encompass tumors arising in Carney–Stratakis syndrome and the Carney triad, in pediatric GISTs, and in a subset of apparently sporadic KIT/PDGFRA WT GISTs.[41,42] These GISTs are characterized by unique clinicopathological features and biological properties: (i) female preponderance; (ii) gastric location (predilection for the distal stomach/antrum); (iii) common multifocality; (iv) a multinodular/plexiform growth pattern; (v) epithelioid cytomorphology, either pure or combined with a spindle-cell component; (vi) SDHA and/or SDHB immunonegativity; (vii) KIT (and DOG1) immunopositivity despite the lack of KIT/PDGFRA mutations; (viii) metastatic potential (often to lymph nodes); (ix) a relatively indolent clinical course, even in the presence of metastatic disease; and (x) insensitivity to imatinib.[41,42]

With regard to RCCs and PAs, the number of reported mutations is too low (30 and 10, respectively) to draw reliable conclusions about any potential genotype–phenotype correlations, although it must be noted that 83% of the mutations identified in RCCs occurred in *SDHB* (**Figure 1a**). *SDH*-deficient RCC is a unique subtype of RCC that has been recently accepted as a provisional entity in the 2013 International Society of Urological Pathology Vancouver Classification.[43] *SDH*-deficient RCCs display a slight male predisposition, present as bilateral tumors in 25% of cases, and have stereotypical morphologic features, that is, solid or focally cystic growth, uniform cytology with eosinophilic flocculent cytoplasm, intracytoplasmic vacuolations and inclusions, round to oval low-grade nuclei, and SDHB immunonegativity.[44,45] SDHA-negative IHC was reported once, but no mutational screening of *SDHA* was performed.[44] Despite the fact that *SDH*-deficient RCC is capable of progression, metastatic disease is rare in the absence of high-grade nuclear atypia or coagulative necrosis.[44,45]

Finally, *SDH*-deficient PAs seem likely to be prolactin-secreting (and/or prolactin-immunopositive) and, to a lesser extent, growth hormone–secreting macroadenomas. They are usually accompanied by a personal history of PCC/PGL and have a slight male predisposition.[46,47]

## In silico analysis association with SDHA/SDHB IHC

The prediction of the impact of an *SDH* mutation was associated with available SDHA and/or SDHB IHC data, as SDHA/SDHB IHC is predictive of the presence of an *SDH* mutation. Indeed, a negative staining for SDHB on IHC indicates a mutation in any of the *SDH* subunits, whereas a negative SDHA staining on IHC exclusively predicts the presence of an *SDHA* mutation. In the majority of cases, bioinformatics analysis, predicting the effect of nonsynonymous mutations, was consistent with the available IHC results (**Supplementary Table S1** online). However, some exceptions were reported. Two previously reported *SDHA* mutations (c.113A>T; p.Asp38Val and c.1969G>A; p.Val657Ile) were predicted as nondeleterious and had a Cscore lower than 15 but were negative for SDHA and SDHB[48,49] (**Table 1**). The p.Val657Ile mutation was reported in COSMIC, and both mutations have been reported in a sample population: p.Asp38Val; rs34635677; minor allele frequency: T = 0.012/26 and p.Val657Ile; rs6962; minor allele frequency: A = 0.161/351 (dbSNP; http://www.ncbi.nlm.nih.gov/SNP/). These discrepancies could reflect the fact that these variants might affect the epitope recognized by the SDHA antibody, explaining the absence of staining on IHC.[48,49] However, this is highly unlikely because both SDHA and SDHB are negative on IHC, indicating a deleterious effect of the variant on the protein.[48,49] Finally, these discrepancies could also be due to staining artifacts. Nonconsistent IHC results were also observed for 9 mutations predicted as damaging and having a Cscore higher than 15 but having positive IHC results (**Table 1**). Three discrepancies occurred in *SDHA* and showed SDHB-negative and SDHA-positive IHC (c.562C>T; p.Arg188Trp, c.818C>T; p.Thr273Ile, and c.1361C>A; p.Ala454Glu).[41,50] Two other *SDHA* mutations (c.778G>A; p.Gly260Arg and c.1873C>T; p.His625Tyr) were positive for SDHB and SDHA on IHC.[51,52] Another one was in *SDHC* (c.380A>G; p.His127Arg), and the last three were in *SDHD* (c.14G>A; p.Trp5*, c.149A>G; p.His50Arg, and c.274G>T; p.Asp92Tyr).[46,53–55] Among these *SDHD* mutations, one resulted in a stop codon (p.Trp5*), which cannot be managed by the prediction tools we used but can be considered to

**Table 1** *SDH*-related tumors showing discrepancies between in silico analysis and SDHA/SDHB immunohistochemistry

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CADD (Cscore) | 10.49 | 4.173 | 21.1 | 19.19 | 18.76 | 18.37 | 20.5 | 20.8 | 13.95 | 17.45 | 23.6 |
| CONDEL | Neutral (0.25) | Neutral (0.259) | Deleterious (1.11) | Deleterious (1.098) | Deleterious (0.977) | Deleterious (0.988) | Deleterious (1.003) | Deleterious (1.029) | — | Deleterious (0.782) | Deleterious (0.96) |
| IHC (SDHB/SDHA) | Neg/neg[47] | Neg/neg[46] | Neg/pos[43] | Pos/pos[50] | Neg/pos[43,48] | Neg/pos[43] | Pos/pos[49] | Pos[52] | Pos/pos[53] | Pos/pos[54] | Pos/pos[51]; neg/neg[44] |
| Mutation Assessor | Low (1.24) | Low (1.07) | High (5.185) | High (5.045) | High (3.63) | High (3.755) | High (3.94) | High (4.24) | — | Medium (2.62) | Medium (3.435) |
| Polyphen2 | Benign (0.002) | Benign (0.123) | Damaging (1.0) | Damaging (1.0) | Damaging (1.0) | Damaging (1.0) | Damaging (1.0) | Damaging (1.0) | — | Damaging (0.804) | Damaging (1.0) |
| SIFT | Tolerated (0.57) | Tolerated (0.62) | Damaging (0) | Damaging (0) | Damaging (0) | Damaging (0) | Damaging (0) | Damaging (1.0) | — | Tolerated (0.13) | Damaging (0) |
| Tumor type | GIST | GIST | GIST | PCC/PGL | GIST | GIST | Colonic polyps | Papillary thyroid carcinoma | PCC/PGL | PCC/PGL | PA |
| Mutation | p.Asp38Val | p.Val657Ile | p.Arg188Trp | p.Gly260Arg | p.Thr273Ile | p.Ala454Glu | p.His625Tyr | p.His127Arg | p.Trp5* | p.His50Arg | p.Asp92Tyr |
| Protein subunit | SDHA[a] | SDHA | SDHA | SDHA | SDHA | SDHA | SDHA | SDHC | SDHD | SDHD | SDHD |

CADD, Combined Annotation Dependent Depletion; CONDEL, Consensus Deleteriousness software; GIST, gastrointestinal stromal tumor; PA, pituitary adenoma; PCC, pheochromocytoma; PGL, paraganglioma; SDH, succinate dehydrogenase.

aSomatic mutation. The predictions were tabulated with a short description and the corresponding score according to the different tools. (i) SIFT: damaging or tolerated, with a reliability score ranging from 0 to 1; (ii) Polyphen2: damaging or benign, with a score ranging from 0 to 1; (iii) Mutation Assessor, with four scores: high (3.5–5.5), medium (2–3.4), low (1–2), and neutral (<1). Neg, negative immunohistochemical result; pos, positive immunohistochemical result. The CONDEL score can be neutral (0–0.5) or deleterious (0.5–1). The last column contains the CADD score: deleterious (Cscore >15) and nondeleterious (Cscore <15).

be damaging because the mutation results in a severely truncated protein. This nonsense variant was identified in a patient suffering from Carney–Stratakis syndrome, with the PGL showing positive SDHB on IHC and the GIST presenting with a negative SDHB on IHC, potentially indicating two different second hit events in the *SDHD* gene.[54] Another *SDHD* mutation, p.Asp92Tyr, was reported in two patients affected by PAs; one PA showed an SDHB-negative IHC and the other PA still expressed SDHB.[46] Nevertheless, this variant was observed in many PCCs/PGLs that had corresponding SDHB-negative IHC.[34] Therefore, these few discrepant results might be due to staining artifacts, a wrong interpretation, an undetected mutation, or another mechanism of inactivation.

Although SDHA and SDHB IHC reliably identifies the presence of *SDH* mutations, the previous findings show that positive staining has been observed in tumors with mutations predicted as damaging. Therefore, in addition to IHC, bioinformatics analysis should be used as a tool to support the definition of the pathogenicity of *SDH* variants of unknown significance. Functional studies could provide more proof to identify the pathogenicity of uncertain variants.

### Mechanisms of biallelic inactivation

*SDH* genes function as tumor suppressor genes (TSGs) with LOH as the predominant mechanism of inactivation (and somatic mutation less frequently) of the WT allele (**Supplementary Table S2** online). However, different mechanisms, including biallelic inactivating *SDH* mutations and epigenetic silencing, can coexist (e.g., *SDH*-deficient GISTs)[48,49,56–60] (**Supplementary Table S2** online). Notably, two sisters with an *SDHA* mutation (c.91C>T, p.Arg31*) were affected by SDHA/SDHB-immunonegative GISTs; one displayed LOH, whereas the other showed retention of heterozygosity, probably indicating a different mechanism of inactivation, such as promoter methylation of the WT allele.[60] However, an undetected mutation seems to be the most likely mechanism. In addition, haploinsufficient and/or dominant negative effects for the *SDH* genes could be another mechanism of inactivation and have been indicated in particular cases: (i) bilateral adrenal medullary hyperplasia associated with a germ-line *SDHB* mutation (c.587G>A, p.Cys196Tyr) showing retention of heterozygosity[61]; (ii and iii) SDHA/SDHB immunonegative GIST and PA in patients with *SDHA* mutations (c.91C>T, p.Arg31*; c.1873C>T, p.His625Tyr) displaying either retention of heterozygosity or paradoxical loss of the mutated *SDHA* allele, respectively[51,60]; (iv) PCCs without loss of the WT *SDHD* allele arising in patients with *SDHA* mutations (c.341A>G, p.Tyr114Cys; c.441delG, p.Gly148Alafs*20)[62]; and (v) somatic *SDHD* inactivation associated with consistent reduction of transcript levels in neural crest–derived, neuroendocrine, and gastrointestinal tumors.[38,63] A recent report showed that somatic *SDHC* hypermethylation might be the cause of the Carney triad, a multitumoral syndrome of unknown etiology affecting five organs: paraganglia (PGL), adrenal gland (adrenocortical adenoma and PCC), lung (chondroma), stomach (GIST), and esophagus

(leiomyoma).[64,65] This suggests that epigenetic silencing of *SDH* could also be an important mechanism involved in tumor development and could explain SDH deficiency in the absence of germ-line mutations.

Tumorigenesis caused by haploinsufficiency seems unlikely because *SDHD*, *SDHD/H19*, and *SDHB* knockout mice, as well as conditional or inducible tissue-specific *SDHD* knockouts, do not develop tumors or any other genotype-related pathology except for a slight carotid body hyperplasia[66–69] (Judith Favier, personal communication).

### Tumor suppressor effect in *SDH*-related tumorigenesis

TSG-related, tissue-specific tumorigenesis may be attributed to a complex interplay, but novel evidence points to a model of TSG loss–driven tumorigenesis, whereas haploinsufficiency might act synergistically with oncogenic signals in a tissue-specific manner.[70–72] With regard to *SDH*-related oncogenesis, it has been suggested that the tumor phenotypes could be affected by the size of *SDH* deletions because they can encompass closely located TSGs and compromise their function, which could lead to unusual phenotypes.[73,74] In addition, some research suggests that germ-line and somatic interactions could account for the clinical variability observed among carriers of an identical *SDH* germ-line mutation, including those within the same family.[46,75–77] In this context, the occurrence of *SDH*-related tumors is extremely rare in the absence of a germ-line mutation. Indeed, only a few cases have been reported: one extra-adrenal PGL harboring a somatic *SDHB* mutation (c.299C>T, p.Ser100Phe) and a somatic *SDHD* mutation in a PCC (c.242C>T, p.Pro81Leu), both displaying LOH; one PA harboring two somatic *SDHA* mutations (c.725_736del; c.989_990insTA); and tumors from four patients with the Carney triad showing a DNA hypermethylation of *SDHC*.[47,65,78,79]

Although familial PCC/PGL syndromes were thought to predispose only to PCCs and PGLs, different tumors such as GISTs, RCCs, and PAs have expanded the tumor spectrum associated with *SDH* mutations.[77] No patient has developed all tumor types, indicating an incomplete penetrance of the syndrome similar to that seen in the Carney triad.[64] To explain the issue of cell specificity, Hoekstra and Bayley[69] pointed toward differences of cell types either in their requirements for adenosine triphosphate, redox agents, and TCA cycle–generated metabolic intermediates during development and/or throughout life or in their response to cancerous transformation. Importantly, both *SDH* mutant PCCs/PGLs as well as GISTs have been recently shown to display a hypermethylator phenotype, exposing a vital interplay among succinate metabolism, epigenetic disruptions, and tissue-specific tumorigenesis.[37,65,80]

### Conclusion

Familial PCC/PGL syndromes were initially thought to predispose only to PCCs and PGLs, but several tumors, including GISTs, RCCs, and PAs, have expanded the *SDH*-associated tumor spectrum. Extensive clinical variability can be expected, even among carriers of an identical *SDH* germ-line mutation, with tumor phenotypes being only partially expressed, as in the Carney–Stratakis dyad.

Previous reports of genotype–phenotype correlations showed that *SDHB* mutations are associated with a high risk of malignancy, and *SDHD* mutations are associated with head-and-neck PGL.[81,82] Our results show an additional strong genotype–phenotype correlation, as *SDHA* mutations seem to be associated with *SDH*-deficient GISTs.

In addition, the absence of SDHA and/or SDHB expression in tumor tissue on IHC is an indicator of the functional absence of one of the *SDH* genes. Therefore, SDHA/SDHB IHC, along with in silico analysis and tumor LOH analysis, seem to be valuable tools in determining the potential pathogenicity of *SDH* variants and therefore have significant implications in the new era of comprehensive next-generation sequencing–based approaches for the analysis of PCC/PGL susceptibility genes. Although questions about some incidental cases remain, based on data from the current comprehensive literature, we demonstrated that the *SDH* genes behave as bona fide TSGs with biallelic inactivation through combinations of germ-line mutations, somatic mutations, LOH, and epigenetic silencing, contributing to a specific spectrum of malignancies.

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