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Re-evaluation of the concept of basaloid follicular hamartoma associated with naevoid basal cell carcinoma syndrome: a morphological, immunohistochemical and molecular study

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ABSTRACT

Naevoid basal cell carcinoma syndrome (NBCCS) is a rare genodermatosis caused by germline mutations in genes of the Sonic Hedgehog (SHH) pathway and is characterised by early onset of multiple basal cell carcinomas (BCCs). Although skin tumours with follicular differentiation, notably basaloid follicular hamartoma (BFH), have been reported in NBCCS, their relations with BCC are poorly defined. In this context, the aim of this study was to clarify morphological, immunohistochemical and molecular features of BFH arising in a context of NBCCS. A total of 140 skin tumours from NBCCS and 140 control BCC tumours were reviewed, blinded to clinical data and classified as BCC or BFH. The morphological characteristics of these two groups were then compared. Twenty cases were submitted for immunohistochemical and molecular analysis. Thirty-three tumours among the exploratory cohort were classified as BFH and were exclusively detected in NBCCS patients. Histopathological criteria that were significantly different from BCC were as follows: a small size (<1.5 mm), connection to a hair follicle, arborescent organoid architecture, lack of cytological atypia and infundibulocystic differentiation. Immunohistochemical analysis confirmed activation of the SHH pathway in these lesions. Targeted next-generation sequencing suggested that *MYCN* and *GLI2/3* amplifications and *TP53* mutations might be involved in progression of these follicular tumours to BCC. Our study confirms the high prevalence of BFH, representing up to 24% of skin tumours in NBCCS and potentially being BCC precursors.

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1. Introduction

Naevoid basal cell carcinoma syndrome (NBCCS)/Gorlin syndrome (OMIM # 109400) is a rare autosomal dominant inherited genodermatosis, with a prevalence estimated between 1 in 31,000 and 1 in 164,000 in Europe.[1,2] Although prior cases might have been reported in the literature,[3] the initial identification of NBCCS as a syndrome was made in 1960 by Gorlin and Goltz.[4] NBCCS is characterised by multiple basal cell carcinomas (BCCs) and/or odontogenic keratocysts of the jaw at a young age in combination with other alterations such as palmar or plantar pits, lamellar calcification of the falx cerebri and medulloblastoma.[2,5,6]

Genetically, NBCCS is caused by germline mutations in genes of the Sonic Hedgehog (SHH) pathway, notably *PTCH1* or *SUFU*, with *SUFU* mutations being less prevalent than *PTCH1* mutations and associated with an increased risk of medulloblastoma.[7] As observed in sporadic BCC and medulloblastoma cases, inactivation of *PTCH1* or *SUFU* gene function leads to the activation of the SHH pathway, finally resulting in tumour formation.[8]

BCC is the only skin tumour currently considered as an NBCCS diagnostic criterion;[5] however, Gorlin *et al.* mentioned in their initial report a combination of trichoepithelioma and BCC in their patients with 'all gradation of activity' from trichoepithelioma to BCC.[4] Accordingly, in transgenic mice models, inactivation of the *Ptch1* mimicking NBCCS genotype led to the formation of microscopic, nascent 'basaloid follicular hamartoma (BFH)-like' tumours, whereas additional secondary genetic hits including *GLI1, GLI2* and *MYCN* amplifications and inactivation of *TP53* were required for macroscopic BCC formation.[9] In line with these findings, recent studies reported high prevalence of BFHs in NBCCS,[10–13] suggesting that BFHs constitute a potential additional diagnostic criterion for NBCCS.[12–14] However, microscopic criteria defining BFH, as well as whether these tumours belong to the same spectrum as BCC or are distinct entities, are controversial.[15–19]

In this context, the aim of this study was to clarify morphological, immunohistochemical and molecular features of BFH arising in the context of NBCCS.

2. Methods

2.1. Patients

Skin tumours from NBCCS patients were diagnosed as BCC between 2004 and 2021 in the Department of Pathology, Hospital of Tours (Local Ethics Committee in Human Research, Tours, France; no. ID RCB 2009-A01056-51). Inclusion criteria of the NBCCS tumour samples were as follows: tumour arising in a patient with a confirmed diagnosis of NBCCS, as previously described[2,6] (Supplementary Table 1), tumours classified as BCC at the initial diagnosis time, and available material for histological examination. After a pathological review was performed for the current study, cases in which the diagnosis was neither BCC nor tumour with follicular differentiation were excluded. A previously described cohort of skin tumours from patients with Gorlin syndrome[11] (Hospital Cochin, Paris) was used for the validation series. Control cases of both exploratory and validation cohorts from non-NBCCS patients including the first 160 BCC and 20 trichoblastoma consecutive cases diagnosed after 1 January 2021 were extracted from the archives of the Department of Pathology, Tours Hospital.

Among all included cases, 140 randomly selected skin tumours from all NBCCS patients diagnosed in the Hospital of Tours as well as 140 cases of sporadic BCC were included in the exploratory cohort (Fig. 1). Twenty

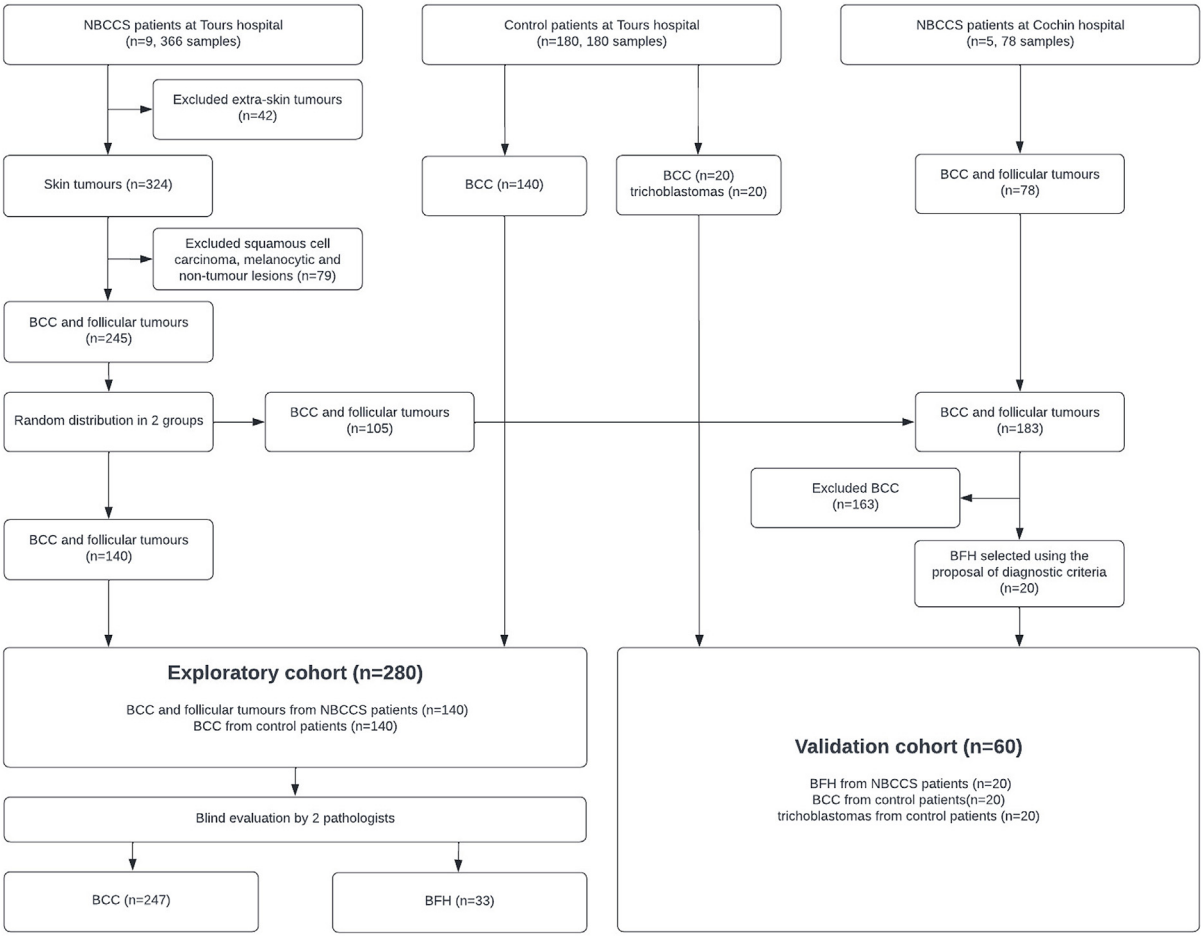

Fig. 1. Flow chart. BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma; NBCCS, naevoid basal cell carcinoma syndrome.

cases of BFH arising in NBCCS patients, 20 sporadic BCC cases and 20 sporadic trichoblastoma cases were included in the validation series.

2.2. Design of the study

Although BCC is the only skin tumour currently included in the criteria for NBCCS diagnosis,[6] several publications reported higher incidence of BFH[4,14,20,21] in NBCCS patients than in the general population. Therefore, based on the literature[16,21–23] and their own experience, a panel of three senior dermatopathologists (SF, MB and BC) established a list of morphological features (Supplementary Table 2), which might be relevant to distinguish BFH in NBCCS patients from sporadic BCC.

Two independent pathologists (MCM and TK), blinded to the clinical data, then (1) classified all cases of the exploratory cohort (including 140 tumours from NBCCS and 140 sporadic BCC from control patients) as BCC or BFH depending on their own appreciation (holistic approach) and (2) evaluated the presence of the previously proposed criteria irrespective of the diagnosis (Supplementary Table 2). When several types of tumours were present on the same slide, only BFH cases were considered. Discordant cases were reviewed collegially. BCC cases were classified as superficial, nodular and infiltrative according to the recently proposed simplified histopathological classification of BCC.[15] The morphological features of the two groups (BFH vs BCC) were compared, and diagnosis performances of these criteria to distinguish one from the other were established using the positive likelihood ratio (LHR). A diagnosis criterion was considered as relevant if the p value was <0.05 and the LHR >5.[24]

In order to validate the performance of criteria established from the exploratory cohort, the latter were assessed by an independent set of pathologists (PS, MLJ, NM, FB, IM, SLR and LD), distinct from those who examined the exploratory cohort, on an independent validation cohort of 60 cases, consisting of BFH from NBCCS patients (n=20), as well as BCC (n=20) and trichoblastomas (n=20) from control patients. The diagnosis had been validated by the first set of dermatopathologists (SF, MB and BC). The evaluation was made on microphotographs using an online platform https://www.survio.com/en/. To this aim, a list of diagnostic criteria as well as illustrations (Supplementary Fig. 1) was provided. Evaluators were asked to indicate whether or not (yes or no) the tumour fulfilled the criteria.

Additional immunohistochemical and molecular characterisation was then conducted on 10 cases of BFH as well as 10 cases of BCC from NBCCS patients, with sufficient formalin-fixed paraffin-embedded (FFPE) material.

2.3. Clinical data

Age, sex and location of the primary tumours were collected from patient files.

2.4. Immunochemistry

Immunohistochemical staining with cytokeratin 20 (CK20), CD10, EpCAM, PHLDA1 and p53 was assessed using a Benchmark XT Platform as instructed. GLI1 staining[25,26] was performed manually. Antibodies and dilutions are displayed in Supplementary Table 3. CK20 was used to evaluate the presence of Merkel cells in the tumour.[18,27,28] The CD10 expression was evaluated on both tumour cells and stroma.[28,29] Intensity of EpCAM staining, a well-established marker of BCC, was classified as diffuse or heterogeneous.[30] Expression of PHLDA1, a hair follicle stem-cell marker, was evaluated on the percentage of positive cells (0, <20%, 20–50%, 50–80%, >80%).[31,32] p53 expression was evaluated as previously described:[33] wild-type profile (heterogeneous expression) or mutated profile, either loss of expression (<5%) or overexpression (>70%).

2.5. Molecular analysis

Molecular analysis was performed on 10 BFHs and on 10 BCCs from five and seven NBCCS patients, respectively. Due to material limitations (small BFH size, sample too old or not sufficient for analysis), we did not perform paired analysis. FFPE tissues were microdissected by needle sampling. DNA samples were then submitted to massive parallel sequencing using a custom-designed panel ATLAS (SureSelect XT HS, Agilent), covering 538 genes. Sequencing was performed on a Nextseq 550 or 2000 System (Illumina), and bioinformatics analysis was based on a skin tumour panel of 92 genes (Supplementary Table 4) using an in-house bioinformatic pipeline allowing detection of single-nucleotide variations (SNVs), copy number variations (CNVs), tumour mutation burden (TMB) and microsatellite instability score estimation.

The selection of the detected SNV was made according to the following criteria: (1) variant allele frequency >5%, (2) at least 10 reads supporting the alteration, (3) frequency in the general population databases (1000G and GnomAD) less than 1%, (4) recurrence in an in-house variant database <20%, (5) strand bias with torrent server metrics <0.95, and (6) variant not described as benign or likely benign in the ClinVar database. Alterations were taken into consideration if at least three of the four bioinformatics prediction algorithms (CADD, Mutation Taster, SIFT and Polyphen2) were in favour of a pathogenic variation (or two of four if the variation was associated with a COSMIC or ClinVar identifier ‘pathogenic’ or ‘likely pathogenic’).

2.6. Statistical analyses

Continuous data were described by medians and ranges and categorical data with numbers and percentage of interpretable cases. Associations were assessed using two-tailed Fisher exact tests for categorical data. A p value <0.05 was considered statistically significant. The diagnostic accuracy of index tests was compared with the reference standard by using the positive LHR as a measure of accuracy, combining sensitivity and specificity. Index tests with a positive LHR >5 were considered efficient. Agreement between evaluators was assessed with the percent agreement and Fleiss Kappa coefficient, interpreted using the standard Landis and Koch.[34]

3. Results

3.1. High prevalence of BFH in NBCCS patients

Nine NBCCS patients were included in the exploratory cohort. Briefly, all cases had a history of multiple BCCs with more than 100 tumours in one case (case #1), four patients had prior history of odontogenic keratocysts and a medulloblastoma arose in one case (#1). A genetic characterisation of the germline mutation was available in four patients revealing mutations in *PTCH1* and *SUFU* in three cases (#1, #3, #4) and one case (#5), respectively. Clinical features of these patients are available in Supplementary Table 5.

To confirm that skin tumours with follicular differentiation morphologically distinct from that of BCC are recurrently observed in NBCCS patients, 140 skin tumours initially diagnosed as BCC from the NBCCS patients were compared to 140 sporadic BCCs, whilst blinded to the clinical data (see Fig. 1). Clinical and microscopic features of both groups are reported in Supplementary Table 6. Among this exploratory cohort of 280 cases, 33 tumours were classified as BFH by the two pathologists after microscopic examination. Microscopic features of these tumours are detailed in Figure 2, Table 1 and Supplementary Figure 1. Importantly, such lesions were exclusively observed in NBCCS cases (24% of the samples in this population), confirming that tumours with a follicular differentiation morphologically distinct from sporadic BCC are recurrently observed in this population.

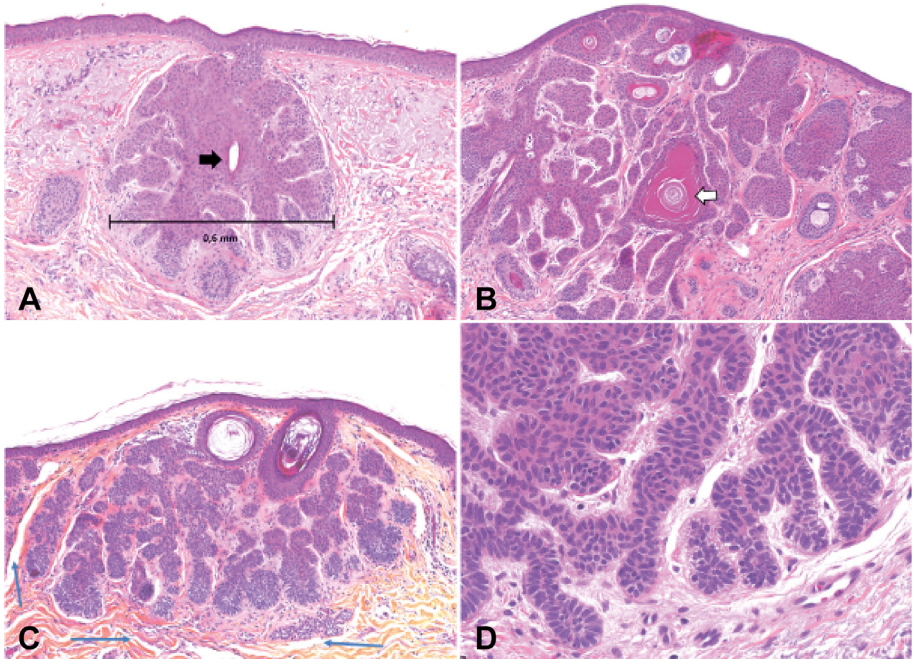

Fig. 2. Proposal for basaloid follicular hamartoma diagnostic criteria. These tumours are characterised by a small size (<1.5 mm), an organoid arborescent architecture, symmetric silhouette centred on a hair follicle (A, black arrow), frequent infundibulocystic differentiation (B, white arrow), stromal–dermal clefting (C, blue arrows), bland cytology with no nuclear palisading and low inflammation.

3.2. Identification of morphological criteria to distinguish BFH from BCC

Comparison of these BFH with BCC either sporadic or from NBCCS patients (Table 1) revealed that the BFHs observed in NBCCS were smaller lesions ($p<0.001$), frequently centred on a hair follicle ($p<0.001$). These tumours harboured an arborescent organoid architecture with anastomosing strands and cords ($p<0.001$) of non-atypical/eosinophilic cells ($p<0.001$). Infundibulocystic differentiation ($p<0.001$) and retraction cleft between stroma and dermis ($p<0.001$) were more frequently observed in these cases than in BCC, whereas peripheral palisading ($p<0.001$) and inflammation ($p<0.001$) were less often detected in this subset. An association with a BCC on the same slide was observed in 54% of the BFH cases ($n=18/33$) (Supplementary Fig. 2). Subgroup analysis comparing BFH to either sporadic BCC or BCC arising in the context of NBCCS is available in Supplementary Table 7.

Of note, additional comparison of BCC in controls and NBCCS patients further revealed a higher incidence of superficial BCC in the NBCCS patients, whereas the nodular BCCs were predominant in the control patients ($p<0.001$). NBCCS BCCs were also smaller than sporadic cases ($p=0.003$), with a more frequent infundibulocystic differentiation ($p=0.048$), less frequent necrosis ($p=0.031$) or inflammation ($p<0.001$) (Supplementary Table 8).

3.3. BFH recognition is relevant to the NBCCS diagnosis

To evaluate whether the morphological identification of BFH in current practice might contribute to the recognition of NBCCS patients, diagnostic performance of the morphological criteria determined in the present study was assessed individually using an LHR, with only criteria harbouring an LHR >5 considered as relevant (Table 1, Fig. 2 and Supplementary Fig. 1). To this purpose, a second independent panel of dermatopathologists ($n=6$) were asked to determine whether the cases were BFH or not using the proposed diagnostic criteria in a second independent validation series including 20 BFHs arising in NBCCS patients, 20 sporadic BCCs and 20 follicular tumours (trichoblastomas) (Fig. 3). Considering the majority response of the pathologists as a consensus, sensitivity and specificity of our criteria were 60% and 100%, respectively. Moreover, the percentage of interobserver agreement was 75%, with a κ coefficient of 0.683 ($p<0.0001$) reflecting a substantial agreement and confirming the reproducibility of our criteria.

3.4. BFH arising in NBCCS harboured activation of the SHH pathway and immunohistochemical features distinct from BCC

The high prevalence of BFH in NBCCS patients in comparison to the general population strongly suggested that development of these tumours is related to the genetic characteristics of the NBCCS, i.e., germline mutations activating the SHH pathway. Thus, to confirm that development of these neoplasms is related to this activation, we then evaluated by immunohistochemistry the expression of GLI1, the main downstream effector of the SHH pathway. Such analysis revealed strong and diffuse expression of GLI1 (Supplementary Fig. 3) in all BFH cases.

We then evaluated whether such BFH might harbour immunohistochemical features distinct from BCC. Indeed, numerous immunohistochemical markers have been proposed to distinguish BCC from adnexal tumours with follicular differentiation.[28,29,32] In this context, to further characterise the phenotype of BFH, expression of some of these markers, i.e., CK20, CD10, EpCAM, PHLDA1 and p53, was assessed in this population ($n=10$) using BCC as controls ($n=10$). Results are shown in Table 2 and Supplementary Fig. 4.

The CK20 staining highlighted the presence of Merkel cells only in BFH (four cases with 10, 21, 45 and 50 cells/mm^2). The CD10 stained either stroma (five cases) or tumour cells (three cases) in BFH, whereas only tumour cells were positive in BCC. The EpCAM staining demonstrated more frequently a focal pattern in BFH (seven cases, 70%) than BCC [one case within which the positivity was focal (10% of positive cells)]. PHLDA1 was heterogeneous in both intensity and distribution in both groups. The p53 staining was overexpressed in favour of a mutated profile only in BCC cases (five cases, 50%).

Table 1
Clinical and morphological features of BFH and BCC tumours included in the exploratory cohort

	BFH (n=33)	BCC (n=247)	p value	Se/Sp	LHR[a] (95% CI)
Size <1.5 mm					
Yes	23 (70%)	42 (17%)	<0.001	0.35/0.95	**7.6 (3.8–15.1)**
No	10 (30%)	205 (83%)			
Connection with the epidermis					
Yes	27 (82%)	229 (93%)	0.048	0.11/0.75	0.4 (0.2–0.9)
No	6 (18%)	18 (7%)			
Intra-epidermal component					
Yes	0 (0%)	6 (2%)	1	0/0.88	–
No	33 (100%)	241 (98%)			
Connection with a hair follicle					
Yes	22 (67%)	8 (3%)	<0.001	0.73/0.96	**16.7 (9–30.9)**
No	11 (33%)	239 (97%)			
Arborescent architecture					
Yes	26 (79%)	36 (15%)	<0.001	0.42/0.97	**13.1 (6–28.6)**
No	7 (21%)	211 (85%)			
Cleft between tumour and stroma					
Yes	13 (39%)	166 (67%)	0.003	0.07/0.80	0.4 (0.2–0.7)
No	20 (61%)	81 (13%)			
Cleft between stroma and dermis					
Yes	16 (48%)	18 (7%)	<0.001	0.0.47/0.93	**6.8 (3.8–12.2)**
No	17 (52%)	229 (93%)			
Peripheral palisading					
Yes	5 (15%)	206 (83%)	<0.001	0.41/0.98	**17.1 (6.9–42.6)**
No	28 (85%)	41 (17%)			
Infundibulocystic differentiation					
Yes	26 (79%)	72 (29%)	<0.001	0.27/0.96	**6.9 (3.1–15.3)**
No	7 (21%)	175 (71%)			
Stromal inflammation					
Yes	6 (18%)	194 (79%)	<0.001	0.34/0.97	**11.3 (4.8–26.2)**
No	27 (82%)	53 (21%)			
Pigment					
Yes	4 (12%)	58 (23%)	0.181	0.13/0.94	2.1 (0.8–5.6)
No	29 (88%)	189 (77%)			
Necrosis					
Yes	2 (6%)	45 (18%)	0.086	0.13/0.95	3.13 (0.8–12.6)
No	31 (94%)	202 (82%)			
Nuclear atypia					
Yes	7 (21%)	243 (98%)	<0.001	0.87/0.97	**31 (14.7–65.1)**
No	26 (79%)	4 (2%)			
Monster cells					
Yes	1 (3%)	5 (2%)	0.532	0.12/0.83	0.7 (0.1–4.3)
No	32 (97%)	242 (98%)			
Mucin					
Yes	5 (15%)	76 (31%)	0.068	0.14/0.93	2.3 (0.9–5.7)
No	28 (85%)	171 (69%)			
Calcification					
Yes	2 (6%)	28 (11%)	0.55	0.12/0.93	1.9 (0.5–7.4)
No	31 (94%)	219 (89%)			
Cholesterol crystals					
Yes	0 (0%)	13 (5%)	0.376	0.12/1	–
No	33 (100%)	234 (95%)			

BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma; LHR, likelihood ratio; Se, sensitivity; Sp, specificity.
[a] The LHR is calculated from the underlined criterion.

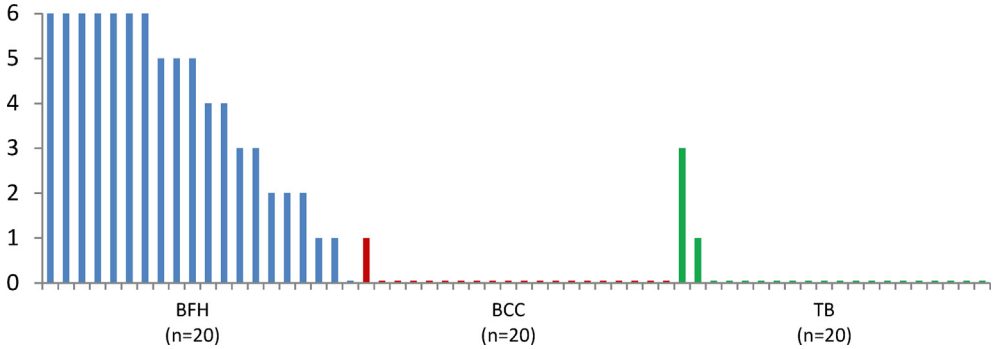

Fig. 3. Evaluation of diagnostic criteria on a validation cohort. Sixty cases including basaloid follicular hamartomas (BFHs) (n=20), sporadic basal cell carcinomas (BCCs) (n=20) and sporadic trichoblastomas (TBs) (n=20) were evaluated by an independent set of dermatopathologists (n=6). For each case, the number of evaluators who consider that the tumour fulfilled the proposed diagnostic criteria is shown.

Table 2
Immunohistochemical features of BFH and BCC

	BFH (n=10)	BCC (n=10)	p value
Cytokeratin 20			
(number of Merkel cells/mm^2)			
31–60	2	0	0.087
16–30	1	0	
6–15	1	0	
1–5	0	0	
0	6	10	
CD10			
Tumour cells	3	7	0.011
Stroma	6	0	
No	1	3	
EpCAM			
Diffuse	3	9	0.02
Heterogeneous	7	1	
No	0	0	
PHLDA1			
(percentage of positive cells)			
>50%	0	0	0.15
20–50%	8	3	
<20%	1	3	
0	1	4	
p53			
Wild-type profile	10	5	0.033
Mutated profile	0	5	
GLI1			
Diffuse	9	10	1
No	0	0	
Unavailable	1	0	

BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma.

3.5. Amplification of MYCN and GLI2/3 and TP53 mutations may contribute to the transformation from BFH to BCC

To determine whether BFH shared the same genetic background as BCC or may harbour a specific genetic profile, DNA from 10 BFHs and 10 BCCs arising in the same NBCCS patients were submitted to targeted next-generation sequencing (Fig. 4). Such analyses revealed high TMB in all cases, with a mean TMB value of 58.6 mutations per megabase for BFH and 65.5 mutations per megabase for BCC. Among the whole cohort, most common point mutations were observed in *PTCH1* (n=10), *KMT2D* (n=10), *FAT1* (n=8), *SUFU* (n=7), *NF1* (n=7), *LATS1* (n=7), *TP53* (n=6), *NOTCH1* (n=6) and *PIK3CA* (n=6) genes. Recurrent CNVs such as amplification of *MYCN* (n=6) and *CCND1* (n=5) or loss of *RASA1* (n=3) were detected. Interestingly, mutations in the *NOTCH3* (n=3), *PIK3CG* (n=3), *BRAF* (n=2), *PDGFRA* (n=2) and *TRAF7* genes (n=2) were the recurrent alteration detected only in BFH (n=3) and not in BCC. By contrast, recurrent pathogenic alterations restricted to the BCC were mutations in *CARD11* (n=3) and amplification in *MYCN* (n=6), *GLI 2* (n=3) and *FGFR3* (n=2). Moreover, *GLI3* amplification was detected in one BCC and not in BFH. *TP53* mutation was detected in five BCCs and only in one BFH (p=0.05). Among these alterations, only *MYCN* (p=0.01) amplification was statistically more prevalent in BCCs than in BFHs.

4. Discussion

BCC is the only skin tumour currently recognised as a NBCCS diagnostic criterion[6] and mentioned in the NBCCS chapter of the current World Health Organization classification.[35] However, several publications, including the initial description by Goltz and Gorlin, reported in

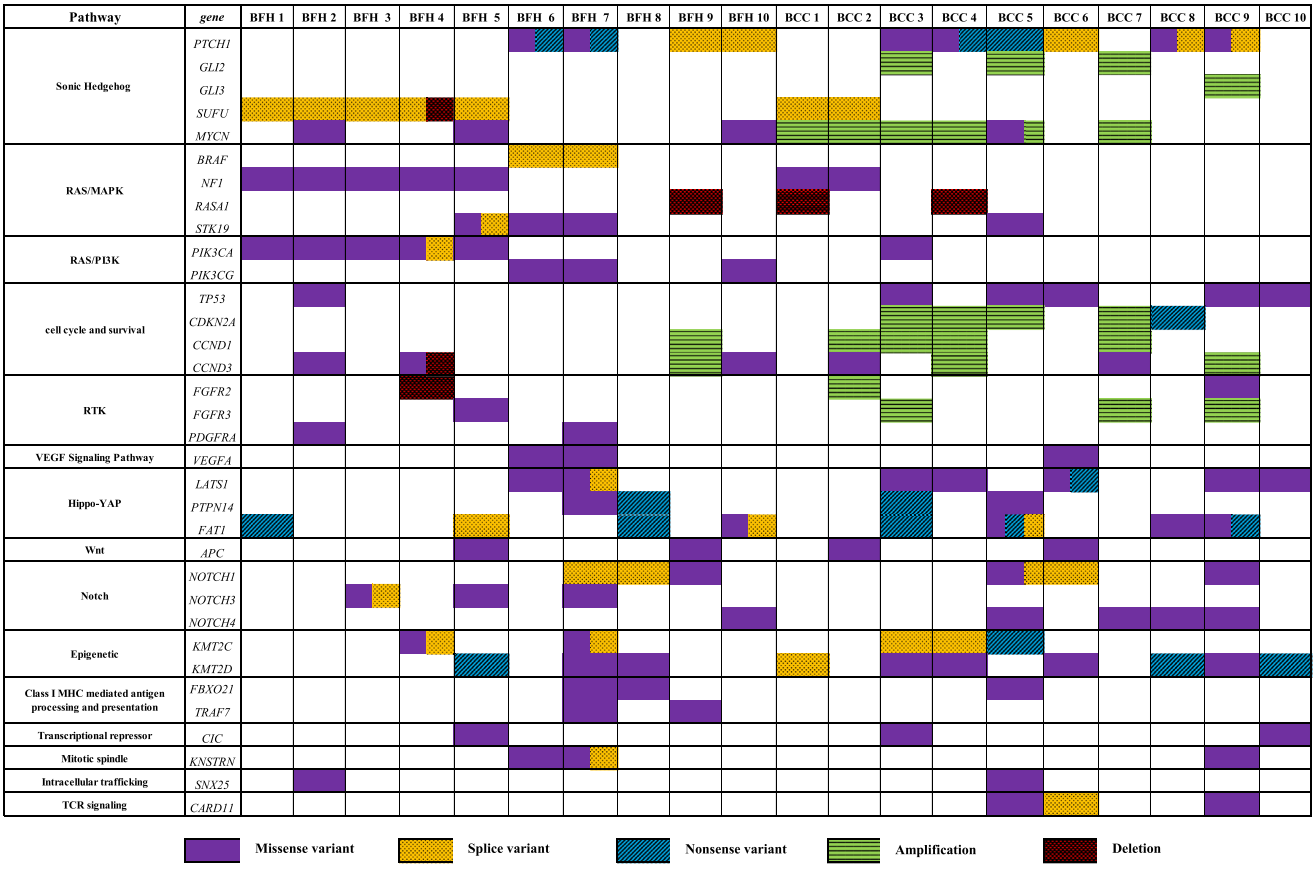

Fig. 4. Molecular features of 10 BFH and 10 BCC cases. BCC, basal cell carcinoma; BFH, basaloid follicular hamartoma.

addition to BCC the recurrent onset of tumours with follicular differentiation in NBCCS patients, either referred as trichoepithelioma or follicular basaloid hamartoma.[4,12,21,23] The present study confirmed that BFHs represent up to 24% of skin tumours in NBCCS and further suggested that recognition of BFHs in current practice might contribute to NBCCS recognition. In this setting, it is interesting to note that all of these cases were initially diagnosed as BCC by the first pathologists, and over the same period we did not identify any tumours diagnosed as BFH, trichoepithelioma or trichoblastoma in this population. These findings might be explained by the fact that Gorlin syndrome was mentioned by the physician in most of the cases, and it is likely that the initial pathologists were prompt to classify the tumours as BCC due to this specific context. However, when searching for cases diagnosed as trichoblastoma/trichoepithelioma in the general population in our institution during the inclusion period used for the control group, only 10 cases were identified, again suggesting that tumours with follicular differentiation are more prevalent in NBCCS patients than in the general population.

In the initial description of BFH by Brownstein in 1992, the author reported isolated and multiple/familial forms of a tumour characterised by: 'small, symmetric, usually 1 to 2 mm in diameter, slightly raised, well-demarcated epithelial proliferations limited to the upper half of the dermis and covered by normal epidermis. Within the dermis were thin anastomosing strands and thicker cords of well-differentiated squamoid cells (about 90%) and basaloid cells (about 10%).'[16] However, this description is close to the one provided by Walsh and Ackerman 2 years earlier for infundibulocystic BCC, and accordingly these authors promptly replied to Brownstein that their tumours were actually infundibulocystic BCCs[17] and that cases with multiple tumours were in fact NBCCS patients. Indeed, a generalised form sometimes congenital or associated with myasthenia gravis and alopecia,[36–38] a unilateral linear variant[39,40] or an isolated form[41,42] of BFH have been reported. Whilst no genetic characterisation of sporadic lesion is currently available, recent sequencing analysis of linear[43] and generalised[12,44,45] variants of BFH revealed frequent alterations of the SHH pathway, suggesting that BFH and BCC including the infundibulocystic variant belong to a unique spectrum.

Indeed, mutations of *PTCH1*, *SMO* or *SUFU* resulting in the uncontrolled activation of the SHH pathway are the genetic hallmarks of BCC.[46,47] Physiologically, the SHH pathway is notably required in the skin for hair follicle development.[48] Accordingly, inactivation of *PTCH1* or activation of *SMO* in the epidermis of transgenic mice[8,49–51] result in both BCC and trichoepithelioma-like tumour formation, with the latter harbouring close morphological similarities with BFH observed in NBCCS patients. In this context, frequent association in NBCCS patients of BFH and BCC[4] strongly suggests that BFHs are actually BCC precursors and that BFH to BCC transformation is dependent on the accumulations of additional alterations.[9] Of note, inactivation of *SUFU* or presence of an oncogenic *SMO* mutation in transgenic mice led to the development of 'BFH-like' tumours without subsequent BCC formation.[52,53] Accordingly, Trieu *et al.* recently demonstrated that inactivation of *PTCH1* led to the formation of microscopic 'nascent BCC-like proliferations' without tumour formation in mice skin. They also observed that additional amplification of *MYCN* and *GLI1* and *GLI2* as well as *TP53* mutations are required for the formation of macroscopic tumours similar to BCC, with these findings being in accordance with previous demonstration of lower SHH activation levels in BFH than in BCC.[53,54] In line with these results, we observed *MYCN* and *GLI2/3* amplifications in BCC, suggesting that in addition to the *PTCH1/SUFU* mutations, these acquired secondary events may enhance SHH activity, thereby contributing to the transformation from BFH to BCC.

5. Conclusions

To conclude, our study confirms the recurrent onset of BFH in NBCCS patients. Since no consensual definition has been available until now, herein we characterised the morphological, immunohistochemical and genetic features of these BFHs and established a list of diagnostic criteria. Evaluation and validation of these criteria on an independent cohort suggest that they might be used by pathologists in routine practice and therefore might contribute to better recognition of NBCCS tumours.

Ethics approval

The local Ethics Committee of Human Research of Tours (France) approved the study (no. ID RCB2009-A01056-51).

Conflicts of interest and sources of funding

The authors state that there are no conflicts of interest to disclose. This work was supported by Société Française de Dermatologie (Bourse Recherche).

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Supplementary data

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