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Hepatobiliary Conditions

Congenital hepatic hemangiomas: Clinical, histologic, and genetic correlation☆,☆☆

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ABSTRACT

Background: The guide for monitoring and treatment of congenital hepatic hemangiomas (CHH) will depend on the subtype and the postnatal clinical behavior. Our aim is to present a series of CHH and characterize its clinical, histologic and genetic correlation, compared to cutaneous congenital hemangiomas (CCH).
Material and methods: A retrospective review of CHH patients diagnosed between 1991 and 2018 was performed. Clinical, morphological and histological data were analyzed and deep high-throughput sequencing was performed.
Main results: Sixteen patients with CHH were included. Five patients were followed up with serial ultrasounds while pharmacological treatment (corticosteroids and propranolol) was decided in five. Surgical resection was performed in five owing to hemorrhage and suspicion of malignancy, and the last patient underwent embolization. Histologic analysis was available in 7 patients and confirmed CHH, showing two different histological patterns that could be associated with the presence of somatic pathogenic variants in *GNAQ* and/or *PIK3CA* detected in the genetic testing. Review of 7 samples of CCH revealed some histologic differences compared to CHH.
Conclusion: CHH resemble its cutaneous homonym with similar clinical behavior. Histologic analysis can differentiate two subgroups while genetic testing can confirm mutations in *GNAQ* and in *PIK3CA* in a subset of CHH.
Type of study: Treatment study.
Level of evidence: IV

Hemangiomas are the most frequent benign liver tumors in childhood [1]. The term "hemangioma" has been used for a variety of hepatic vascular anomalies or hypervascular tumors in the past, including the so-called hepatic hemangiomas in adults which are in fact venous malformations. This misuse of the term "hemangioma" has led to confusion in diagnosis, treatment and outcome of the true hepatic hemangiomas in children. In addition, the former term "infantile hemangioendothelioma of the liver" applied to the pediatric population by the pathologists has also contributed to even more confusion among clinicians. The International Society for the Study of Vascular Anomalies (ISSVA) recommends keeping the term "hemangioendothelioma" restricted to those with kaposiform, epithelioid, retiform, pseudomyogenic or composite components.

Classic classification of hepatic hemangiomas from 2007 included three subtypes: focal, multifocal and diffuse. Focal lesions were considered the hepatic form of the cutaneous rapidly involuting hemangiomas (RICHs), which do not respond to propranolol, while multifocal and diffuse lesions were related to the true infantile hemangioma (IH) [2]. In 2012, this classification was validated through a registry (Liver Hemangioma Registry from Boston Children's Hospital) and confirmed that focal lesions corresponded with congenital hemangiomas. Two subtypes were observed: rapidly involuting congenital hemangiomas, most of which reduced 80% in size around 12 months of age matching cutaneous RICH; and one case of noninvoluting congenital hemangioma, which did

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not change in size [3]. Hepatic noninvoluting congenital hemangiomas (NICHs) have not been characterized so far.

These results were adopted by the ISSVA by including in the classification of hepatic hemangiomas two different types: infantile hepatic hemangiomas (IHHs) and congenital hepatic hemangiomas (CHHs) [4].

Diagnosis of CHH may occur on prenatal imaging, as an incidental finding or through evaluation of a mass or heart failure in the newborn. Although the finding of a unifocal congenital liver mass is highly suspicious of CHH, differential diagnosis with hepatoblastoma or metastatic neuroblastoma must be made [4]. A consensus for monitoring and treatment has not been reached and currently it is guided depending on the subtype of CHH and the postnatal clinical behavior.

Cutaneous congenital hemangiomas (CCHs) arise owing to somatic activating mutations in *GNAQ* or *GNA11* genes, but this has not been confirmed in a long series of visceral congenital hemangiomas [5].

The aim of our study is to present our series of CHH and characterize the clinical, histologic and genetic correlation of CHH, compared to its cutaneous counterpart.

1. Materials and methods

1.1. Data collected

A retrospective review of patients with solitary hepatic hemangiomas between 1991 and 2018 was performed in two pediatric centers. Patients were included through clinical records review when presenting with a diagnosis of focal hepatic hemangioma. Collected variables included basic characteristics of patients (sex, birth date, prenatal history, familial history, cutaneous hemangiomas), type of lesion (location, size), diagnosis (age at diagnosis, symptoms, imaging tests, suspect diagnosis, biopsy), follow-up, treatment (corticosteroids, propranolol, embolization, surgical resection), histological findings and genetic testing.

1.2. Diagnosis, follow-up and treatment

The finding of a unifocal congenital liver mass and suspicion of CHH by clinical history and imaging would initially determine conservative treatment and follow-up with serial ultrasounds in asymptomatic patients. When expert panel of radiologists experienced in liver disease imaging was not able to conclude on a clear diagnosis or when growth of the lesion was determined in consecutive controls, an ultrasound-guided percutaneous needle biopsy was performed in order to rule out malignancy, which led to definite diagnosis. CHHs were then classified in three groups according to clinical history and involution time through serial imaging: rapidly involuting congenital hemangioma (RICH), partially involuting congenital hemangioma (PICH) and noninvoluting congenital hemangioma (NICH).

Follow-up time was defined by the interval from the initial presentation to the last imaging study.

1.3. Histological findings

The samples were processed according to a standard hematoxylin and eosin staining protocol and examined through light microscopy. After appropriate antigen retrieval, immunohistochemical staining was performed on an Agilent DAKO Envision Flex visualization system. Sections were incubated with monoclonal and polyclonal antibodies against CD31 (DAKO, GA610), CD34 (DAKO, GA632), Glut-1 (Spring Bioscience E2840, dilution 1:100), WT1 (DAKO, IR055) and D2-40/Podoplanin (DAKO, IR072). For histological comparison of CHH to its cutaneous counterpart, the slides of 7 cases of CCH were obtained from the archives and examined through light microscopy.

1.4. Genetic testing

The molecular study was performed in the Institute of Medical and Molecular Genetics (INGEMM) at La Paz Hospital, Madrid, Spain, according to the Hospital Ethics Committee, with written informed consent.

DNA was extracted from available formalin-fixed paraffin-embedded (FFPE) hepatic tumor samples using the truXTRAC FFPE DNA Kit (Covaris, EE.UU.) according to the manufacturer's protocol.

High-throughput sequencing (or next generation sequencing, NGS) was performed on each DNA sample using a 56 vascular anomalies-related genes (Refseq) custom design panel that includes all coding exons (>98%), the UTR sequences and 20–25 bp flanking intron regions per exon (Appendix 1). Libraries were created according to the standard protocols KAPA HTP Library Preparation Kit for Illumina platforms, SeqCap EZ Library SR (Roche NimbleGen) and NEXTflex-96 Pre Capture Combo Kit (Bioo Scientific, USA) for indexing. The captured DNA samples were sequenced on a NextSeq 500 instrument (Illumina, USA) according to the manufacturer's recommendations for paired-end 150 bp reads.

Data generated were analyzed using our previously described in-house pipeline by Rodríguez-Laguna *et al*, designed to detect single nucleotide variants and small insertions/deletions (indels) present in low allele frequencies (VAFs) or low mosaics [6].

Candidate variants obtained by the high-throughput deep sequencing were validated in DNA samples isolated from new FFPE extractions by using a droplet digital polymerase chain reaction (ddPCR), able to detect and quantify mosaic variants at frequencies as low as 0.1%. The QX200 Droplet Digital PCR System (Bio-Rad, EE.UU.) was used according to the manufacturer's protocol (FAM channel for mutant allele and VIC channel for wild type).

1.5. Data analysis

A descriptive analysis was performed expressing data in percentage from total and medians with their ranks. A literature review was also performed. Electronic databases (PubMed and Medline) were systematically searched for articles in English and Spanish language referring to congenital hepatic hemangiomas, with the terms: “vascular anomalies”, “vascular tumors”, “hepatic hemangioma”, “congenital hepatic hemangioma”, “hemangioendothelioma of the liver”.

2. Main results

2.1. Patient characteristics

Sixteen patients with solitary hepatic hemangiomas were included in the study. The male–female ratio was 1:1. One patient was premature (36 weeks) and there were no patients with low birth weight. Three patients (18.75%) were first-born and there was no familial history of liver vascular tumors. One patient also presented with a classic infantile hemangioma in the upper eyelid, which started to involute at four months of age without treatment.

Patient and tumor characteristics data is summarized in Table 1.

2.2. Clinical characteristics

The diagnosis of a solitary hepatic vascular lesion was made during the first week of life in eight patients (50%), in the first three months of life in six patients (37.5%) and after three months in two patients (12.5%). The warning sign that motivated the study was hepatomegaly in six patients (37.5%), abdominal distension in three patients (18.75%) and heart failure in one patient (6.25%). It was an incidental finding in ultrasound in six patients (37.5%), two of whom were diagnosed at the prenatal ultrasound.

Every patient underwent complementary imaging tests, including ultrasound, CT-scan and/or MRI. Description of the lesion by ultrasound

Table 1
Patient's and tumor's characteristics.

Patient's characteristics						Tumor's Characteristics			
Cases	Prenatal diagnosis	Gestational age (weeks)	First born	Sex	Cutaneous hemangioma (IH)	Age at diagnosis (days)	Signs or symptoms	Localization (segments)	Size (cm)
1	No	37	No	Female	No	5	Distension	IV, V, VIII	8 × 5
2	No	41	No	Female	No	70	Hepatomegaly	II, III	8.3 × 8 × 5.6
3	No	40	No	Female	No	1	Hepatomegaly	I, II, III, IV, VIII	14 × 11 × 9
4	No	40	No	Female	No	90	Hepatomegaly	VI	5.8 × 4.5
5	No	40	No	Female	No	2	Hepatomegaly	II, III	5 × 5 × 4
6	No	38	No	Female	No	356	Hepatomegaly	V, VI	7 × 6 × 6
7	No	40	No	Male	No	4	Heart failure	II, III, IV	6.3 × 5.3 × 2.2
8	No	37	No	Male	No	45	Incidental	II, III, IV	1.7 × 1.4 × 1.4
9	No	40	No	Male	No	150	Incidental	VI	0.5 × 0.5 × 0.5
10	No	39	No	Male	No	90	Hepatomegaly	V, VIII	4 × 4 × 3
11	No	39	No	Female	No	20	Incidental	V, VI	1 × 1 × 0.6
12	Yes	41	Yes	Male	No	Prenatal	Incidental	V, VI	4 × 3 × 3
13	Yes	38	No	Male	No	Prenatal	Incidental	II, III	4.7 × 3 × 3
14	No	37	No	Male	Yes (upper eyelid)	18	Incidental	II, III	2 × 1
15	No	39	Yes	Male	No	1	Distension	IV, V, VIII	11 × 10 × 5.5
16	No	36	Yes	Female	No	1	Distension	IV, V, VIII	7.5 × 6.5 × 5.5

IH: Infantile hemangioma.

and CT-scan featured a unifocal heterogeneous liver mass, well-differentiated from the normal liver parenchyma, with intralesional hemorrhage, calcification and/or necrosis. MRI studies showed peripheral enhancement and typically hyperintensity on T2. Two patients (12.5%) presented self-limited thrombocytopenia without need of transfusion. Alpha-feto protein levels were within normal range in all patients. Two patients (12.5%) underwent biopsy after complementary tests because of uncertain diagnosis.

Patient and tumor characteristics data are summarized in Table 1.

2.3. Follow-up and treatment

Follow-up with serial ultrasound, without biopsy and without any other treatment was performed in five patients (31.25%), who presented total resolution of the lesion in two (at 4 months and 4 years of age) and partial resolution in three (at 3, 9 and 34 months of age).

Four patients (25%) were treated with oral propranolol and one (6.25%) with corticosteroids; with one total resolution (at 48 months of age), two partial resolutions (at 9 and 18 months of age) and two without changes (after 24 and 72 months respectively). The two patients who remained without changes and did not involute (case #1 and #6), underwent percutaneous biopsy confirming the diagnosis of congenital hemangioma withdrawing the previous treatment. Case #6, after the genetic testing described below, started treatment with sirolimus (mTOR inhibitor) and showed mild downsizing of the lesion 4 months later.

Five patients (31.25%) underwent surgical resection with partial hepatectomy (at 1, 2, 3, 4 and 4 months of age) owing to hemorrhage of the tumor in one patient and suspicion of hepatoblastoma in four. In this group, one patient received four cycles of cisplatin owing to an initial diagnosis of hepatoblastoma. One patient (6.25%) was treated with embolization (at 9 months of age) owing to a large intrahepatic shunt, although asymptomatic.

Table 2
Patient's treatment and outcome.

	Conservative treatment						
Cases	Suspect diagnosis	Previous biopsy	Type of treatment	Age of treatment (days)	Resolution	Age of resolution (months)	Definite diagnosis
8	Congenital hemangioma	No	Serial ultrasound	–	Total	4	RICH
9	Congenital hemangioma	No	Serial ultrasound	–	Partial	3	PICH
11	Congenital hemangioma	No	Serial ultrasound	–	Partial	9	PICH
12	Congenital hemangioma	No	Serial ultrasound	–	Partial	34	PICH
14	Congenital hemangioma	No	Serial ultrasound	–	Total	48	RICH
	Pharmacological treatment						
Cases	Suspect diagnosis	Previous biopsy	Type of treatment	Age of treatment (days)	Resolution	Age of resolution (months)	
1	Hepatoblastoma	Yes	Corticosteroids	30	No	–	NICH
6	Hepatoblastoma	Yes	Propranolol	510	No	–	NICH
7	Congenital hemangioma	No	Propranolol	7	Partial	18	PICH
15	Congenital hemangioma	No	Propranolol	5	Total	48	RICH
16	Congenital hemangioma	No	Propranolol	1	Partial	9	PICH
	Surgical and Interventional treatment						
Cases	Suspect diagnosis	Previous biopsy	Type of treatment	Age of treatment (days)	Resolution	Age of resolution (months)	
2	Hepatoblastoma	No	Left hepatectomy	75	–	–	–
3	Congenital hemangioma[a]	No	Extended left hepatectomy	90	–	–	–
4	Hepatoblastoma	No	Segmentectomy	120	–	–	–
5	Hepatoblastoma	No	Left hepatectomy	120	–	–	–
10	Hepatoblastoma	No	Right hepatectomy	110	–	–	–
13	Intrahepatic shunt	No	Embolization	270	–	–	–

RICH: rapidly involuting congenital hemangioma. PICH: partially involuting congenital hemangioma. NICH: noninvoluting congenital hemangioma.
[a] Sepsis and tumor enlargement owing to hemorrhage, which prompted surgery.

According to the presence or absence of involution, patients were finally classified under the definitive diagnosis of RICH, PICH or NICH.

Data on patient treatment and outcome are summarized in Table 2.

2.4. Histological findings

Two biopsies and five partial hepatectomies were available for study. Every sample received diagnosis of congenital hepatic hemangiomas (former "hemangioendotheliomas"). In all cases, the endothelium stained for CD31 and CD34, and was negative for Glut-1 and D2-40. Negativity for GLUT-1 excluded IHH. However, only four cases provided abundant material for thoroughly histopathological examination. Two patterns were identified.

The first pattern, present in cases 2, 3 and 4, showed a vascular proliferation with very well delimitated borders, showing clear demarcation and even a capsule between the lesions and normal surrounding hepatic tissue (Fig. 1A). The lesions contained no normal hepatic tissue other than some ducts. At a closer examination, they were constituted by capillaries, often elongated, that showed thin walls, with prominent and abundant nonatypical endothelia that contained erythrocytes. These vessels were not grouped in lobules. There were numerous large vessels admixed which also presented similar characteristics (Fig. 1B). In some areas, the small vessels predominated and in other areas, the large ones did. There were few intermediate-sized vessels. Thick-walled vessels were not identified. All vessels were surrounded by fibrous tissue. The lesions exhibited mostly proliferative areas with many vessels and little stroma, while involutive areas had vessels separated from each other with bigger lumen and less endothelial cells. Finally, there were large areas of myxoid stroma, where vessels were very few and distant (Fig. 1C). Large vessels had occasional thrombi. No hobnail endothelium was observed. Along with the lesions, a few small stromal calcium deposits were observed. WT1 was positive in small vessels and negative in the large ones.

In contrast, the second pattern represented by case 5 (which had suffered four previous cycles of cisplatin but showed no noteworthy histological chemotherapy effects) was poorly circumscribed from the normal hepatic tissue around, and showed large lobules of hepatocytes at the periphery of the lesion, admixed with it (Fig. 2A). As in the first pattern, the center of the lesion had no hepatocytes, but contained ducts. It was equally composed of capillary-looking vessels, with prominent and cellular nonatypical endothelia that, in contrast to previous pattern, in many vessels was hobnailed (Fig. 2B). Vessels were of all sizes, including solid areas made of small vessels with no lumen, in which the vascular origin of the lesion was difficult to determinate. Somewhat larger vessels were round and not elongated, and showed a frequent "back-to-back" image, with very little fibrous tissue around (Fig. 2C). Areas with larger vessels had a "honeycomb" appearance and there were not involutive areas. As in the first pattern, there were no lobules, no thick-walled vessels and some of the large vessels showed thrombi. There were extensive areas of fibrous or myxoid stroma, much of it with large calcium deposits, as well as wide areas of necrosis. WT1 was positive in all vessels.

Histologic review of 7 samples of CCH from the archive was also performed in order to compare the CHH to its cutaneous counterpart. CCHs examined had presented no involution and were diagnosed as NICH. They were constituted by lobules surrounded by fibrous tissue without normal cutaneous tissue in between the sample. Thick-walled vessels were identified, but there were no thrombi in larger vessels or calcium deposits throughout the tissue sample.

2.5. Genetic testing

Genetic testing was performed in FFPE tissue samples from six (37.5%) available samples (cases 1 to 6) using a 56 vascular anomalies-related genes custom NGS panel, a custom bioinformatic pipeline for somatic mosaicism, and Droplet Digital PCR (ddPCR) for variant validation. Average on-target coverage on NGS ranged from 32 to 800 sequencing reads per bp, after removing duplicates. Variants were excluded as disease candidates by their presence in >0.01 population frequency (1000 Genomes project (http://www.internationalgenome.org/), Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org)), by pathogenicity predictors, and by description in the scientific literature.

Our molecular analysis identified pathogenic *GNAQ* variants in three of six (50%) tissue samples studied (cases 1, 5 and 6) as well as pathogenic *PIK3CA* variants in four of the six (67%) tissue samples studied (cases 1, 2, 3 and 4). Note that case 1 presented variants in both genes. All pathogenic *GNAQ* variants were somatic missense single-nucleotide variations with a range of mosaicism between 3% and 18% (Fig. 3). We identified two different *GNAQ* variants: c.626A > T; p.Gln209Leu (cases 1 and 6) and c.626A > C; p.Gln209Pro (case 5).

In the case of *PIK3CA*, all the samples presented the somatic missense-variant c.3140A > T; p.His1047Leu in mosaic ranging from 1 to 26%. No pathogenic variant was detected in the *GNA11* gene in any of the samples studied. *GNA14* gene was not included in the NGS panel.

Histologic and genetic findings are summarized in Tables 3A and 3B.

3. Discussion

The right diagnosis of CHH, as well as specific recognition of each subtype of CHH, is crucial for a correct disease management. It will ultimately aid in establishing a consensus concerning the follow-up and treatment of these patients. We report a series of CHH showing three types of behavior, similar to those CCH: complete involution (RICH), partial involution (PICH), or noninvolution (NICH). Histologic analysis allowed the confirmation of CHH, showing two clearly differentiated patterns. Genetic testing detected *GNAQ* and/or *PIK3CA* mosaic pathogenic variants in all the samples studied, although analysis of a larger number of samples is needed to determine whether there is a correlation between genetic variants and histological findings.

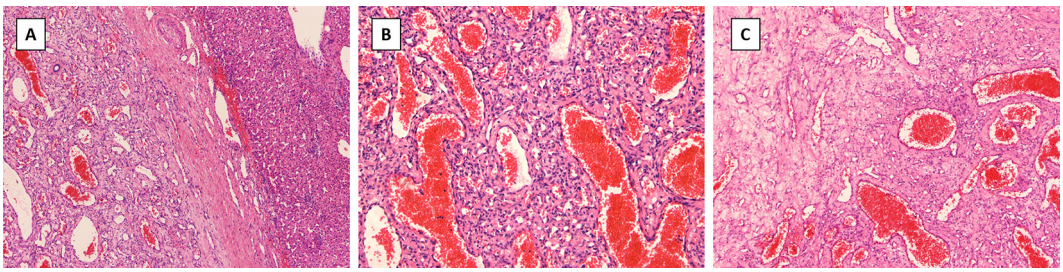

Fig. 1. (A) Case 4. This GNAQ-negative vascular proliferation was very well circumscribed, with a capsule between the normal hepatic tissue (right) and the vascular lesion (left). (B) Case 4. The same hemangioma, at higher magnification, was composed of small and large vessels that were surrounded by fibrous tissue. This picture shows a proliferative area with many vessels. (C) Case 4. Other areas of the same lesion were less proliferative. They showed more fibrous tissue and separated and less cellular vessels (right). The left side of the photograph exhibits a myxoid area.

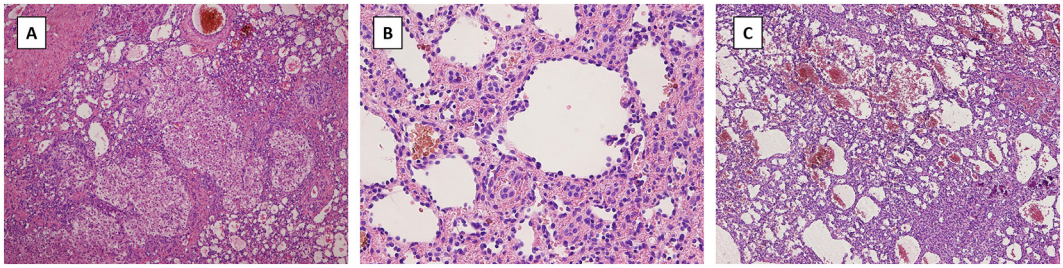

Fig. 2. (A) Case 5. The hemangioma that was GNAQ-positive had very poorly demarcated borders, and the periphery of it showed an admixture of hepatic tissue and the vessels of the hemangioma. (B) Case 5. At a closer look, many of the vessels had a hobnail endothelium. (C) Case 5. The lesion was composed of vessels of all sizes, with little stroma around them and solid areas. At the right, there is some calcium deposit.

3.1. Clinical history

CHH are solitary liver masses that are usually asymptomatic or present with abdominal distension or hepatomegaly without any other accompanying symptoms. CHH can present with mild anemia and minor thrombocytopenia, although in our series most lesions were detected after the period in which self-limited hematological abnormalities have corrected. Rare forms of presentation of CHH may include cardiac failure owing to intralesional shunting or coagulopathy, although disseminated intravascular coagulation is uncommon and liver function is generally preserved [7]. In our series, most of our patients were asymptomatic; only two presented with self-limiting thrombocytopenia and one with heart failure. Although clinical findings are essential in differential diagnosis, imaging plays a deciding role.

3.2. Complementary imaging

Doppler ultrasound is the recommended initial imaging study to assess these patients. The classic appearance of a congenital hemangioma is a solitary mass that in certain cases may contain calcifications or fibrous areas that confers a heterogeneous appearance. If the clinical presentation, the clinical history or the imaging features do not support the diagnosis of congenital hemangioma, magnetic resonance imaging (MRI) should be considered [4].

On MRI, congenital and infantile hemangiomas are typically hyperintense on T2 and hypointense relative to the normal liver parenchyma on T1. CHHs are unifocal and usually present intralesional calcifications in opposition to IHH. Peripheral enhancement is also typical from CHH, differentiating them from hepatoblastoma. These patterns have been well described in the Liver Hemangioma Registry from Boston Children's Hospital and concur with our series [3].

3.3. Histology and genetics

Histologically, congenital hemangiomas are clearly described in skin [8]. Immunostaining with GLUT-1 negative will exclude IHH without a doubt [9].

In our series of CHH, there was a slight histological difference between the three cases showing the *PIK3CA*: p.His1047Leu mosaic variant and the case positive for the *GNAQ*: p.Gln209Leu mosaic variant. The group of the three cases with the *PIK3CA* variant was characterized by lesions with a very well delimitated contour with a "pushing" border, little variety in the size of vessels (that were either small or large), quite fibrous tissue around them and no hobnail endothelium. Different phases of proliferative and involutive areas could be identified throughout the lesions. This mutation in PIK3CA is a gain-of-function pathogenic variant previously described in cancer (Catalogue of Somatic Mutations in Cancer database; http:// cancer .sanger .ac .uk/), *PIK3CA*-related overgrowth syndrome (PROS; Mirzaa *et al*, 2016; Kuentz *et al*, 2017) and in vascular malformations (Luks *et al*, 2015) [10–12].

In contrast, the case with the *GNAQ* variant had infiltrative borders in which the lesion was admixed with hepatic tissue, had vessels of all diameters with little fibrous tissue around them, was uniform with no involutive areas and had hobnail endothelium. Both *GNAQ* variants have already been described, associated with uveal melanoma (Van Raamsdonk CD *et al*, 2009), anastomosing hemangiomas (Bean GR *et al*, 2017) or hepatic small vessel neoplasm (Joseph NM *et al*, 2018), and have shown to constitutively activate MAPK and/or YAP signaling

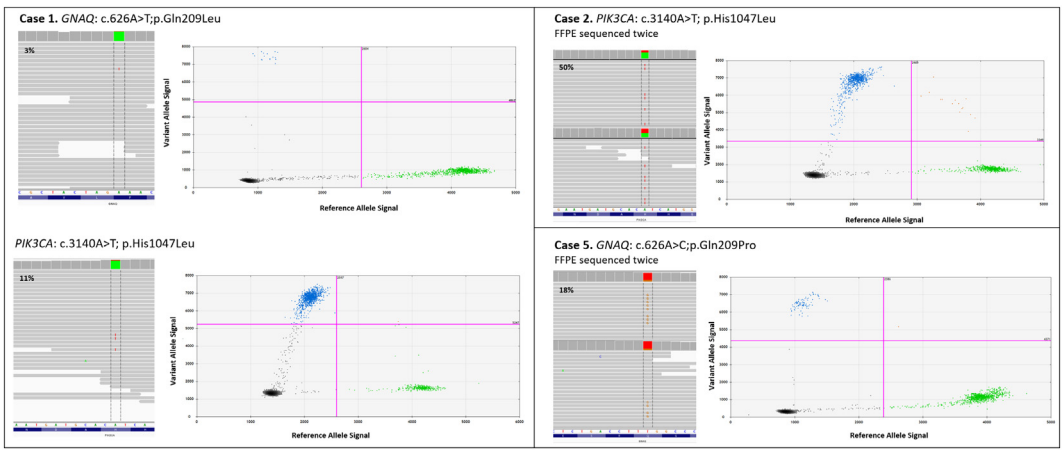

Fig. 3. Detection of GNAQ and PIK3CA mosaic mutations in FFPE from cases 1, 2 and 5. The visual analysis using the IGV tool shows the presence of the pathogenic variants obtained by NGS in mosaic ranging from 3% to 50% for these cases. Droplet Digital PCR (ddPCR) results confirmed the presence of those variants.

Table 3A
Patient's histologic and genetic findings.

	Histologic findings		Genetic Findings	
Cases	Immunostaining	Subgroup	*GNAQ* variants	*PIK3CA* variants
1	GLUT-1 negative		*GNAQ*: c.626A > T;p.Gln209Leu	*PIK3CA*: c.3140A > T; p.His1047Leu
2	CD31, CD34, WT1 positive. GLUT-1, D2–40 negative.	1	No mutation found	*PIK3CA*: c.3140A > T; p.His1047Leu
3	CD31, CD34, WT1 positive. GLUT-1, D2–40 negative.	1	No mutation found	*PIK3CA*: c.3140A > T; p.His1047Leu
4	CD31, CD34, WT1 positive. GLUT-1, D2–40 negative.	1	No mutation found	*PIK3CA*: c.3140A > T; p.His1047Leu
5	CD31, CD34, WT1 positive. GLUT-1, D2–40 negative.	2	*GNAQ*: c.626A > C;p.Gln209Pro	No mutation found
6	CD31, CD34, WT1 positive. GLUT-1, D2–40 negative.		*GNAQ*: c.626A > T;p.Gln209Leu	No mutation found
10	GLUT-1 negative		Insufficient sample	Insufficient sample

(Lim Y *et al*, 2016) [13–16]. In fact, case 5 is very similar to those reported by Joseph NM et al. as "hepatic small vessel neoplasms", in which frequent *GNAQ* or *GNA14* mutations were reported, including a case with both *GNAQ*: p.Gln209His and *PIK3CA*: p.His1047Arg variants [15].

In our series, case 1 also showed recurrent mutations in both *GNAQ* and *PIK3CA*. Unfortunately, the lack of material available from this sample did not allow us to perform the histological study; therefore we cannot assign this sample to any of the histological subgroups. Similarly, case 6 presented mutation in *GNAQ* but could not be histologically evaluated. The small number of patients makes it difficult to establish if there are really two different histologic subgroups or they are part of a histologic spectrum of CHH, as well as its association to mutations in *GNAQ* and *PIK3CA*.

Despite the mentioned histological differences between the two groups, when compared to 7 CCHs, all lesions are composed of benign vascular proliferations of capillaries. However, cutaneous and hepatic lesions have different characteristics. The main differences are that lesions in the liver do not form lobules, thick-walled vessels are absent, they exhibit thrombi in some of the larger vessels, they show hepatic tissue (ducts) in the middle of the lesions (there is no evidence of normal skin tissue in the lobules of capillaries of the skin), they have extensive areas of fibrous or myxoid stroma, and they exhibit calcium deposits.

CCHs show mutually exclusive mosaic missense mutations in Gln209 in *GNAQ* or *GNA11* genes [5]. However, in our series we have not detected pathogenic variants in *GNA11*, which may be because of the number of samples studied. The absence of mutations in *GNA11*, together with the clear histological differences between cutaneous and hepatic congenital hemangiomas, could also be because of specific effects associated with the biological context or the specific cells affected during vasculogenesis. Moreover, we cannot rule out the presence of pathogenic variants in *GNA14*, also described for the hepatic small vessel neoplasms. Nevertheless, this seems unlikely since all of our negative cases for *GNAQ* were not only positive for *PIK3CA* but also showed differential histological features, which have not been described for the hepatic small vessel neoplasms. We have not found histological differences in the samples with mutually exclusive mutations in *GNAQ* or *GNA14*, including the single case with mutations in both *GNAQ* and *PIK3CA*. On the other hand, for case 1 in our series and for the case reported in the literature, the consequences of the co-occurrence in the same sample of two variants in *GNAQ* and *PIK3CA* genes remain to be clarified. These variants are widely known to be pathogenic, recurrent, and frequently found as cancer drivers.

In the literature, there are many other vascular entities with similar mutations to those of the CCH: isolated and syndromic capillary malformations (*GNA11* or *GNAQ* p.Arg183Gln), hepatic small vessel neoplasm (*GNA14* p.Gln205Leu, *GNAQ* p.Gln209His, *GNAQ* p.Gly48Leu), tufted angioma (*GNA14* p.Gln205Leu), kaposiform hemangioendothelioma (*GNA14* p.Gln205Leu), anastomosing hemangioma (*GNAQ* p.Gln209His, *GNAQ* p.Gln209Leu) and lobular capillary hemangioma (GNA11 p.Arg183Cys, *GNA14* p.Gln205Leu, *GNAQ* p.Arg183Gln) [13–15,17,18]. Is it possible that these entities and CHH are related to each other, represent a spectrum of the same pathology or even correspond to the same lesion? A proposal to include all these entities under the category of G-protein receptor mutations, affecting the closely related *GNAQ*/*GNA11*/*GNA14* genes [(*e.g.*, GNA-vascular anomaly (GNAVA)] has already been suggested [19].

Table 3B
Histologic subgroups.

Congenital hepatic hemangioma		Cutaneous congenital hemangioma
Subgroup 1	Subgroup 2	
Delimitated borders	Poorly delimitated borders	
No lobules	No lobules	Lobules surrounded by fibrous tissue
Hepatic ducts presence	Hepatic ducts presence	No normal cutaneous tissue
No thick-walled vessels	No thick-walled vessels	Thick-walled vessels
No hobnail endothelium	Hobnail endothelium	
Proliferative and involuting areas	No involuting areas	
Thrombi in larger vessels	Thrombi in larger vessels	No thrombi in larger vessels
Small stromal calcium deposits	Large stromal calcium deposits	No calcium deposits
WT1 positive in small vessels	WT1 positive in all vessels	

3.4. Evolution and follow-up

Once the diagnosis of CHH is certain, follow-up recommendations through serial blood analysis and ultrasound are based on possible complications such as intratumoral bleeding, thrombocytopenia, hypofibrinogenemia and cardiac failure. There is lack of consensus regarding the exact frequency of follow-up; however, the hepatic hemangioma registry recommends ultrasound monitoring with an initial 2-week interval adding 2 weeks to the interval after each stable evaluation [4]. For the patients who underwent observation and medical treatment in our series, control ultrasound was performed initially every month and every 3 months after stabilization or beginning of shrinkage.

3.5. Treatment

Treatment of CHH is only needed if the patient is symptomatic, even though there is no evidence that interferon alpha-2a, β2-adrenergic antagonists or corticosteroids contribute to reduction of the lesion. If there is heart failure, medical treatment of the cardiac failure is the first option before embolization. Surgery for resectable lesions should only be considered when all other measures have failed. Asymptomatic CHH should only be monitored until ultrasound studies show stable size and vascularity in at least two consecutive tests [4].

Our series is in part retrospective and many patients were treated before CHH diagnosis was made. This contributes to the heterogeneity in the management of these lesions. It is possible that patients classified

as NICH who underwent surgical resection could have continued to involute if given more time and then reclassified in RICH or PICH.

However, surgical resection has allowed us to further study these tumors, learning valuable information in the histological and molecular areas. These results have implications in a future use of specific inhibitors of G-proteins or *PIK3CA*, or even combined therapies targeting multiple downstream pathways as has been suggested for uveal melanoma [20]. Our case #6 has shown mild response after 4 months on sirolimus treatment, which supports the idea that inhibition of mutated genes may become a future option for the management of these lesions. However further studies with larger number of patients are needed.

4. Conclusions

In summary, CHH behavior resemble its cutaneous homonym with three different behavior patterns: total involution (RICH), partial involution (PICH) and noninvolution (NICH). Histology can confirm the diagnosis, but biopsy is not essential except when there is uncertain diagnosis, and CHH should be followed-up without intervention when asymptomatic. Genetic testing has showed the presence of somatic mutations in *GNAQ* and/or *PIK3CA* genes, not described before. In our series the CHH showed two slightly different histological patterns that could be associated with the presence of somatic mutations in *GNAQ* and/or *PIK3CA* genes, although the small number of patients makes it difficult to establish if there are really two different histologic subgroups or they are part of a histologic spectrum of CHH. The finding of mutations in *GNAQ* and/or *PIK3CA* genes could contribute to the use of specific (or combined) molecular targets for treatment in the future. These histological and genetic subgroups in our series did not present any differences in terms of tumor characteristics, symptoms or involution.

There are many other entities, including anastomosing hemangioma and hepatic small vessel neoplasm, with similar mutations in G-proteins. It is possible that these entities and CHH can be related to each other and represent a spectrum of the same pathology or even correspond to the same lesion. In fact, it is reasonable to suspect that hepatic small vessel neoplasm could eventually correspond to a previously nondiagnosed NICH of the liver. Whether this could be influenced by the presence of somatic mutations in *GNAQ* or *PIK3CA* remains to be resolved.

Appendix 1. Appendix

High-throughput sequencing (or next generation sequencing, NGS) was performed on each DNA sample using a 56 vascular anomalies-related genes (Refseq) custom design panel that includes all coding exons (>98%), the UTR sequences and 20–25 bp flanking intron regions per exon.

ACVRL1, ADAMTS3, AKT1, AKT2, AKT3,
CCBE1, CCM2, CCND2, CDKN1C, CELSR1, COL3A1,
ELMO2, ENG, EPHB4, FAT4, FGFR3, FLT4, FOXC2,
GATA2, GDF2, GJC2, GLMN, GNA11, GNAQ,
HGF, HRAS, IKBKG, KDR, KIF11, KRAS, KRIT1,
MAP2K2, MAP3K3, MAPK1, MTOR, NF1, NOTCH1, NRAS,
PDCD10, PIEZO1, PIK3CA, PIK3R1, PIK3R2, PKD1, PTEN, PTPN11, PTPN14,
RAF1, RASA1, SMAD4, SOX18, STAMBP, TEK, TSC1, TSC2, VEGFC.

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