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New case of syncytial giant-cell variant of hepatocellular carcinoma in a pediatric patient with *HNF1B* deficiency: does it fit with the syndrome?

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ABSTRACT

Background *Hepatocyte nuclear factor 1B (HNF1B)* is a member of the homeodomain-containing family of transcription factors located on 17q12. *HNF1B* deficiency is associated with a clinical syndrome (kidney and urogenital malformations, maturity-onset diabetes of the young, exocrine pancreatic insufficiency) and to an underdiagnosed liver involvement. Differently from *HNF1A*, the correlation between hepatocellular carcinoma (HCC) and germline *HNF1B* deficiency has been poorly evaluated.

Case report Here, we report a novel case of a syndromic *HNF1B*-deficient paediatric patient that developed HCC with unique histopathological features characterised by neoplastic syncytial giant cells, which was observed only in one additional case of paediatric cholestatic liver disease of unknown origin.

Conclusions Our case highlights the influence of *HNF1B* deficiency in liver disease progression and its putative association with a rare yet specific HCC histotype. We hypothesised that HCC could be secondary to the repressive effect of *HNF1B* variant on the *HNF1A* transcriptional activity.

INTRODUCTION

Hepatocyte nuclear factor 1B deficiency (*HNF1B*-d) is a rare monogenic disorder most frequently affecting the kidney (renal cystic disease) and the pancreas (maturity-onset diabetes of the young (MODY)). The liver could also be involved, but is frequently neglected. As part of a syndromic ciliopathy, *HNF1B*-d mainly affect bile duct development and maturation, leading to an asymptomatic rise of transaminases or to cholestatic abnormalities. Indeed, *HNF1B*-d has been recently suggested to be included in the diagnostic workup of neonatal/infantile cholestasis, and we recently reported a case of paediatric cholestasis with paucity of the interlobular bile ducts and a variable degree of periportal fibrosis due to a pathogenetic variant of *HNF1B*.[1] Nevertheless, its proper role in liver disease aetiopathogenesis, and particularly its association with paediatric hepatocellular carcinoma (P-HCC), is largely unexplored.

In this report, we describe a novel paediatric case of *HNF1B*-d presenting a syncytial giant-cell variant of P-HCC.

CASE DESCRIPTION

We report a male patient who was admitted to our neonatal intensive care unit for mechanical invasive ventilation until 6 weeks of life due to severe growth restriction and small for gestational age (Apgar score: 2/4 at 1/5 min of life). In the first years of his life, he presented severe retinopathy and bilateral cecity, multicystic dysplastic kidneys, chronic kidney disease (interstitial nephropathy, eGFR of 30–35 mL/min/1.73 mq, persistent polyuria/polydipsia, and mild hypokalaemia/hyponatraemia), bilateral cryptorchidism and an autism spectrum disorder.

At the age of 10 years, routine ultrasound (US)-abdominal study and CT identified two nodular hepatic lesions (48×53×61 mm and 57×75×52 mm) suspicious for HCC (figure 1). Liver function tests and hepatic elastography were normal, alpha-fetoprotein was 199 IU/mL. Based on biopsy findings of the major lesion (hepatocellular tumour consistent with HCC), the hepatic lesions were resected eventually resulting HCCs and revealing a relevant component of intratumour giant cells (figure 2A). These cells were

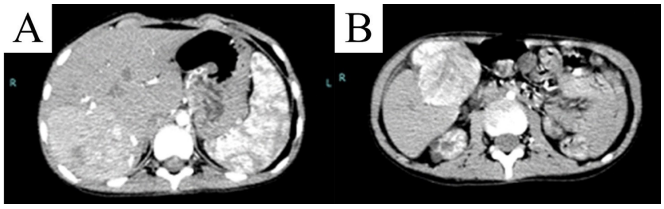

Figure 1 CT images of the two hepatic lesions hepatocellular carcinoma (HCC) in (A) S7 and (B) S5-S6 liver segments, respectively.

characterised by a hepatocellular-like morphology that was further confirmed by immunohistochemical (IHC) stains (figure 2B–D). Altogether, these features were diagnostic of a syncytial giant-cells variant of P-HCC developed in a liver with no signs of fibrosis (figure 2E), but characterised by a proliferation of reactive ductular cells (RDCs) and activation of the hepatic progenitor cells (HPCs) compartment, while intermediate hepatobiliary cells (IHBCs) were virtually absent (figure 2F). Despite differing from the Alagille syndrome phenotype (where

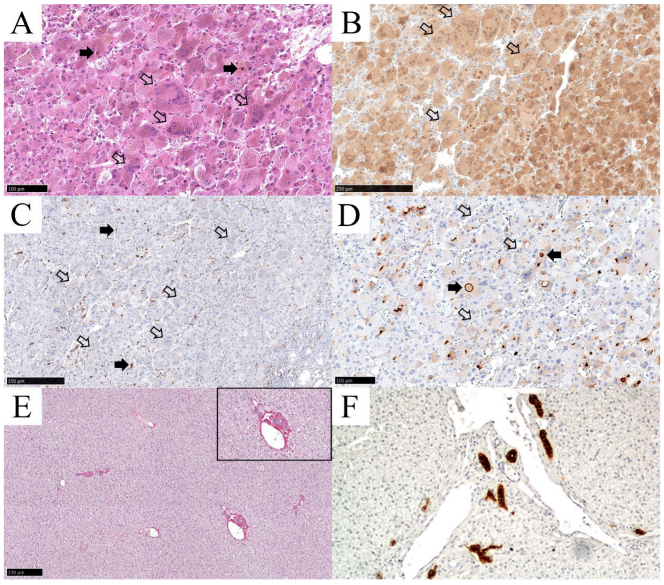

Figure 2 Histopathological features of the paediatric hepatocellular carcinoma (P-HCC) and the surrounding liver. (ie, moderately differentiated HCC, pT3m, with a solid and trabecular architecture, expansive pattern of growth, no vascular nor perineural invasion, and low mitotic index (<1 mitosis/10 high-power field)). The HCC shows a combined solid-acinar architecture with interspersed intratumoral cholestasis (black arrows). The lesion presents several neoplastic giant cells (empty arrows) (A). Immunohistochemical analysis confirmed the hepatic nature of the neoplastic giant cells, showing a diffuse and intense Arginase-1 expression (B), whereas CD68 was negative (C). The neoplastic hepatocellular giant cells present a canalicular expression of CD10 (as observed in HCC), but no expression in the cytoplasm (D). Perilesional parenchyma shows no signs of increased fibrosis (E; Sirius red stain) and a proliferation of reactive ductular cells with activation of the hepatic progenitor cells compartment, while intermediate hepatobiliary cells were virtually absent (F; cytokeratin 7 immunohistochemical stain).

marked ductopenia is accompanied by enrichment in IHBC), we suspected a genetic syndrome (sporadic P-HCC seemed unlikely) and planned a closer follow-up, with no neoplastic recurrence (5-year follow-up).

At the age of 13, the patient accessed our Pediatric Emergency Department for gait disturbances and drowsiness: he showed poor general conditions, moderate-to-severe dehydration, and diffuse hypotonia/hyposthenia. Infectious processes were excluded, and laboratory tests showed significant hyperglycaemia associated to normal acid/base balance, suggesting a severe hyperglycemic hyperosmolar state (HHS). Therefore, based on the HHS onset and the absence of type 1 diabetes mellitus (DM) autoantibodies and type 2 DM clinical features, we hypothesised a MODY. After insulin and fluid intravenous therapy, the patient recovered and normalised the glycaemic profile. A slight elevation of transaminases (3× ULN) and a marked asymptomatic rise of pancreatic enzymes (amylase 408 UI/l; lipase 708 UI/l) were recorded, but with no further signs of liver or pancreatic injury.

The renal involvement, MODY, urogenital malformation and neurological impairment led us to suspect *HNF1B*-d syndrome. We performed whole exome sequencing and identified a likely benign *HNF1B* homozygous synonymous variant (c.36C>T; p.Leu12Leu) inherited from the healthy father that led us to hypothesise a heterozygous deletion of one allele of the gene. Our hypothesis was confirmed through direct multiplex ligation-dependent probe amplification analysis, identifying a whole-gene deletion in the context of 17q12 microdeletion syndrome (OMIM #614527) (online supplemental data). Of note, subsequent IHC for HNF1A and HNF1B on tumour samples revealed a diffuse nuclear positivity in tumour cells, while the non-tumorous hepatocytes were mostly negative (figure 3).

DISCUSSION

We here described a rare case of *HNF1B*-d associated P-HCC presenting a unique morphological feature, which is the presence of neoplastic hepatocellular syncytial giant cells. Multinuclear giant cells represent a rare feature of HCC and are mostly observed in the sarcomatoid variant of adult HCC as osteoclastic-like cells with a mesenchymal phenotype. Differently, our case presented syncytial giant cells of hepatocellular nature, which, according to the AFIP Tumors of the Liver Atlas,[2] qualifies the diagnosis of a syncytial giant cells P-HCC, an extremely rare subtype of P-HCC with only one case reported so far.[3] It was observed in a 9-month-old girl with cholestasis, hyperparathyroidism, growth retardation and delayed motor development. Interestingly, the perilesional liver showed injured/completely absent bile ducts, suggesting a non-syndromic or syndromic ductopenia, but no genetic analysis was performed. Analysing the clinical history and liver involvement of this report, we believe

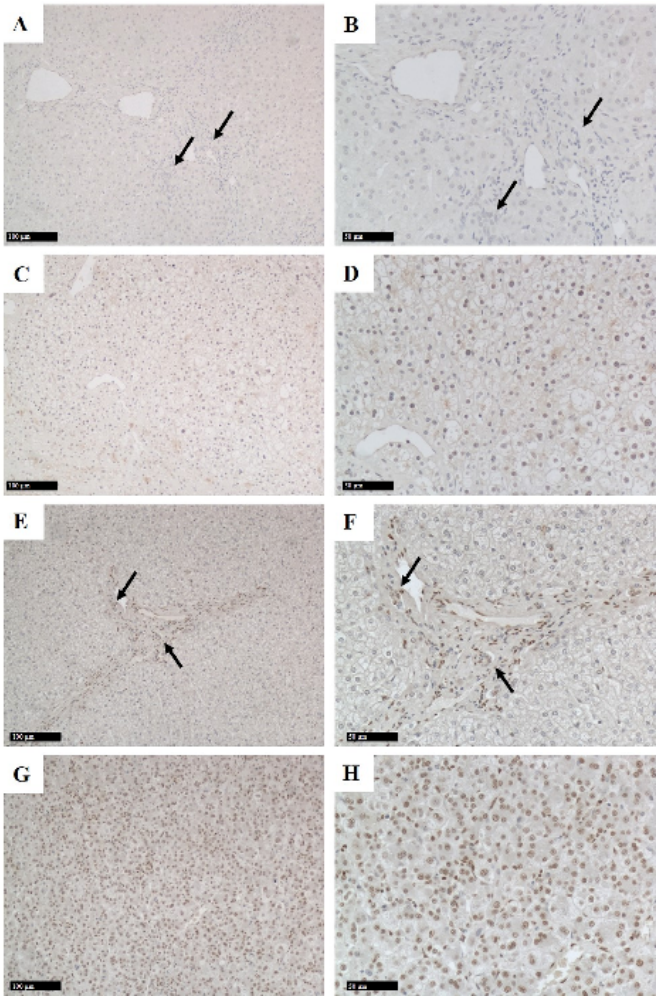

Figure 3 Immunohistochemical expression of hepatocyte nuclear factor 1A (HNF1A) and hepatocyte nuclear factor 1B (HNF1B) in paediatric hepatocellular carcinoma (P-HCC) and peritumoral tissue. Immunohistochemistry for (A–D) HNF1A and (E–H) HNF1B showed and intense and diffuse nuclear staining for both transcritption factors in neoplastic hepatocytes, while peritumoral hepatocytes are mostly negative. (E, F) HNF1B, but not (A, B) HNF1A decorates the nuclei of the intraportal bile ducts (black arrows).

that this patient might represent a case of misdiagnosed *HNF1B*-d. The similar clinical profile and overlapping P-HCC subtype with our case led us to hypothesise that the syncytial giant-cell variant of P-HCC could be related to the *HNF1B* oncogenetic mechanism, as it has never been reported elsewhere.

To date, only one case of P-HCC was associated to germline heterozygous deletion of *HNF1B*[4] and the association of HCC with germline *HNF1B-d* is largely unexplored.[1 5] A speculative hypothesis is that P-HCC could be secondary to the repressive effect of *HNF1B* variant on *HNF1A* transcriptional activity (*HNF-1B* H153N mutant had a promoter-specific and tissue-specific repressive effect on *HNF1A* through the inhibition of the DNA binding of *HNF1A*), as suggested by the increased *HNF1A/HNF1B* ratio in well differentiated compared with poorly differentiated HCC.

We believe that *HNF1B*-d presented a more prevalent and complex phenotype than previously reported and our finding may lead to better clarify the pathophysiology of the *HNF1B*-d-related liver involvement. Indeed, the mechanisms regulating regenerative and reparative response to biliary damage determine the long-term outcome of cholangiopathies: three epithelial cell phenotypes, namely HPCs, IHBCs and RDCs compose the hepatic regenerative/reparative machinery and, in case of liver damage, HPCs can differentiate into cells committed toward the hepatocellular (IHBC) or biliary (RDC) lineage. Differently from most cholangiopathies, Alagille syndrome is characterised by the absence of ductular hyperplasia, associated to an increase of IHBCs that do not express the biliary-specific transcription factor *HNF1B*, reduced portal fibrosis, and deposition of sinusoidal fibrosis. Differently from Alagille syndrome, our case showed ductular hyperplasia, absence of IHBCs, and aberrant de novo expression of both *HNF1A* and B in neoplastic hepatocytes. All these peculiar features kindle further studies to investigate the presence of redundant mechanism controlling the activation and modulation of the hepatic reparative complex in *HNF1B-d*, potentially leading to the restriction towards a biliary lineage. To this end, we are developing a *HNF1B* KO rodent model and mouse organoids to characterise the diffusion and pathophysiology of liver disease associated with variants in the *HNF1B* signalling pathway, tracing the progressive natural course of the disease over the sequential stages.

In conclusion, *HNF1B* is emerging as an oncogenic biomarker, but the causative mechanisms remain poorly explored. Our case, by highlighting the putative association of *HNF1B*-d with the syncytial giant cells histotype of P-HCC, supports the oncogenetic role of *HNF1B* and kindles further mechanistic studies, eventually leading to novel clinical intervention, such as the inclusion of *HNF1B*-d in the aetiological workup of HCC and a periodic abdominal US monitoring in patients with *HNF1B*-d.

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Contributors Study concept and design: MP and AG; acquisition of data: MP, AG, LG, CC, SK, SD, DT, LP, MC, LF, PLC, RR; analysis and interpretation of data: MP, AG, LG, CC, SK, SD, DT, LP, MC, LF, PLC, RR; drafting of the manuscript: MP and AG; critical revision of the manuscript for important intellectual content: MP, AG, LG, CC, SK, SD, DT, LP, MC, LF, PLC, RR; study supervision: PLC and RR.

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Competing interests None declared.

Patient consent for publication Consent obtained from parent(s)/guardian(s)

Ethics approval This study involves human participants and was approved by The study was approved by the Institutional Review Board (Comitato Etico Interaziendale AOU Città della Salute e della Scienza di Torino, Italy; IRB number: 678.555). Participants gave informed consent to participate in the study before taking part.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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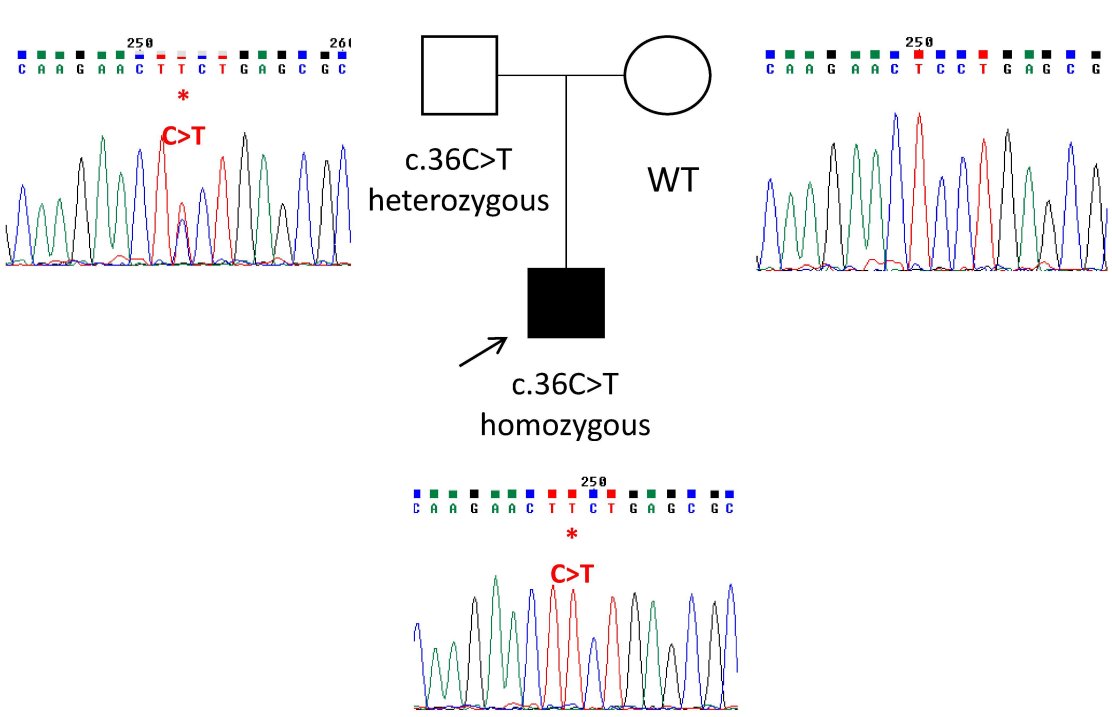
Supplementary data

Genetic analysis

NGS analysis on the proband resulted in the identification of a homozygous SNV (c.36C>T). (A) Family segregation studies showed that the mother was heterozygous for the same variant, while the father was WT. This led to the hypothesis that the second allele in the proband was missing. This hypothesis was confirmed by MLPA analysis, which showed a heterozygous CNV (B), which was originated de novo in the proband.

A



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