Case Report

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

# An adult male patient with multiple adenomas and a hepatocellular carcinoma: Mild Glycogen Storage Disease type Ia

David Cassiman[1,2,*,†], Louis Libbrecht[3,†], Chris Verslype[1], Wouter Meersseman[2], Roberto Troisi[4], Jessica Zucman-Rossi[5], Hans Van Vlierberghe[6]

[1]*Dept. of Hepatology, University Hospital Gasthuisberg, Leuven, Belgium;* [2]*Metabolic Center, University Hospital Gasthuisberg, Leuven, Belgium;* [3]*Dept. of Pathology, University Hospital Ghent, Belgium;* [4]*Dept. of General and Hepatobiliary Surgery, Ghent University Hospital, Belgium;* [5]*Inserm U674, Génomique fonctionnelle des tumeurs solides, Université Paris Descartes, Paris, France;* [6]*Dept. of Gastroenterology and Hepatology, Ghent University Hospital, Belgium*

The development of hepatocellular adenomas and – more rarely – carcinoma in the liver of patients with Glycogen Storage Disease type Ia (GSDIa) is a well-known complication of the disease. The pathophysiology of adenoma and carcinoma development in these patients is, however, hitherto largely unknown and is thought to be related to the metabolic control of the patient and/or the type of mutations in the G6PC gene. We report here on a very illustrative case of adenoma and carcinoma formation in a previously undiagnosed 42 year old male GSDIa patient (enzymatically and genetically proven). He had two episodes of mild hypoglycaemia in childhood, never required formal treatment, showed normal growth, and only mild lactate increases after prolonged starvation. He was a long-distance runner for most of his adult life, without the need for more than normal carbohydrate intake before/during exertion. To gain a better view on the type of adenoma formed in this patient, molecular studies were performed. We show here that in this patient with mild GSDIa without recurrent hypoglycaemic episodes, adenoma and carcinoma formation still occurred and that malignant transformation of adenoma here is associated with CTNNB1 mutations and a typical mRNA profile of a β-catenin activated lesion.


## Introduction

Glycogen Storage Disease type Ia (GSDIa) or Von Gierke's disease (OMIM 232200) is an autosomal recessive disease caused by mutations in the G6PC gene encoding for glucose-6-phosphatase, an enzyme essential in the mobilisation of glucose from liver glycogen during fasting. Patients with this disease in general present in infancy, with ketotic hypoglycaemia, lactic acidosis, poor growth, hepatomegaly, hyperuricemia, hypertriglyceridemia, and renal insufficiency. Traditionally, patients have to be kept in an anabolic state by nocturnal nasogastric tube feeding and/or ingestion of uncooked corn starch to prevent life-threatening hypoglycaemia and hyperlactacidemia and to achieve normal development and growth, for the entirety of their lives. In the second or third decade, a large majority of GSDIa patients develop hepatocellular adenomas, some of which evolve into hepatocellular carcinoma (HCC) [1,2]. Because GSDIa is a rare disorder, the molecular subtype of the typical adenomas in these patients is largely unknown. On the other hand, to be able to distinguish adenomas associated with malignant degeneration from the adenomas that will not turn malignant would be very worthwhile, in order to allow adequate selection of patients with GSDIa and adenomas for tumor resection or liver transplantation.

Outside the context of GSDIa, it is becoming clear that adenomas that carry risk of malignant transformation are those carrying CTNNB1 mutations leading to an activation of β-catenin, as opposed to the steatotic adenomas associated with HNF1A (Hepatocyte Nuclear factor 1 α) mutations and the inflammatory adenomas associated with IL6ST mutation, activating gp130 [3–5].

Until now, mutation analysis and chromosomal studies of a total of 13 GSDIa-associated adenomas have been reported. Three of these adenomas were CTNNB1 mutated [3,6,7]. The only adenoma associated with an HCC, was CTNNB1 mutated [7].

## Materials and methods

### *Patient*

The patient signed a written informed consent form, approved by the local Ethical Committee (Leuven University Hospitals, Belgium).

### *Pathology*

The resection specimen was received fresh and eight biopsies taken from the lesion were snap-frozen in liquid nitrogen-cooled isopentane and stored at −80 °C for molecular evaluation. Standard histopathological examination and histochemical stains were performed as described previously [8]. Immunohistochemistry for β-catenin and glutamine synthetase was performed according to a recently described protocol [9].

Keywords: Glycogen storage disease; Adenomatosis; β-Catenin; Adult.
*Received 16 November 2009; received in revised form 16 January 2010; accepted 7 March 2010*
* Corresponding author at: Dept. of Hepatology and Metabolic Center, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. Tel.: +32 16344299.
*E-mail addresses:* David.cassiman@uzleuven.be, david.cassiman@med.kuleuven.ac.be (D. Cassiman).
[1] These authors contributed equally to this work.

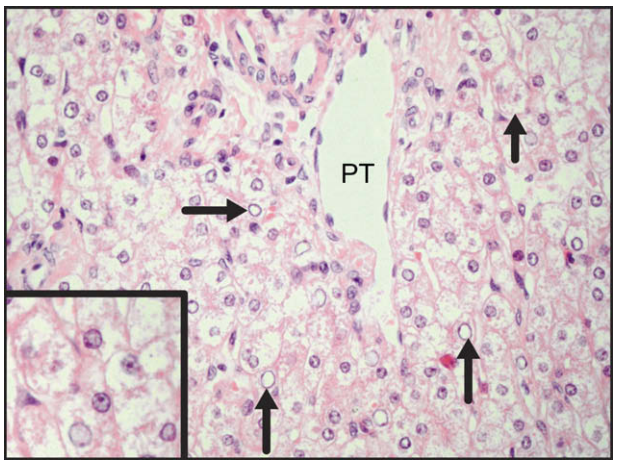


**Fig. 1. Several hepatocytes show swollen, vacuolated nuclei.** Hepatocytes with such glycogenated nuclei are mainly located in the area surrounding the portal tract (PT) (long arrows). The cytoplasm of hepatocytes is frequently swollen and is pale with small clumps of eosinophilic material. This "plant-like" aspect is most pronounced in the area indicated by the small arrow and enlarged in the inset. Original magnification 400×.

*Molecular studies*

Molecular studies (CTNNB1, HNF1A, and IL6ST mutation analysis and real-time quantitative RT-PCR for LFABP1, UGT2B7, CRP, SAA, GLUL, GPR49, ANGPT1, ANGPT2, NTS, and HAL) were performed as described, on all eight biopsies [3,5,7].

## Case description

*Patient history*

A 42 year old male patient presented at the outpatient clinic, with abdominal pain and cramps for 2 weeks. Clinically, hard hepatomegaly was noted. On subsequent CT scan, a large ($23 \times 12 \times 14$ cm) lesion was detected in the left liver lobe, showing an irregular margin, necrotic areas, and calcifications. On MRI with contrast, multiple focal lesions mildly hyperintense on T2-weighted imaging, arterially vascularised, and without late retention, were noted. The largest lesion was in segment 5/6 (3.5 cm), 5 (3.0 cm), 7 (1.7 cm), and multiple similar lesions were in segments 5, 6, 7, and 8 (all smaller than 2 cm). Based on the radiological appearance, preference for adenomas was accepted. Carcino-embryonic antigen and α-fetoprotein were not determined.

There were no metastases within or outside of the liver, so the lesion was resected. The patient underwent a laparoscopic left hepatectomy with preservation of the middle hepatic vein. This was done in a standard fashion using the surgical aspirator (CUSA Excel, Integra Life Science Ltd, IDA Business and Technology Park, Ireland) with minimal blood loss provided by a unilateral vascular inflow exclusion before starting the resection. The pathology report of the resection specimen is described below.

As a child, between 1 and 2 years of age, the patient experienced two episodes of syncope with suspected hypoglycaemia, during viral illness. The treatment consisted of oral intake of glucose. Clinically at that time there was hepatomegaly. A diagnosis of glycogenosis was suggested but not pursued, followed-up, or treated accordingly. On the contrary, the patient was started on a diet for celiac disease, which he was still following at the age of 42. He did not have a history of recurrent hypoglycaemia, nocturnal feeding, or lactic acidosis. The patient went through a late puberty and growth spurt, but later displayed excellent catch-up growth to reach 170 cm height (weight 61 kg). He never experienced any problems with fasting or exertion. On the contrary, the patient is a long-distance runner, running marathons without apparent need for extra carbohydrates before or during exertion. After a 12 h fast, lactate was at 4.49 mM (0.4–2.0; in fed state 1.22 mM on one occasion, 2.01 mM on another), glucose after 12 h fast was at 64 mg/dl (55–100), triglycerides at 328 mg/dl (⩽180), cholesterol at 151 mg/dl (⩽190), and uric acid at 5.0 mg/dl (3.5–7.2). Glycaemia measured at home in the mornings, in a fasting state, or when feeling weak or hungry, was consistently normal. Creatine kinase and liver tests were normal. Both echocardiography and the glomerular filtration rate was normal as well. There was no evidence of proteinuria, nor was there any history of kidney stones. During work-up for a gastroenteritis episode, seven years prior to his presentation with the

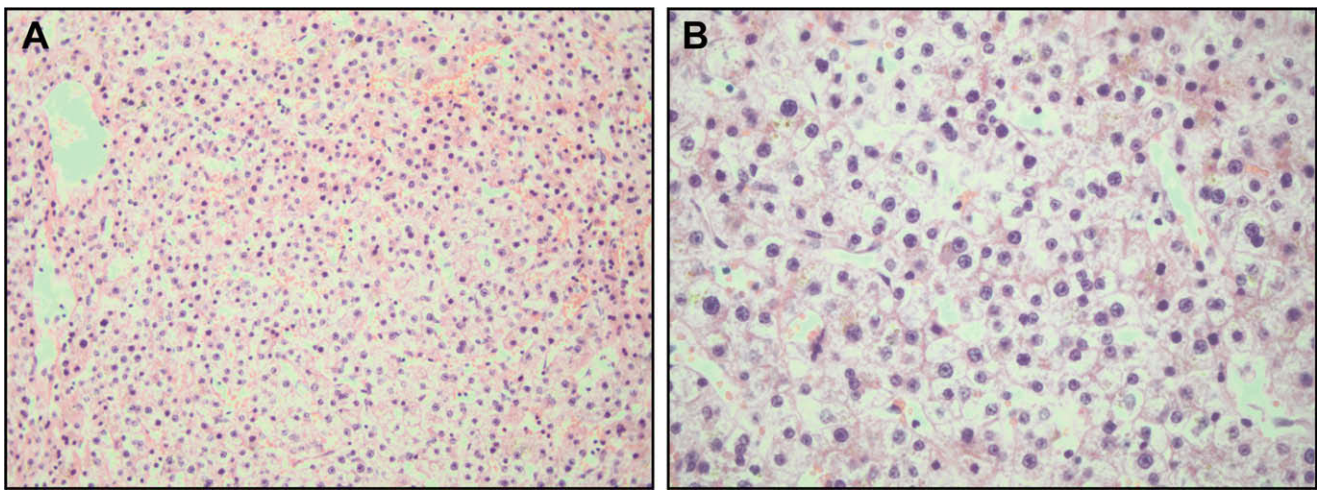


**Fig. 2. The adenomatous component of the multinodular lesion consists of trabecules of hepatocytes that show discrete nuclear atypia.** The morphological features are consistent with a β-catenin activated adenoma. (A) Original magnification 200× and (B) same field at 400× magnification.

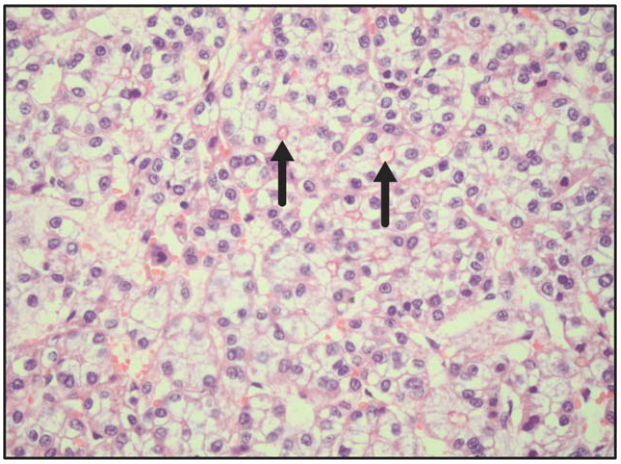

**Fig. 3. In contrast to the adenomatous part, the areas that transformed into well-differentiated hepatocellular carcinoma show more distinct nuclear atypia and crowding due to an increased nucleo-cytoplasmic ratio and thickening of the trabecules.** Original magnification 400×.

current lesion, ultrasound revealed a 'somewhat enlarged, homogeneous liver' (no pictures stored for review).

The patient's family history indicates no genetic predisposition. The patient has no children, has one healthy sister (routine biochemistry and liver ultrasound were normal), and both parents are alive and well around the age of 70.

At one year follow-up after the resection, the patient has resumed his work and sports without restraint. A malignant lesion measuring 23 cm is well outside Milan criteria for transplantation. The patient has declined the theoretical option of transplantation. Control MRIs, taken every four months, showed no recurrence of HCC in the liver and a stable size of the remaining adenomas. A chest CT revealed no lung metastases.

*Pathology report of the resection specimen*

The partial hepatectomy specimen measured 19 × 13 × 9 cm and contained a multinodular tumor with a maximal diameter of 18 cm. The tumor had a lightbrown color in some areas and a yellow to greenish aspect in other areas. Biopsies taken from the small rim of non-tumorous liver showed normal portal tracts and some fibrosis due to compression of the tumor. Very focally, there was some mixed steatosis. The periportal parenchyma contained numerous hepatocytes with a glycogenated nucleus (Fig. 1). Several hepatocytes also had a swollen, pale cytoplasm with wisps and small clumps of eosinophilic staining, giving the impression of so-called "plant-like" hepatocytes, which is suggestive of glycogen storage disease which is not of type 0 or 4 (Fig. 1) [11]. An intense, cytoplasmic hepatocytic signal was not seen on the PAS-staining, most likely due to wash-out of glycogen during processing of the tissue.

The lesion consisted of several nodules of well-differentiated hepatocellular carcinoma against a background of hepatocellular adenoma. The adenomatous areas consisted of sheets of non-steatotic hepatocytes organised as one to two cell thick plates separated by non-dilated sinusoids and traversed by some blood vessels. Reticulin surrounding these plates was preserved. Portal tracts, ductular reaction, and inflammation were all absent. The nuclei of the hepatocytes showed mild atypia: they were slightly irregular and contained chromatin clumps and a distinctive nucleolus. Based on these morphological features (Fig. 2A and B), the adenoma was suspected to be of the β-catenin activated subtype [9]. This was confirmed by immunohistochemistry for glutamine synthetase which showed diffuse and strong staining of the adenomatous areas (Fig. 4A), while the surrounding, non-lesional liver only showed a signal in hepatocytes surrounding centrolobular veins (Fig. 4B). Nuclear expression of β-catenin was observed in only very few hepatocytes in the adenoma. Positive staining of the hepatocytic cell membrane for β-catenin served as an internal control.

The malignant nodules were sharply demarcated from the adenomatous areas and consisted of hepatocyte-like cells organised in trabecular and pseudoglandular structures. The trabecules were separated from each other by sinusoids and they were frequently more than three cells thick. The reticulin pattern was clearly lost. The nuclei were sometimes peripherally located within the cell and were slightly more atypical than in the adenomatous areas. The nucleo-cytoplasmic ratio was clearly

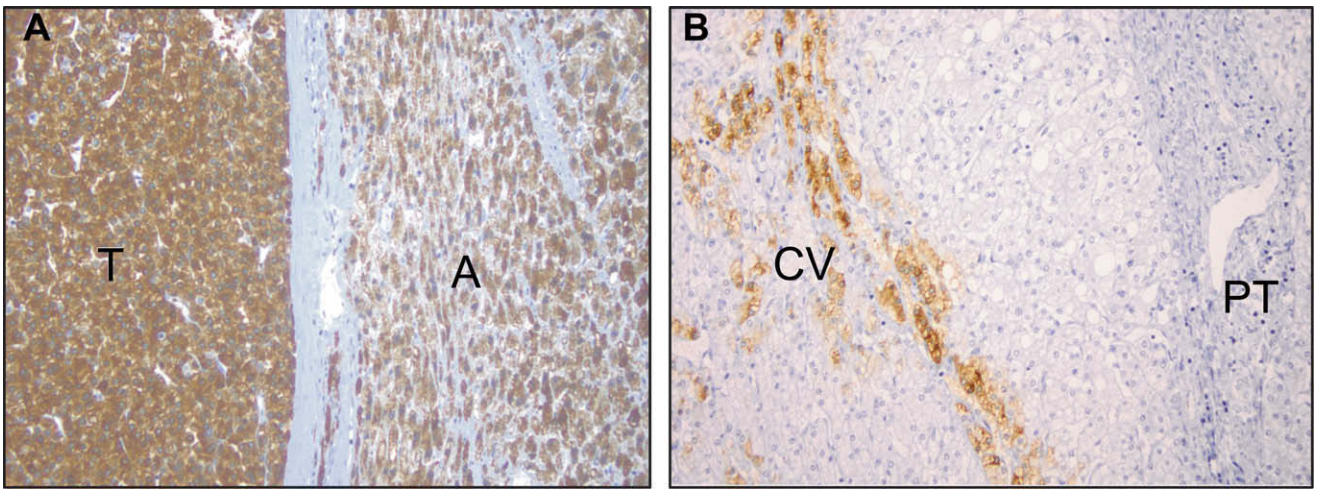


**Fig. 4. Mutation analysis** (A) Both the adenomatous areas (A) as the parts that consist of malignant tumor (T) show diffuse and strong cytoplasmic staining for glutamine synthetase staining; (B) surrounding non-lesional tissue serving as an internal control only shows some staining in the area around the centrolobular vein (CV). Original magnification 200×.

increased. Bilirubin droplets were present in some of the malignant nodules. Based on these features (Fig. 3), the diagnosis of well-differentiated hepatocellular carcinoma arising in a β-catenin activated hepatocellular adenoma was established. The malignant areas showed diffuse and strong staining for glutamine synthetase (Fig. 4A). While all malignant hepatocytes showed positivity of the cell membrane for β-catenin, there were very few positive nuclei.

*Diagnostic work-up for suspected glycogenosis*

Glycogen phosphorylase kinase enzymatic activity on fresh red blood cells was normal. On snap-frozen liver biopsy, glycogen was 4.8% of wet liver weight (3.53 ± 0.45), enzymatic activity of phosphorylase and hexose diphosphatase were normal, glucose-6-phosphatase activity was clearly reduced to 0.36 U/g (7.95 ± 0.51).

Mutation analysis of the G6PC gene revealed compound heterozygosity for p.Arg170X (c.508C>T) in exon 4 and p.Ala192Val (c.575C>T) in exon 5. p.Arg170X has been described as a pathogenic mutation [10], while p.Ala192Val has not, but affects a phylogenetically highly conserved amino acid in a transmembrane domain of the protein and is thus predicted to be pathogenic.

*Molecular studies of the HCC (eight biopsies)*

Four random biopsies, taken from the large malignant lesion, showed the same mutation in CTNNB1: in-frame deletion Del7L-131L, while four other random biopsies showed another mutation in CTNNB1: in-frame deletion del21G-98M + ins21CC. No IL6ST or HNF1A mutations were found in any of the samples. We performed RT-PCR analysis testing the expression of 10 genes (LFABP1, UGT2B7, CRP, SAA, GLUL, GPR49, ANGPT1, ANGPT2, NTS, and HAL) (Table 1). We found an mRNA expression profile typical of a β-catenin activated adenoma in all samples, with overexpression of two genes targeted by β-catenin (GLUL and GPR49) when compared to normal liver tissues (fold change T/N ranging from 3- to 374-fold, depending on the biopsies). Taken together, these results clearly demonstrate the presence of somatic CTNNB1 in-frame deletions activating β-catenin in this lesion.

## Discussion

We report on a mild clinical presentation of GSDIa, where the diagnosis was missed early in life and the patient developed normally, without symptoms or complaints and without treatment or follow-up. Nevertheless, in adult age, the patient presented with multiple liver adenomas, one of which had developed into an HCC.

In the case presented here, the presence of hepatocytes with swollen and pale cytoplasm and with glycogenated nuclei in the tissue surrounding the resected hepatocellular carcinoma, together with a vague history of hypoglycaemia and hepatomegaly and an increased fasting lactate, set off the search for an underlying glycogenosis. The fact the patient has an enzymatically and genetically proven GSDIa and presented with adenomatosis and HCC in adult age, should prompt liver specialists and oncologists to look for GSDIa in patients presenting with adenomatosis and/or HCC, even when adult.

**Table 1. Real-time quantitative RT-PCR results on the eight biopsies taken from the resected lesion.**

| Nodules ID | HNF1 Mutation | Mutation gp130 | CTNNB1 mutation (nucleotide) | CTNNB1 mutation (AA change) | GLUL* | LGR5* | FABP1* | UGT2B7* | ANGPT1/ANGPT2** | NTS/HAL** | CRP* | SAA2* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biopsy 1 | nm | nm | 21_395del | 7L-131Ldel | 3.7 | 2.5 | 1 | 3.1 | 0.6 | 0.6 | 0.2 | 1.2 |
| Biopsy 2 | nm | nm | 21_395del | 7L-131Ldel | 39.9 | 26.9 | 0.3 | 0.9 | 0.6 | 0.7 | 0.00 | 0.02 |
| Biopsy 3 | nm | nm | 21_395del | 7L-131Ldel | 12.5 | 5.3 | 0.3 | 0.4 | 1.6 | 0.3 | 0.01 | 0.00 |
| Biopsy 4 | nm | nm | 21_395del | 7L-131Ldel | 74.9 | 7.1 | 0.3 | 0.2 | 0.4 | 0.3 | 0.00 | 0.00 |
| Biopsy 5 | nm | nm | 64-291del | 21G-98Mdel+insCC | 68 | 58 | 0.2 | 0.3 | 2.0 | 16.0 | 0.01 | 0.01 |
| Biopsy 6 | nm | nm | 64-291del | 21G-98Mdel+insCC | 99.5 | 39.5 | 0.4 | 1 | 1.25 | 26 | 0.01 | 0.01 |
| Biopsy 7 | nm | nm | 64-291del | 21G-98Mdel+insCC | 142.2 | 62.3 | 0.5 | 1.5 | 2.4 | 38 | 0.02 | 0.01 |
| Biopsy 8 | nm | nm | 64-291del | 21G-98Mdel+insCC | 374.2 | 196.7 | 1.2 | 4.4 | 3 | 36 | 0.1 | 0.3 |
| H-HCA ($n$ = 7) | M | nm | nm | nm | 1.3 | 0.1 | 0.01 | 0.009 | 4.7 | 3.1 | 1.3 | 0.002 |
| βHCA ($n$ = 3) | nm | nm | M | M | 96 | 56 | 0.6 | 1.7 | 4.3 | 13 | 0.2 | 0.58 |
| IHCA gp130 mutated ($n$ = 6) | nm | M | nm | nm | 2.7 | 2.1 | 0.85 | 3 | 2.3 | 2.6 | 36.00 | 35.00 |
| Focal nodular hyperplasia ($n$ = 3) | nm | nm | nm | nm | 18.4 | 21.8 | 0.3 | 2.3 | 12.1 | 386 | 0.1 | 0.2 |

nm, non-mutated; M, mutated; AA, amino acid; H-HCA, adenoma inactivated for HNF1A; βHCA, adenoma mutated for β-catenin; IHCA, inflammatory adenoma.

* Quantitative RT-PCR data expressed in 2^-DDCT, arbitrary unit, normal liver = 1.

** Quantitative RT-PCR data, ratio of expression.

The presence of hepatocytes with swollen and pale cytoplasm is a well-known feature of GSD [11] Although glycogenated nuclei in the periportal area are known to represent a disturbance of glycogen metabolism, as is seen in, e.g., NASH [12], they have hitherto not been reported as a typical feature of GSD. Therefore, it is possible that they are related to the atypical clinical presentation of this patient. If not, the presence of glycogenated nuclei would be an additional and easy feature to prompt pathologists and clinicians to explore underlying glycogenoses.

The association of CTNNB1 mutations with malignant degeneration, is well known in sporadic liver adenoma [3]. Out of the 13 GSDIa-associated adenomas reported so far, three were CTNNB1 mutated [3,6,7]. Only one out of 13 was associated with an HCC and was CTNNB1 mutated. We describe another case here, where the resected malignant lesion carried different CTNNB1 mutations and an mRNA expression profile typical of a β-catenin activation associated lesion (Table 1).

The discrepancy between the rarity of a nuclear signal for β-catenin and the diffuse signal for its down-stream target gene glutamine synthetase in the lesion described here is remarkable. This has also been observed recently by Bioulac-Sage et al. in three CTNNB1 mutated sporadic adenomas [9]. This case report suggests that glutamine synthetase is a more sensitive marker than β-catenin itself, when screening for possible β-catenin mutations is performed by immunohistochemistry. This needs to be studied and confirmed in a larger series of adenomas.

Finally, several types of CTNNB1 mutations were present in one malignant lesion here and all eight random biopsies carried CTNNB1 mutations. This suggests that at least two adenomas with distinct mutation types have undergone confluence, either before or after malignant transformation, but that all lesions giving rise to the malignant lesion were CTNNB1 mutated.

In a setting of organ shortage and in view of the risks associated with liver transplantation, it would be useful to gain a better insight into the type of adenomas in GSDIa patients that are likely to undergo malignant transformation and therefore would strengthen the case for liver transplantation [13]. By inference from what is know about sporadic adenomas, one would expect these to be the CTNNB1 mutated adenomas, i.e., glutamine synthetase positive or β-catenin positive on immunohistochemistry. This is the second case of malignant transformation of a CTNNB1-mutated GSDIa-associated adenoma. If this association confirms itself in other patients, it would affect our treatment choices in these patients.

In conclusion, we describe a case of mild GSDIa, presenting with liver lesions in adult age. The HCC resected from this patient was CTNNB1 mutated, which confirms the premalignant nature of CTNNB1 mutated adenomas, also in GSDIa. Molecular studies for CTNNB1 mutations or routine immunohistochemistry for glutamine synthetase and β-catenin, performed on punction biopsies taken from adenomas in GSDIa livers, could be useful to select patients with large and/or growing lesions for transplantation.

## Conflicts of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

## References


[1] Conti JA, Kemeny N. Type Ia glycogenosis associated with hepatocellular-carcinoma. Cancer 1992;69:1320–1322, [Review. PubMed PMID: 1311620].

[2] Franco LM, Krishnamurthy V, Bali D, Weinstein DA, Arn P, Clary B, et al. Hepatocellular carcinoma in glycogen storage disease type Ia: a case series. J Inherit Metab Dis 2005;28:153–162, [PubMed PMID: 15877204].

[3] Zucman-Rossi J, Jeannot E, Nhieu JT, Scoazec JY, Guettier C, Rebouissou S, et al. Genotype–phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. Hepatology 2006;43:515–524.

[4] Van der Borght S, Libbrecht L, Katoonizadeh A, Aerts R, Nevens F, Verslype C, et al. Nuclear beta-catenin staining and absence of steatosis are indicators of hepatocellular adenomas with an increased risk of malignancy. Histopathology 2007;51:855–856, [Epub 2007 September 28. PubMed PMID: 17903198].

[5] Rebouissou S, Amessou M, Couchy G, Poussin K, Imbeaud S, Pilati C, et al. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. Nature 2009;457:200–204, [Epub 2008 November 19. PubMed PMID: 19020503; PubMed Central PMCID: PMC2695248].

[6] Kishnani PS, Chuang TP, Bali D, Koeberl D, Austin S, Weinstein DA, et al. Chromosomal and genetic alterations in human hepatocellular adenomas associated with type Ia glycogen storage disease. Hum Mol Genet 2009;18:4781–4790.

[7] Bioulac-Sage P, Laumonier H, Couchy G, Le Bail B, Sa Cunha A, Rullier A, et al. Hepatocellular adenoma management and phenotypic classification: the Bordeaux experience. Hepatology 2009;50:481–489, [PubMed PMID: 19585623].

[8] Meuleman P, Libbrecht L, De Vos R, de Hemptinne B, Gevaert K, Vandekerckhove J, et al. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology 2005;41:847–856, [PubMed PMID: 15791625].

[9] Bioulac-Sage P, Rebouissou S, Thomas C, Blanc JF, Saric J, Sa Cunha A, et al. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. Hepatology 2007;46:740–748, [PubMed PMID: 17663417].

[10] Rake JP, ten Berge AM, Verlind E, Visser G, Niezen-Koning KE, Buys CH, et al. Glycogen storage disease type Ia: four novel mutations (175delGG, R170X, G266V and V338F) identified. Mutations in brief no. 220. Online. Hum Mutat 1999;13:173, [PubMed PMID: 10094563].

[11] Portmann BC, Thompson RJ, Roberts E, Paterson AC. Genetic and metabolic liver disease. 5th ed. In: Burt AD, Portmann BC, Ferrell LD, editor. MacSween's pathology of the liver. Churchill Livingstone Elsevier; 2007. p. 199–326 [973 paginas].

[12] Liew PL, Lee WJ, Wang W, Lee YC, Chen WY, Fang CL, et al. Fatty liver disease: predictors of nonalcoholic steatohepatitis and gallbladder disease in morbid obesity. Obes Surg 2008;18:847–853, [Epub 2008 May 6. PubMed PMID: 18459024].

[13] Reddy SK, Austin SL, Spencer-Manzon M, Koeberl DD, Clary BM, Desai DM, et al. Liver transplantation for glycogen storage disease type Ia. J Hepatol 2009;51:483–490.