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NATURAL HISTORY OF HEPATOCELLULAR ADENOMA FORMATION IN GLYCOGEN STORAGE DISEASE TYPE I

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Abstract

Objective—To characterize the natural history and factors related to hepatocellular adenoma (HCA) development in glycogen storage disease type I (GSD I).

Study design—Retrospective chart review was performed for 117 patients with GSD I. Kaplan-Meier analysis of HCA progression among two groups of patients with GSD Ia (five-year mean triglyceride concentration ≤ 500 mg/dL and > 500 mg/dL); analysis of serum triglyceride concentration, body mass index (BMI) standard deviation score (SDS), and height SDS between cases at time of HCA diagnosis and age- and sex-matched controls.

Results—Logrank analysis of Kaplan-Meier survival curve demonstrated a significant difference in progression to HCA between the five-year mean triglyceride groups ($p = 0.008$). No significant difference was detected in progression to adenoma event between sexes. Serum triglyceride concentration was significantly different at time of diagnosis of adenoma (737 ± 422 mg/dL) compared with controls (335 ± 195 mg/dL) ($p = 0.009$). Differences in height SDS ($p = 0.051$) and BMI SDS ($p = 0.066$) approached significance in our case-control analysis.

Conclusion—Metabolic control may be related to HCA formation in patients with GSD Ia. Optimizing metabolic control remains critical, and further studies are warranted to understand the pathogenesis of adenoma development.

Keywords

Glycogen storage disease I; Hypertriglyceridemia; Hepatocellular adenoma

Glycogen storage disease type I (GSD I) is an autosomal recessive inborn error of metabolism caused by defects in the glucose-6-phosphatase complex. Deficient activity of the glucose-6-phosphatase- α (G6Pase) catalytic unit characterizes glycogen storage disease type Ia (GSD Ia), whereas type Ib (GSD Ib) is characterized by defects in the glucose-6-phosphate transporter protein. Over 81 mutations in the G6PC gene on chromosome 17q21 have been described.⁽¹⁾ This key enzyme in glucose homeostasis catalyzes the final step for

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glycogenolysis and gluconeogenesis, and all endogenous glucose synthesis is impaired in affected individuals. In addition, defective activity of G6Pase manifests with metabolic derangements including hypertriglyceridemia, hyperuricemia, and hyperlactatemia due to shunting of glucose-6-phosphate into alternative pathways. The mainstay of treatment remains dietary therapy with uncooked cornstarch as a low glycemic index carbohydrate to prolong periods of euglycemia and ameliorate metabolic abnormalities.(2)

With the introduction of continuous glucose therapy in 1971 and uncooked cornstarch in 1982, the prognosis for children and adults with GSD I has improved dramatically. Despite advancements in therapy, hepatic pathology continues to occur frequently in the setting of GSD Ia. Disordered glucose metabolism results in marked hepatomegaly due to glycogen deposition and accumulation of lipids. Hepatocellular adenoma (HCA) formation is a common sequela of GSD Ia, with reported prevalence rates up to 75%.(3) Notably, the incidence of adenoma formation has not significantly changed over the past three decades despite improvement in survival and decreasing rates of other complications. Management of HCA includes serial imaging and surgical resection of concerning lesions to avoid risks of bleeding and/or malignant transformation to hepatocellular carcinoma.

Our goal for this study is to clarify the role of metabolic control in HCA formation and propose further hypotheses for the pathophysiology of adenoma development.

Methods

Institutional review board approval and informed written consent was obtained from patients participating in the study at the University of Florida Glycogen Storage Disease Program. Subjects have been followed in a longitudinal and prospective manner at our institution, and all patients with GSD Ia and GSD Ib were included in the analysis. Patients were excluded if imaging studies were unavailable. A total of 97 subjects with GSD Ia and 20 subjects with GSD Ib were included in these investigations. The diagnosis for GSD Ia and GSD Ib was established biochemically or by assessment of enzyme testing from a liver biopsy. The diagnosis was confirmed for subjects by mutation analysis where available.

Patients were evaluated annually with laboratory testing for markers of metabolic control and ultrasound screening for hepatic and renal lesions. Because fasting laboratory studies cannot be performed in patients with GSD, baseline studies were obtained in the period immediately prior to the morning cornstarch administration. Patients discovered to have hepatic adenomas were subsequently monitored with serial imaging through either magnetic resonance imaging or ultrasonography as recommended in the literature.(3)

A retrospective review of the clinical records for all patients with GSD Ia and GSD Ib was performed. Time to adenoma formation was analyzed with the Kaplan-Meier method.(4) Patients were censored at the time of their latest negative imaging study. An event was recorded if a patient had ultrasonographic imaging demonstrating hepatic adenoma after previously negative imaging.

Because serum glucose, lactate, and pH fluctuate rapidly and serum urate is an unreliable marker in the setting of allopurinol treatment, we targeted serum triglyceride concentration as a surrogate marker of metabolic control. Mean serum TG concentrations from five years preceding adenoma discovery or censorship were analyzed in a Kaplan-Meier survival curve. Patients without laboratory testing from this period were excluded from comparison. A predetermined triglyceride concentration of 500 mg/dL from the consensus panel discussion at the 2010 Association for Glycogen Storage Disease Conference was used to stratify patients into two groups. A total of 83 subjects (62 five-year mean TG $\leq$ 500 mg/dL, 21 five-year mean TG $>$ 500 mg/dL) were available for survival comparison.

Serum triglyceride concentration, height, and body mass index (BMI) were compared on a 1:1 case-control basis. Height and BMI were represented with the standard deviation score (SDS) which was calculated based on age-, sex-, and geographically-matched controls. A total of 28 patients with GSD Ia and hepatic adenomas were known to us. Each patient's records were reviewed to confirm time of initial adenoma diagnosis by imaging. Patients without previously negative imaging records were excluded, as were patients without laboratory values at time of adenoma discovery. This resulted in 12 cases. Controls were matched by both sex and record availability for the equivalent case age. A total of 24 subjects (12 cases and 12 controls) met these criteria.

Statistical analysis

Statistical results were descriptive and were reported as either mean $\pm$ standard deviation (SD) or median with range, as appropriate. Differences in variables between cases and controls were analyzed with unpaired two-tailed Student *t*-tests. Logrank analysis of Kaplan-Meier survival curves was performed using MedCalc for Windows, version 11.3.0.0 (MedCalc Software, Mariakerke, Belgium). A *p*-value <0.05 was used to define significance.

Results

Patients with GSD Ia (59 male and 38 female) were included in the Kaplan-Meier analysis of adenoma-free progression. The median age of most recent imaging follow-up was 12.5 years (range 1.3-38.8 years); 28 of the 97 patients developed adenomas (28.9%) at a median age of 16.3 years (range 3.6-30.7). Logrank analysis did not detect a significant difference in adenoma development between sexes ($p = 0.9442$). Median age of adenoma development in females was 16.0 years (range 10.1-30.7 years) and 17.3 years in males (range 3.6-25.6 years). For analysis of five-year mean TG concentrations, 33 females and 50 males were included. Logrank analysis of the Kaplan-Meier curve demonstrated a highly significant difference in adenoma progression between five-year mean TG ≤ 500 mg/dL and >500 mg/dL groups ($p = 0.008$) (Figure 1).

Twelve patients with adenomas met inclusion criteria for case-control analysis, with 5 females and 7 males in each group (Table). The median age at adenoma diagnosis was 17 years (range 3.6-30.7). Six patients in the case group were being treated with allopurinol at time of adenoma diagnosis, as opposed to two controls. Three patients in the case group were treated with lipid-lowering medications compared with none in the control group. Patients had a highly significant difference in serum TG concentration at time of adenoma discovery compared with age-matched controls ($p = 0.009$) (Figure 2). Mean TG concentration was 737 mg/dL (SD ± 422) in the case group and 335 mg/dL (SD ± 195) in the control group. Height SDS approached significance between case (mean -1.71, SD ± 1.57) and control (mean -0.41, SD ± 1.19) groups ($p = 0.051$) (Figure 3). BMI SDS was not significantly different between case (mean 0.76, SD ± 0.94) and control (mean 1.55, SD ± 0.87) groups ($p = 0.066$) (Figure 2).

Patients with GSD Ib (9 females and 11 males) were included in Kaplan-Meier analysis of adenoma-free progression in comparison with patients with GSD Ia ($p = 0.107$) (Figure 3). Median follow-up was 14.4 years (range 1.9-38 years). Three of the 20 patients (15%) developed adenomas, at a median age of 15 years (range 13.5-22).

Discussion

Hepatic adenomas were initially reported in the setting of GSD in 1955 and have been described in patients from ages 3 to 54 years.(3,5) However, the natural history and

pathophysiology remains poorly understood, and significant concerns arise from possible malignant degeneration and attendant risks of both surgical resection and liver transplantation.(6-8) Metabolic control has been hypothesized to play a role in adenoma development, but studies have shown conflicting results.(9,10) Using serum TG concentration as a surrogate marker of metabolic control, our survival analysis and case-control study highlights significant differences correlated with progression to HCA development.

Previous studies have shown that adenomas develop predominantly during and after puberty, and our Kaplan-Meier analysis is concordant with these findings. The earliest adenoma discovery was at 3.6 years, though only two patients developed adenomas before 10 years of age; 21 of 28 patients (75%) developed adenomas between ages 10 and 20 years of age, and the incidence of new adenomas appears to decrease after 20 years of age. The increased incidence of HCA development during adolescence may be related to suboptimal metabolic control during this age period or to pubertal hormone secretion. Although it was previously thought that the prevalence of adenomas in males was twice as frequent as females, this study reproduces the 1:1 ratio seen in the European Study on Glycogen Storage Disease Type I (ESGSD I).(3,6,7) A small case series indicated that male patients develop adenomas later in life, though our data indicate there are no significant differences in time of adenoma onset between sexes.(8)

Our Kaplan-Meier survival analysis detected a highly significant difference in adenoma development dependent on five-year mean TG concentrations. Further evidence from our analysis of patients at time of adenoma discovery demonstrated significantly higher serum triglyceride concentrations compared with age- and sex-matched controls. Notably, three out of 12 patients (25%) in the case group were treated with lipid-lowering medications, compared with none in the control group. Though it has been speculated that metabolic control is an important factor in adenoma development, a previous case-control study did not find significant differences in markers of control in adenoma cases.(9) This study found similarly high triglyceride concentrations among patients developing adenomas, but our control group demonstrated lower values with a smaller range. Because these studies analyzed different populations, disparities in these studies may be due to genetic factors or differences in treatment.

Linear growth of patients with GSD I has been studied in the ESGSD I and may serve as a useful indicator of long-term metabolic control.(7) We analyzed the height SDS in our case-control study and found a trend towards significance, though larger numbers of patients will be required to confirm these differences. One previous study found BMI to be significantly lower in patients with adenomas compared with age-matched controls.(9) Even though our comparison of BMI SDS did not achieve significance due to the relatively small sample size, the trend of lower BMI in the case group is consistent with previous findings. It is possible that patients with better metabolic control have higher BMI and additional studies are required to understand the role of metabolic control in growth and development of patients with GSD I.

Patients with hyperlipidemia commonly exhibit hyperlactatemia. Because lactate concentrations fluctuate rapidly, they are difficult to use as a marker of chronic control. A prior study found higher mean daily lactate concentrations in patients with adenomas, but future studies will be required to elucidate the role of other metabolic markers in the pathogenesis of HCA.(10)

Mutation analysis did not demonstrate a significant difference in genotypes between cases and controls. The R83C mutation was the most frequent mutation in both case and control

groups. Because patients harboring a variety of different mutations have been reported to develop hepatic adenomas, a genetic component of adenoma pathophysiology cannot be excluded. Differences between our findings and those reported by Di Rocco et al may partially be explained by varying genotypes between the investigated populations.(9)

The discrepancy of HCA development in different GSD types provides further clues for adenoma pathophysiology. HCA develops commonly in GSD Ia, less commonly in GSD Ib, and rarely in types III, VI, IX, and XI even though these types are all characterized by excessive glycogen storage.(8,11) Corresponding evidence from the ESGSD I shows that 73% of patients with GSD Ia develop severe hypertriglyceridemia compared with 43% in patients with GSD Ib(7). Our internal records indicate that only 15% of patients with GSD Ib have five-year mean TG levels greater than 500 mg/dL (unpublished data). A study of patients with GSD type III found the prevalence of hypertriglyceridemia to be 67% in early childhood, but significant TG elevation is uncommon as patients' age.(12) Lipid abnormalities in types 0, VI, IX, and XI are usually relatively mild. Notably, considerable alteration of lipid metabolism in the liver is a shared feature among forms of GSD associated with HCA formation.

The pathophysiology of hyperlipidemia in GSD I differs from other types of GSD and is thus a salient feature related to HCA investigation. Bandsma demonstrated a 40-fold increase in *de novo* fatty acid synthesis among patients with GSD I.(13) A recent review highlights the central role of *de novo* FA biosynthesis in tumorigenesis.(14) Increased *de novo* FA synthesis is required in tumor cells for construction of highly lipidic membranes, and studies with chemical inhibitors of fatty acid synthase (FAS) show decreased proliferation and increased apoptosis of tumor cells.(15) Post-translational modification with lipid moieties is a key process regulating oncoprotein transport and function.(14) Taken together, there is compelling evidence that oncogenes require metabolic modification in the form of increased *de novo* FA synthesis to transform the cell. Though there has been no study of *de novo* FA synthesis in human hepatic adenomas, evidence in mouse models show strong FAS over-expression in precursor lesions to HCA.(16)

Future investigations will also be required to further characterize the histological subtype of adenomas in this population. It is known that the CTNNB1 mutation confers higher risk of malignant degeneration to HCC than other subtypes, and this subtype has been identified in previous studies of GSD I adenoma tissue.(17-20) These studies can offer insight into the pathophysiology of HCA formation and may also help guide management of patients with known adenomas.

In contrast to prior studies, our investigations illustrate that poor metabolic control appears to play a role in HCA formation. It is important to note that the pathogenesis of adenoma development is likely multifactorial and there are almost certainly genetic and other unknown factors involved. Histological and genetic analyses assessing differential gene expression between HCA and surrounding hepatic tissue may help clarify adenoma pathogenesis. Differences between patient genotypes are difficult to assess due to the rarity of these mutations, but collaborations with the International Study for GSD may allow for better determination of a genotype/phenotype correlation. Optimizing metabolic control remains critical in the management of patients with GSD I, and further studies are needed to determine whether pharmacologic therapy aimed at improving metabolic derangements can help prevent the common complication of HCA formation.

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List of Abbreviations

BMI	body mass index
ESGSD I	The European Study on Glycogen Storage Disease Type I
FA	fatty acid
FAS	fatty acid synthase
GSD I	glycogen storage disease I
G6Pase	glucose-6-phosphatase- α
GSD Ia	glycogen storage disease type Ia
GSD Ib	glycogen storage disease type Ib
HCA	hepatocellular adenoma
SD	standard deviation
SDS	standard deviation score
TG	triglyceride

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Figure 1.
Kaplan-Meier survival curve of adenoma-free progression in GSD Ia based on five-year mean triglyceride concentration.

Figure 2.

Box-and-whisker plots comparing serum triglyceride concentration and body mass index between case and control groups. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. The horizontal line extends from the minimum to the maximum value, excluding “outside” and “far out” values which are displayed as separate points. An outside value is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range. A far out value is defined as a value that is smaller than the lower quartile minus 3 times the interquartile range, or larger than the upper quartile plus 3 times the interquartile range.

Figure 3.
Kaplan-Meier survival curve of adenoma-free progression in GSD types Ia and Ib.

Table 1

Demographics of case-control analysis

Cases					Controls				
Age (y)	Sex	GSD diagnosis (m)	Medication	Genotype	Age (y)	Sex	GSD diagnosis (m)	Medication	Genotype
3.6	M	1	A	?	3.8	M	8		R83C/Q347X
11.9	F	5	A	R83C	11.6	F	3		R83C
12.5	M	6		?	13.5	M	5		R83C/1G>C
14.9	F	9		35X/D38V	15.7	F	1		R83C
15.5	M	6		W77/?	14.9	M	7		R83C/35X
16.7	F	4	A, G	R83C	13.7	F	12		G188R/Q347X
17.3	M	7	A, G	R83C/R170Q	17.4	M	9		R83C/Q347X
17.5	M	4	A	R83C	17.3	M	6	A	W69X/?
17.7	F	7		R83C	17.7	F	2		R83C
18.7	M	1	N	R83C	17.9	M	9	A	delF327
25.6	M	12		35X/Q347X	25.8	M	1		?
30.7	F	20	A	?	27.7	F	1		?

A, allopurinol; G, gemfibrozil; N, niacin