Cross-Sectional Analysis of Progressive Familial Intrahepatic Cholestasis in Puerto Rican Children

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Objective: Specific inherited disorders may be more common in island communities. Prior case reports suggest that cholestatic liver diseases may constitute a group of these inherited disorders in Puerto Rico. A cross-sectional survey of liver diseases in children was conducted to assess this hypothesis.

Methods: A cross-sectional analysis was performed in patients with chronic cholestasis at "Hospital Pediátrico Universitario" in San Juan, Puerto Rico. Ten potential participants with high gamma-glutamyl transpeptidase (GGTP) cholestasis were identified. Therapeutic response to ursodeoxycholic acid (UDCA) was assessed by the examination of alanine aminotransferase (ALT) and GGTP changes.

Results: Four participants, who were under 1 year of age, presented with pruritus, abnormal liver biochemistries, and/or hepatomegaly. Older patients had similar presentations but also had splenomegaly, jaundice, and/or esophageal varices. Three had progression of liver disease, 2 required liver transplantion after partial external biliary diversion. The third patient developed hypersplenism despite having a normal liver profile on UDCA. Six of 10 patients had normalization of their liver profiles (ALT<40 IU/L; GGTP<100 IU/L) after UDCA administration (before: ALT = 182 ± 61 and GGTP = 353 ± 192; after: ALT = 30 ± 15 and GGTP = 21 ± 13; p-value<0.001)

Conclusion: The response to UDCA in a sub-group of patients in Puerto Rico with high GGTP cholestatic liver disease is described. The findings suggest the possibility that *ABCB4*-related disease is an important genetic disorder in Puerto Rico. Future investigations utilizing the genome sequence are important to the further understand liver disease in Puerto Rico. [*P R Health Sci J 2016;35:220-223*]

Key words: Liver, Cirrhosis, Bile acid, Ursodeoxycholic acid, Pediatric

The Puerto Rican ancestry is a mix of African, European, and American Indian, "los Tainos." Several genetic diseases, including Hermansky–Pudlak syndrome (HPS 1 and 3), are found with relatively high frequency on the island of Puerto Rico (1)(2). Steel syndrome (3) and primary ciliary dyskinesia-11 (4) have also been described as founder mutation events that segregate in the Puerto Rican population. Spondylocostal dysostosis is also more prevalent on the island than anywhere else, comprising 49% of described cases (5).

Clayton first described severe fatal intrahepatic cholestasis in 6 members of 4 sibships of the Old Order Amish (6). To our knowledge, the term "progressive familial intrahepatic cholestasis" was first used by Ballow in his 1973 report (7). In that report, 2 brothers of unrelated Puerto Rican parents who developed early-onset jaundice, pruritus, malabsorption, and rickets were described. The brothers both had conjugated hyperbilirubinemia with elevated bile acids. At that time, a defect in the hepatic excretory mechanism for bile acids was postulated, with a possible autosomal recessive pattern of inheritance.

Leevy described 4 Puerto Rican sisters who had prolonged recurrent cholestasis of pregnancy, accompanied by pruritus with every pregnancy (8). In addition, their pruritus was provoked by oral contraceptives. Two had gallstones and 2 had increased hepatic copper deposition. A genetic basis for their disorder is highly likely, perhaps MDR3 (ABCB4) deficiency, which has been associated with gallstones and intrahepatic cholestasis of pregnancy, among other problems (9). Leevy's report preceded the molecular identification of MDR3 deficiency.

In this report we describe 10 children from Puerto Rico with elevated gamma-glutamyl transpeptidase (GGTP) cholestasis. We explored aspects of their clinical presentations as well as their responses to ursodeoxycholic acid (UDCA) therapy.

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Methods

A cross-sectional analysis including a review of existing medical records and the conducting of personal interviews were performed in patients with chronic cholestasis at "Hospital Pediátrico Universitario" in San Juan, Puerto Rico. Patients were identified by their primary gastroenterologists for possible enrollment in the study. For each, an informed consent was obtained at the time of the appointment with his or her primary gastroenterologist. Exclusion criteria as well as a data collection questionnaire were adapted with permission from the protocol for the NIH/NIDDK-sponsored study of the Childhood Liver Disease Research Network (ChiLDReN): Longitudinal Study of Genetic Causes of Intrahepatic Cholestasis (NCT00571272). The study had 2 sets of inclusion criteria, with the first consisting of evidence of liver disease and the second consisting of evidence of cholestasis; to take part in the study a given patient had to meet a minimum of 1 condition in each set. Criteria were as follows:

Set 1: clinical or biochemical hepatic abnormalities for at least 6 months; clinical or histological stigmata of liver disease; a sibling affected by progressive familial intrahepatic cholestasis or benign recurrent intrahepatic cholestasis; recurrent and episodic cholestatic disease (without identifiable cause) occurring on more than 2 occasions, the episodes of which are separated by at least 3 months.

Set 2: a fasting serum bile acid level greater than 3x the upper limit of normal (ULN) for age; a direct or conjugated bilirubin level greater than 2mg/dL; fat soluble vitamin deficiency without identifiable cause; a GGTP level greater than 3x ULN for age; intractable pruritus secondary to liver disease.

Individuals with other known causes of chronic cholestasis were excluded. The study was approved by the IRBs of both the University of Puerto Rico Medical Sciences Campus and the University of Pittsburgh School of Medicine. Informed consent/assent was obtained from all of the participants and/or their parents/guardians.The clinical data were collected by reviewing medical records and from the personal interviews conducted at the scheduled 1-time visit with the participants' primary gastroenterologists. The clinical information included family history; age and clinical presentation at the onset of symptoms; disease evolution; diagnostic workup including bloodwork, imaging, liver biopsy findings; and previously attempted therapeutic measures (with a focus on the use of UDCA) and surgical interventions such as partial external biliary diversion or liver transplantation. The clinical onset of disease was determined based upon each patient's history; the initial laboratory data included the first testing done after that patient's referral to their pediatric gastroenterologist, while the last set was the one obtained closest to and prior to the time of each participant's enrollment. Liver biopsy pathology results were extracted from reports without an independent review of histology. Informed consent was obtained for 23 patients. Thirteen participants met the inclusion criteria. As the vast majority of participants had a GGTP level of more than 100 IU/L, this analysis was focused on the 10 enrolled participants making up that majority.

The differences in the biochemical parameters were assessed using standard statistical parameters, unpaired t-test results were used.

Results

This cohort included 10 children with high GGTP cholestasis, operationally defined as having a GGTP level greater than 100 IU/L at initial testing (prior to UDCA treatment) (Table 1). The relevant clinical information from the review of the medical records and the medical interviews is summarized in Tables 1 and 2. Participants had been followed for a minimum of 6 months at the time of study enrollment, with a median follow-up of 7.5 years after the clinical presentation. Of note 2 of the participants, siblings 5 and 6, were referred as low GGTP cholestasis, although their GGTP measurements prior to instituting UDCA therapy were not available for review. One of these siblings had a GGTP level over 100 IU/L while receiving UDCA, and as such both siblings were classified as having high GGTP cholestasis. These 2 children had a poor response to UDCA and ultimately required partial external biliary diversion. They had a partial response to surgery, and over 5 to 10 years developed end-stage liver disease manifesting with variceal hemorrhage and ascites. They underwent liver transplantation at 11 and 12 years of age, respectively.

Response to UDCA could be assessed in 8 children. For this analysis a significant improvement ("normalization") after the use of UDCA was defined as an ALT ≤ 40 IU/L and a GGTP ≤ 100 IU/L. The liver profile in six patients normalized, and in two patients improved in response to UDCA. (Table 3). Overall, differences in both ALT and GGTP in response to UDCA therapy were statistically significant (ALT before: 182 ± 61; and after: 30 ± 15; p-value<0.0001; GGTP before: 353 ± 192; and after: 21 ± 13; p = 0.0005).

The family histories revealed that 1 participant had 1 set of consanguineous grandparents, 2 participants had positive family histories for gallstones, and 1 had a grandparent with decompensated liver disease of unknown etiology. In this study, participant 3 had increased copper deposition on liver biopsy, and participant 9 suffered from pruritus during pregnancy, with complete resolution after delivery.

Discussion

In this study 10 Puerto Rican patients with high GGTP chronic intrahepatic cholestasis are described. Genetic mutations of *ABCB4* may lead to chronic cholestasis, chronic "hepatitis," cryptogenic cirrhosis, intrahepatic lithiasis, gallstones, intrahepatic cholestasis of pregnancy (ICP), or progressive

Table 1. Initial clinical data in 10 patients with high GGT cholestasis

Case number	Sex age at onset	Clinical presentation	Age presentation	ALT	AST	TB	DB	GGTP	Liver Biopsy (age)
1	M/8m	Hepatomegaly, cholelithiasis and abnormal liver panel	8m	98	146	0.7	0.1	223	N/D
2	F/5m	Pruritus, abnormal liver panel	9m	118	111	1.1	0.8	313	Canalicular cholestasis with ductular proliferation, periportal bridging fibrosis (10m)
3*	M/5y	Hepatosplenomegaly	7y	268	268	1.4	-	650	Cirrhosis and moderate amount of copper deposition (7y)
4	F/12y	Abnormal liver panel	13y	165	110	0.7	0.2	653	Proliferation of bile ductules at periphery. Ductular metaplasia of periportal hepatocytes. Portal-portal bridging fibrosis (14y)
5	F/6m	Pruritus	4y	47	75	1.7	1	126	Mild portal lymphocytic infiltrate, portal-portal bridging fibrosis. Signs of early cirrhosis. Mild bile ductular proliferation with number of bile ducts slightly diminished with no cholestasis (3y)
6	F/6m	Pruritus	10y	37	56	4	-	33	Micronodular cirrhosis with scant patchy hepatocellular and canalicular cholestasis. Immunostaining positive for BSEP/MRP2. CD10/GGT found along few canaliculi but elsewhere absent (explant-12y)
7	M/3 y	Abnormal liver panel	3y	226	99	0.6	-	154	Portal areas with chronic inflammatory cells with interface involvement. Normal bile ducts (3y)
8**	F/13y	Abnormal liver panel and jaundice	13y	135	147	6	-	230	N/D
9	F/9y	Abnormal liver panel, pruritus	9y	219	129	-	-	290	No interface hepatitis or lobular degeneration. Porto-portal fibrosis was positive by trichrome/ reticulin. Paucity of bile ducts with ductular proliferation (10y)
10	F/14y	Abdominal pain, esophageal varices, sporadic pruritus	14y	223	188	0.6	-	310	N/D

*patient 3 had an initial platelets count of 102x10^3 µL and a ceruloplasmin of 67 mg/dL; ** patient 8 had an initial platelets count of 78x10^3 µL; Legend (for table 1 and 2): ALT= alanine aminotransferase (U/L), AST= aspartate aminotransferase (U/L), TB= total bilirubin (µmol/L), DB= direct bilirubin (µmol/L), GGTP= gamma-glutamyl transpeptidase (U/L), BSEP= bile salt export pump, MRP2= multidrug resistance-associated protein 2, OLT= orthotopic liver transplant, N/D= not done.

Table 2. Last clinical data in 10 patients with high GGT cholestasis

Case number	UDCA initiation	Age	ALT	AST	TB	DB	GGTP
1	12m	17m	36	41	-	-	13
2	11m	12m	35	-	0.7	-	24
3	7y	8y	25	37	0.3	-	48
4	14y	14y10m	20	30	0.5	0.15	18
5	infancy	18y PostOLT	24	28	-	-	15
6	infancy	17y Post-OLT	13	17	3	0.6	14
7*	4y	13y	12	24	0.5	-	12
8**	13y	19y	24	26	0.8	-	25
9°	10y	18y	59	35	0.3	-	8
10	14y	20y	37	49	1.8	0.4	54

*last platelets count was 274x10^3 µL, **last platelets count was 89x10^3 µL; °last platelets count was 33x10^3 µL, albumin 4.5g/dL

familial intrahepatic cholestasis type 3 (PFIC3), all of which are typically associated with elevated GGTP levels (9). ICP associated pruritus usually resolves after delivery (10). Patients with PFIC3 have elevated serum GGTP and their clinical presentation may vary; including asymptomatic chronic elevations in liver biochemistries, jaundice, pruritus, hepatosplenomegaly, and features of cirrhosis and portal hypertension. These clinical features were observed in this cohort.

Table 3. Changes in serum alanine aminotransferase and gamma-glutamyl transpeptidase measurements after the use of ursodeoxycholic acid.

Case Number	Initial ALT	Final ALT	Initial GGTP	Final GGTP
1	98	36	223	13
2	118	35	313	24
3	268	25	650	48
4	165	20	653	18
5*	N/A	N/A	N/A	N/A
6*	N/A	N/A	N/A	N/A
7	226	12	154	12
8	135	24	230	25
9	219	59	290	8
10	223	37	310	54
Mean (n=8)	182	30	353	21
SD (n=8)	61	15	192	13

Legend: ALT= alanine aminotransferase (U/L), GGTP= gamma-glutamyl transpeptidase (U/L), SD= standard deviation. *Data is not available for subjects 5 and 6.

In patients with PFIC3, especially in individuals with "mild" mutations in *ABCB4*, UDCA may be very effective, leading to improvements in both biochemical parameters and symptoms (11). UDCA is hypothesized to work by altering the biliary bile acid composition in favor of the less toxic hydrophilic bile acids

(9). In general, the UDCA response in this cohort suggests that *ABCB4* (MDR3) deficiency, a relatively rare genetic disorder, may be more prevalent than previously suspected in Puerto Rico.

Elevated hepatic copper has been described in patients with mutations in the *ABCB4* gene after initially been incorrectly diagnosed with Wilson's disease (WD), as we observed in 1 of our subjects. Shneider reported on a 2-year-old patient who presented with cirrhosis and copper overload secondary to heterozygous missense mutations in the *ABCB4* gene (12). Ramraj described 2 patients with elevated hepatic copper. Both patients had full gene sequencing for both the *ABCB4* gene and the ATP7B gene (WD gene) (13). Both patients were compound heterozygous for deleterious mutations in the *ABCB4* gene, with no mutations in *ATPB7*.

This report is limited by the small number of cases and the lack of general availability of clinical genotyping. Despite these limitations, the investigations suggest that *ABCB4* deficiency may be prevalent amongst genetic disorders in the Puerto Rican population. Definitive diagnosis will require genetic testing. Eight of the 10 patients may benefit from selective *ABCB4* gene sequencing, as they presented with the classic clinical signs for MDR3 deficiency with adequate response to UDCA. On the other hand, the 2 sisters may have a different, and possibly more aggressive, familial cholestatic disease. Whole exome sequencing may be an important diagnostic approach for them. A systematic investigation of the genetic causes of specific forms of chronic liver disease in Puerto Rico is warranted.

Resumen

Objetivo: Algunos desórdenes hereditarios podrían ser más comunes en comunidades de islas. Estudios previos de casos han sugerido que las enfermedades colestásicas del hígado podrían formar parte de estas enfermedades hereditarias en Puerto Rico. Para evaluar esta hipótesis se realizó un estudio transversal de enfermedades del hígado en niños en Puerto Rico. Métodos: Un análisis transversal fue llevado a cabo en pacientes con colestasis crónica en el Hospital Pediátrico Universitario en San Juan, Puerto Rico. Se identificaron diez participantes con colestasis y niveles altos de gamma glutamil transpeptidasa (GGTP). Se evaluó la respuesta terapéutica al ácido ursodeoxicólico midiendo el cambio en niveles de alanina aminotransferasa (ALT) y gamma glutamil transpeptidasa (GGTP). Resultados: Cuatro sujetos, antes del primer año de edad, presentaron con picor, anormalidades en las pruebas de hígado y/o hepatomegalia. La presentación clínica de los pacientes mayores fue similar, y además presentaron esplenomegalia, ictericia y/o varices esofágicas. Tres pacientes mostraron progresión de su enfermedad de hígado y dos requirieron trasplante de hígado posterior a la desviación biliar parcial externa. El tercer paciente desarrolló hiperesplenismo a pesar de tener enzimas hepáticas normales mientras tomaba el ácido ursodeoxicólico. Seis de los diez pacientes normalizaron sus pruebas de hígado (ALT< 40 IU/L, GGTP < 100 IU/L) con la administración de ácido ursodeoxicólico (ALT antes 182 ± 61 y GGTP 353 ± 192; ALT 30 ± 15 y GGTP 21 ± 13 después, p <0.001). Conclusiones: Se describe la respuesta al ácido ursodeoxicólico en un subgrupo de individuos con enfermedades colestásicas de hígado con altos niveles de GGTP en Puerto Rico. Los hallazgos sugieren la posibilidad de que enfermedades relacionadas al gen *ABCB4* sean un desorden genético importante en Puerto Rico. Investigaciones en el futuro utilizando la secuencia del genoma podrían ser importantes para entender mejor las enfermedades de hígado en Puerto Rico.

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