Commentary

iMedPub Journals
www.imedpub.com

Journal of Genetic Disorders

2018
Vol.2 No.1:2

Cholestasis with Normal Gammaglutamyl Transferasis from Management to Molecular Investigation with Novel Mutations in ATP8B1 and ABCB11: Tunisian Study

Barkaoui E[1,4*], Alobaidy H[2], Darragi I[4], Ayadi A[3], and Abbes S[4]

1 Protection of Maternal and Infantil Center Ezzouhour Tunis, Tunisia
2 El Khadra Hospital Tripoli, Libya
3 Pediatric Center Mahdia Hospital, Tunisia
4 Laboratory of Cellular Hematology and Molecular Biology, Pasteur Institute Tunis, Tunisia

***Corresponding author:**
Emna Barkaoui

emna_barkaoui@yahoo.fr

Head of Protection of Maternal and Infantil Center Ezzouhour Tunis Tunisia, Laboratory of Hematology And Molecular Biology, Pasteur Institute Tunis, Tunisia.

Tel: 00216 53 73 15 16

Citation: Emna B, Hanna A, Imen D, Abdelkarim A, et al. (2018) Cholestasis with Normal Gammaglutamyl Transferasis from Management to Molecular Investigation with Novel Mutations in ATP8B1 and ABCB11: Tunisian Study. J Genet Disord. Vol.2 No.1:2

Abstract

Introduction: Cholestasis with normal gamma-glutamyltransferasis (GGT) is a heterogeneous group. ATP8B1 and ABCB11 two genes implicated on the respectively phenotypes PFIC1, BRIC1 and PFIC2 BRIC2, lithiasis, and drug cholestasis. Aims: The aim of this study is to report the phenotype, the outcome and to determine the profile of mutations.

Methods & Materials: Eleven patients with persistent normal GGT cholestasis were studied from 93 patients with liver disease referred to us from 2007 to 2014. Eight patients were studied for ATP8B1, five for ABCB11, one family, with two patients, was studied for both genes. Polymerase chain reaction (PCR) was performed using a set of selected primers and direct sequencing by Applied Biosystems. Intronic variants were analyzed by the Human Splicing Finder program.

Results:

Patients phenotype: The age at the diagnosis was ranged from neonatal to 12 months. Consanguinity was found in 10/11 of the patients. The constant features were pruritus and hepatomegaly. No one developed portal hypertension (3 to 22 years). Two patients presented asthma with high level of IgE with negative sweat test. Two patients developed intense pruritus despite ursodesoxycholic acid (UDCA) and rifadin with severe lichenification of the skin, they were successfully treated by sertraline with a considerable improve of the skin. In one patient, we observed a relapse of the pruritus after two months requiring biliary derivation and in one patient, frequent crisis of crazy laughs, sertraline was stopped. Two patients developed liver failure respectively at the age of six months (hepatocarcinoma was found in explant liver) and six years old, received liver transplant.

Molecular Study Results: For ATP8B1, we found two novel exonic mutations, the V310F and D379RfsX51 and two known variants the F305I and R952Q, seven novel intronic mutations, three silent mutations: two new mutations one of them D234D was near splicing site. For ABCB11, we found three exonic mutations: Y354X, two novel mutations N372YfsX23, A986P and two novel intronic variants. The analysis of the seven intronic variants by splicing Finder Program showed the possible role of four of them in the disease.

Conclusions: This study is the first to be performed in Tunisia for patients with normal GGT cholestasis. The molecular results were preliminary but did not find founder effect. We report both novel exonic and intronic mutations. Most patients have more than two related variants, which may be due to high consanguinity. This study should be completed by the transcriptional study for the novel intronic and silent mutations.

Received: January 01, 2017; **Accepted:** January 18, 2018; **Published:** January 25, 2018

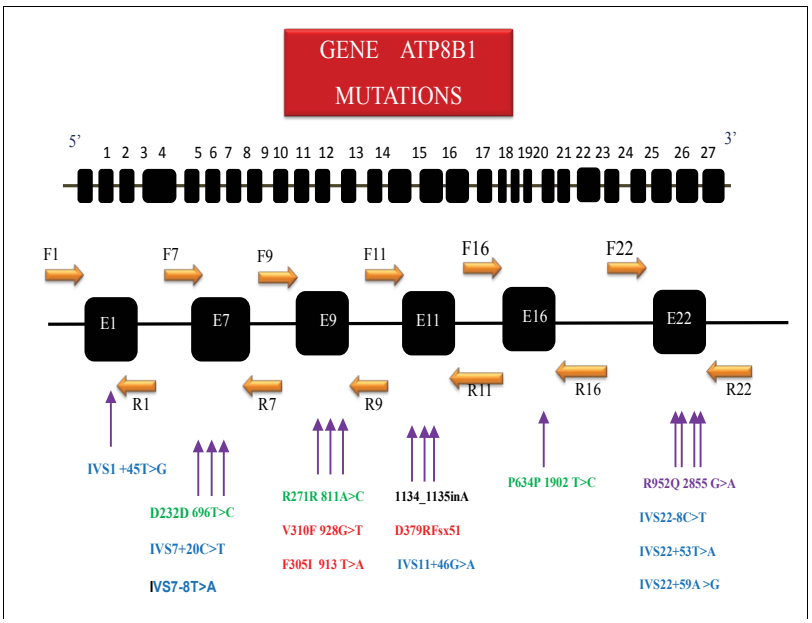

Figure 1 Mutations in ATP8B1.

Keywords: Heterogeneous group; Gammaglutamyl transferasis; Cholestasis; Genetic analysis

Abbreviations: ATPase: Adenosin Tri phosphate; DNA: Deoxyribo Nucleic Acid; HC: Hepatocarcinoma; MCT: Medium Chain Triglycerides; PCR: Polymerase Chain Reaction; PFIC: Progressive Familial Intra Hepatic Cholestasis; UDCA: Ursodesoxycholic Acid

Introduction

Progressive familial intra hepatic cholestasis (PFIC) is a heterogeneous group of biliary acids disorders divided into two groups distinguished by value of gammaglutamyl transferasis (GGT) with three types (PFIC3, PFIC2, PFIC1) [1-3]. The group with high GGT (PFIC3) due to mutation in ABCB4 gene encodes floppies for phosphatidyl choline (PC) [4]. The group with normal GGT is due to two genes [3]. ATP8B1 for PFIC1 and ABCB11 for PFIC2, characterized by jaundice, intense pruritis, hepatomegalia, splenomegalia with normal GGT and cholesterol rates. PFIC1 patients could have extrahepatic symptoms as deafness, pancreatitis and tubulopathy. ATP8B1 plays in translating phosphatidyl serine (PS) from the outer to the inner leaflet cell membrane [5,6] and could affect the membrane integrity [7]. ABCB11 codes for bile salt export pumpprotein (BSEP). The estimated incidence varies between 1/50 000 and 1/100 000 births.

The present research purports to report the preliminary results of the first molecular study for ATP8B1 and ABCB11.

Materials and Methods

From June 2007 to March 2013, 93 patients with liver diseases were referred to our centre (among them18 patients from Libya, three from Algeria, one from Mali).

Patients: Fourteen patients have cholestasis with normal GGT, eleven were genetically investigated. The parents of all patients gave written informed consent for the genetic analysis. The phenotype diagnosis was performed by a combination of clinical (jaundice, hepatomegaly, splenomegaly with pruritus), biological (cholestasis (conjugated bilirubin>17 micromole/L) with normal or low GGT (<40 UI/l) and normal cholesterol rates (<2.50 mmol/l)), high level of serum primary bile acids (>15 ng/l) and familial history. All patients had alpha-foetoprotein (AFP) (normal<10 ng/ml), ear (BER: Brainstem Evoked Responses), pancreatic (amylasemia (normal<100 UI/l), lipasemia (normal<60 UI/l), echography +/-tomodensitometry) and tubular (phosphor reabsorption rate), explorations. The management was standardized and treatment was based on: 1-special nutrition (rich milk medium chain triglycerides (MCT), Malto dextrin), 2-Ursodesoxycholic acid UDCA, 3-Parenteral vitamins ADEK, 4-Rifampycin for persistent pruritus, 5-Sertraline (Zoloft) used in refractory pruritus (as known protocol), 6-Bilary diversion for one patient, 7-Liver transplantation in two patients for developing liver failure respectively at the age of 6 months (with hepatocarcinoma in liver explant) and 6 years. Six unrelated families were screened for ATP8B1 and four families for ABCB11. One family was studied for both ATP8B1 and ABCB11.

Molecular study: DNA was extracted from peripheral blood leucocytes with a standard phenol chloroform technique. Polymerase chain reaction (PCR) was performed using a set of selected primers for coding exon according to http://pharmagenetics.ucsf.edu (from 2 to 28 but considered from 1 (exon 2) to 27 (exon 28) as coding exons). PCR products were purified using the High Pure PCR purification system before direct sequencing by version 2.0 Big Dye Terminator cycle sequencing kit (Applied Biosystems). Bioinformatic software was used for genetic analysis (« *Sequencer»* and « *Human splicing Finder site»)*. For ATP8B1: First, we analyzed common variations previously described in PFIC, for coding exons 6, 7, 8, 9, 15, 17, 18 and 22. The total exons were screened for only two patients (P1, P6). For ABCB11, the only exons bearing the common mutations were

Table 1 Phenotype characteristics of patients.

Patient/ Sample	Origin/ Region	Consanguinity/ Family history	Age at onset	First phenotype classification	Outcome/ Particularity
P1/10	Sfax Hancha Center	+/ Brother dead with normal GGT PFIC	Neonatal	PFIC1 Pancreatic crisis	Bilary diversion 14 years Old/ Elevated GGT after bilary diversion
P2/13	SfaxHancha Center	+	Neonatal	PFIC1	PFIC1 to BRIC1 Good outcome 22 years Old
*P3/16	Kef North west	+	6 months	PFIC1 PFIC2	Ataxia by vitamin E defect Short stature Normal liver parameters 12 years Old
*P4/17	Kef North west	+	Neonatal screening	PFIC1 PFIC2 High level of AFP	Normal liver parameters 10 years Old
P5/9	Medenine South	+	6 months	FIC1	Liver transplantation at 6 years old Good outcome 8 years Old
P6/34	Kairouan Center	+	7 months	PFIC1	Stable
P7/40	Mahdia Center	+	NP	PFIC1	Stable
P8/43	Mahdia Center	+	NP	PFIC1	Stable
P9/64	Guebelli South	+	Neonatal	PFIC2 High AFP	Indication of liver transplant 3 years Old
P10/71	Mali	-	Neonatal	PFIC2 High AFP	Returned to Mali?
P11/87	Medenine South	+	Neonatal	PFIC2 High AFP	Liver transplant HCC Good outcome 6 years Old

Table 2 Variants in ATP8B1.

Patient/ Sample	Region	Genomic position	DNA change	Protein change	Zygosity	Predicted effect
P1/10	Exon7	g.105677 T>C	c.696 T>C	p.D232D	Homozygote	Silent mutation
	+	+	+	+		
	Exon9	g.107994 A>C	c.811 A>C	p.R271R	Homozygote	Silent mutation
	+	+	+	+		
	Exon11	g.111405InsA	c.1134_1135inA	p.D379RFsX51	Homozygote	Truncated protein
	Exon16	g.131799 T>C	c.1902 T>C	p.P634P	Heterozygote	Silent mutation

P2/13	Exon9 + Exon9	g.107994 A>C + g.108610 G>T	c.811 A>C + c.928 G>T	p.R271R + p.V310F	Homozygote Heterozygote	Silent mutation Missense
*P3⁄16	Exon9 + Exon22	g.107994 A>C + g.148026 G>A	c.811 A>C + c.2855 G>A	p.R271R + p.R952Q	Homozygote + Homozygote	Silent mutation Polymorphisme
*P4//17	Exon9 + Exon22	g.107994 A>C + g.148026 G>A	c.811 A>C + c.2855 G>A	p.R271R + p.R952Q	Homozygote + Heterozygote	Silent mutation Polymorphisme
P5/9	Exon9	g.107994 A>C	c.811 A>C	p.R271R	homozygote	Silent mutation
P6/34	Exon7 + Exon9 + Exon11 + Exon16	g.105677 T>C + g.107994 A>C + g.108598 T>A + g.111405InsA + g.131799 T>C	c.696 T>C + c.811 A>C c.913 T>A + c.1134_1135inA + c1902 T>C	p.D232D + p.R271R p.F305I + D379RFsX51 + p.P634P	Homozygote Homozygote Heterozygote Homozygote Heterozygote	Silent mutation Silent mutation Missense Truncated protein Silent mutation
P7//40	Exon9	g.107994 A>C	c.811 A>C	p.R271R	homozygote	Silent mutation
P8/43	Exon9 + Exon22	g.107994 A>C + g.148026 G>A	c.811 A>C + c.2855 G>A	p.R271R + p.R952Q	Homozygote Heterozygote	Silent mutation Polymorphism

*: Sister and brother

Table 3 Intronicvariations in ATP8B1**.**

Exon/Intron	Sample/Patient	Nucleotide change	Zygosity	Predicted effect
1/1	34 (P6)	IVS1+45T>G	Heterozygote	Potential alteration of splicing site
8/7	12 (P1) 9 (P5) 13 (P2)	IVS7-8T>A IVS7-8T>A IVS7-8T>A	Heterozygote Heterozygote Heterozygote	Polymorphism Not specific of a family
7/7	34 (P6) 10 (P1)	IVS7+20C>T IVS7+20C>T New Donor site	Heterozygote Homozygote	Activation of an intronic cryptic donor site. Potential alteration of splicing site.
11/11	10 (P1) 34 (P6)	IVS11+46G>A ESS Site broken	Heterozygote Heterozygote	Alteration of an intronic ESS site. Potential alteration of splicing
22/21	17 (P4)	IVS22-8C>T	Heterozygote	Polymorphism? Specific of the family No significant splicing Motif alteration detected
22/22	34 (P6) 40 (P7)	IVS22+53T>A New ESE site	Heterozygote Homozygote	Creation of an intronic ESE site. Potential alteration of splicing
22/22	34 (P6)	IVS22+59A >G	Heterozygote	Specific of the family Polymorphism

screened: exon7, exon9, exon11, exon14 and exon23.

Results

Patient's phenotype profile (Table 1): The Consanguinity was found in 10/11 of the patients. The constant features were pruritus and hepatomegaly. No one developed portal hypertension. Two patients (P1, P6) developed intense pruritus despite UDCA and rifadin with severe lichenifaction of the skin were treated by sertraline. On sertraline the two patients' skin and pruritus improved considerably with disappearance of the lichenifactions but P1 relapsed after two months requiring biliary derivation and P6 developed frequent crisis of crazy laughs, the sertraline was then stopped. The skin improvement remained constant after cessation of the treatment. Patient P1 presented also abdominal pain with episodes of pancreatitis treated by low fat diet. Two patients developed liver failure respectively at the age of 6 months and 6 years old and received liver transplant in France (P5, P11). Patient (P11) was referred to us for neonatal cholestasis, the investigations at 1.5 months showed positive cytomegalovirus (CMV) PCR with high level of alphafoetoproteine (AFP) (>200 000 ng/ml) with persistent normal GGT level, he developed sudden and early liver failure after normalisation of liver parameters on UDCA, he received liver transplant at the age of 6 months, hepatocarcinoma was found in explants liver.

Molecular results: In ATP8B1 **(Figure 1)** we found three exonic mutations, two of them were novel: V310F (p.310G>T) in one patient (P2) and D379RFsX51 in two patients (P1, P6), the F305I (p.305T>A) mutation was found in patient P6, one polymorphism R952Q, three silent mutations, and seven intronic variants **(Table 2 and Table 3).** The Three silent mutations were R271R (homozygote in all studied Tunisian patients) and two novel mutations the D232D mutation located near the splicing site and the P634P. The polymorphisms R952Q was found in 9/24 alleles (P4, P8). In ABCB11, We found two novel exonic mutations (N372YfsX23, A986P) and two novel intronic variants (IVS13-43 T>C, IVS13-24 T>C). One patient (P11) had p.Tyr354X mutation.

Discussion

Many mutations were published from many ethnic groups (more than 100) and in 2004, Klomp described 54 mutations [8-12]. Our study was the first in Tunisia and cholestasis with normal GGT constitutes 15% (14/93). The patients were from different regions but most of them from the center. This ascertainment was found for other diseases. One patient was from Mali. The parental consanguinity was found in all Tunisian patients. In Tunisia, the consanguinity was high and estimated at 32% and could be elevated in each disease separately from 80% to 100% [13]. Four patients had permanent jaundice with high level of AFP (P4, P9, P10, and P11) and studied for ABCB11. Spraul et al. [9] found that elevated serum AFP concentrations were above normal limit value in 12/15 PFIC2 patients however authors considered that the combination of alanine aminotransferasis activity (ALT) ≤ 5x upper limit of normal range (ULN) and normal AFP value was present in all PFIC1 patients and (ALT)>5x ULN range and high AFP value was present in 73% of PFIC2 patients [9].

Patient P11 was referred to us for neonatal cholestasis with positive cytomegalovirus (CMV) PCR, high levels of alphafoetoproteine (AFP) (>200 000 ng/ml) and persistent normal GGT levels. For this case the treatment by UDCA normalized all liver parameters and the AFP was rapidly decreased. CMV infection was interpreted as post natal undercurrent infection. For this patient the first diagnosis of PFIC2 was confirmed with Y354X mutation. None of the patients studied developed portal hypertension after long time of outcome **(Table 1)** in our study.

As far as the exonic mutations are concerned we detected three in ATP8B1 and two in ABCB11. Two novel mutations were found for ATP8B1, D379RFsX51 and V310F; D379RFsX51 is predicted to lead to truncation of synthesis at amino acid 379 (of 1251) and significantly change the structure of ATP8B1 protein and could

be associated with PFIC1. The V310F mutation changes valine, aliphatic amino acid and apolar to phenylalanine aromatic and apolar amino acid without predictive effect on structure but its implication for PFIC needs a screen of controls and a functional study. The F305I, a missense mutation was described before the present study in one patient with ICP from a cohort of 182 women [14], in one of 120 healthy control pregnancies [14], in one patient with chronic pancreatitis [15] and in our study it is the first time found in PFIC patient. This mutation results in the substitution of a phenylalanine by isoleucine and creates an EcoRV site and could play a role for PFIC1 [14]. The V310F and F305I mutations, each one is specific to one family. The R952Q (c.2855G>A) known as polymorphism (rs12968116) was equally frequent among ICP patients and Finnish controls (respectively 14.7 and 14% of samples), twice as frequent among CEPH controls (28%) [16], we found this variant in nine of 24 alleles and homozygous in two individuals (P4 and his father). Most of the patients of this study had more than two variants and P6 have five variants two of them with possible association for PFIC1. The intronic mutations and after analysis by « *Human splicing Finder site»,* they showed the possible implication of four variants in splicing site (IVS1+45T>G, IVS7+20C>T, IVS22+53T>A, IVS11+46G>A). These variants are novel. Patients P1 and P7 are homozygote respectively for the IVS7+20C>T and IVS22+53T>A. The patient P1 was homozygote for two mutations exonic and intronic both, patient P7 was only homozygote for intronic mutation. For all the other intronic variants, patients are heterozygote. For the three other intronic mutations, they could be polymorphisms and some of them were specific to the family **(Table 3).** Additional studies are required, functional and transcriptional study should be completed to confirm the role of these variants in relation to the disease.

For ABCB11, the insertion mutation N372YfsX23 was responsible for truncated protein. For the A986P mutation, we must screen it in controls to confirm its association with PFIC2**.** Intronic variants (intron13: IVS13-43 T>C; IVS13-24 T>C) were novel and their analysis with *Splice Site Finder* program found that they could play a role in the splicing site. One patient P11 had a p.Tyr354X mutation, which was found by French laboratory [9]. This nonsense mutation creates a premature stop codon and a truncated protein. This patient was transplanted with early liver failure associated with hepatocarcinoma. Two maternal uncles have developed early hepatocarcinoma associated to hepatitis B. Up to the present day there is no correlation between hepatocarcinoma and mutations. Our case represents a rare example of early hepatocellular carcinoma complicating PFIC2 that need evaluation of clinic symptoms and we highlight that the first presentation with high level of AFP with unexplained early liver failure after response to treatment may be related to high risks of hepatocarcinoma in PFIC2. Knisely et al. reported 7 PFIC2 patients with hepatocarcinoma at age under 5 years [17].

Conclusion

This study is the first to be performed in Tunisia for patients with normal GGT cholestasis. The preliminary molecular results report both novel exonic and intronic mutations with their possible implication for the disease. Most patients have more than two related variants, which may be due to high consanguinity. This study should be completed by the transcriptional study for the novel intronic and silent mutations and all of the patients examined should be screened for all exons and for other genes for negative patients.

Acknowledgement

This article has been financially supported by the Ministry of Higher Education and Scientific Research of Tunisia (LR 05 SP 04).

References

1 Jacquemin E (2012) Progressive familial intrahepatic cholestasis. Clin Res Hepatol Gastroenterol 36: S26-35.

2 Jansen P, Muller M (1998) Progressive familial intrahepatic cholestasis types 1, 2, and 3. Gut 42: 766-767.

3 Bull LN, van Eijk MJT, Pawlikowska L, DeYoung JA, Juijn JA, et al. (1998) A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet 18: 219-223.

4 Crawford AR, Smith AJ, Hatch VC, Oude Elferink RP, Borst P, (1997) Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. Visualization by electron microscopy. J Clin Invest 100: 2562-2567.

5 Paulusma CC, Folmer DE, Ho-Mok KSD, Rudi de Waart, Hilarius PM, et al. (2008) ATP8B1 requires an accessory protein for endoplasmic reticulum exit and plasma membrane lipid flippase activity. Hepathology 47: 268-278.

6 Ujhazy P, OrtizD, MisraS, Li S, Moseley J, et al. (2001) Familial intrahepatic cholestasis 1: Studies of localization and function. Hepathology 34: 768-775.

7 Groen A, Romero MR, Kunne C, Hoosdally SJ, Dixon PH, et al. (2011) Complementary functions of the Flippase ATP8B1 and the Floppase ABCB4 in maintaining canalicular membrane integrity. Gastroenterology 141: 1927-1937.

8 Klomp LWJ, Vargas JC, van Mil SWC, Pawlikowska L, Strautnieks SS, et al. (2004) Characterization of mutations in ATP8B1 associated with hereditary cholestasis. Hepatology 40: 27-38.

9 Spraul AD, Fabre M, Branchereau S, Baussan C, Gonzales E, et al. (2010) ATP8B1 and ABCB11 analysis in 62 children with normal Gamma-glutamyl transferase progressive familial intrahepatic cholestasis (PFIC): Phenotypic differences between PFIC1 and PFIC2 and natural history. Hepatology 51: 1645-1655.

10 Pawlikowska L, Strautnieks S, Jankowska I, Czubkowski P, Emerick K, et al. (2010) Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol 53: 170-178.

11 Li-yan Liu, Xiao-hong Wang, Zhong-lin Wang, Qi-rong Zhu, Jian-She Wang (2010) Characterization of ATP8B1 gene mutations and a hot-linked mutation found in Chinese children, with progressive intrahepatic cholestasis and low GGT. JPGN 50: 179-183.

12 Numakura C, Abukawa D, Kimura T, Tanabe S, Hayasaka K (2011) A case of progressive familial intrahepatic cholestasis type 1 with compound heterozygous mutations of ATP8B1. Pediatrics International 53: 107-110.

13 Riou S, El Younsi C, Chaabouni H (1986) Consanguinité´ dans la population du nord de la Tunisie. Tun Med 67: 167-172.

14 Muellenbach R, Bennett A, Tetlow N, Patel N, Hamilton G, et al. (2005) ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. Gut 54: 829-834.

15 Wendy L, van der Woerd, Désirée Y, van Haaften-Visser, Stan FJ, et al. (2013) Mutational analysis of ATP8B1 in patients with chronic pancreatitis. Plos One.

16 Painter JN, Savander M, Ropponen A, Nupponen N, Riikonen S, et al. (2005) Sequence variation in the ATP8B1 gene and intrahepatic cholestasis of pregnancy. Eur J Hum Genet 13: 435-439.

17 Knisely AS, Strautnieks SS, Meier Y, Stieger B, Byrne JA, et al. (2006) Hepatocellular carcinoma in ten children under five years of age with bile salt export pump deficiency. Hepatology 44: 478-486.