ARTICLE ORIGINAL

# Cholestase intrahépatique progressive familiale (PFIC) à révélation néonatale : première étude moléculaire tunisienne

## Neonatal-onset Progressive Familial Intrahepatic Cholestasis (PFIC): first molecular study in Tunisian patients

Ines Selmi[1], Franck Broly[2], Haifa Ouarda[1], Emna Marmech[1], Zied Khlayfia[1], Jihed Kanzari[1], Ons Azzabi[1], Nadia Siala[1]

*1-Hôpital Mongi Slim La Marsa, Faculté de médecine de Tunis*
*2-Institut de Biochimie et Biologie Moléculaire, CHU de Lille*

**RÉSUMÉ**

La cholestase intrahépatique progressive familiale est un groupe hétérogène d'hépatopathies rares, de transmission autosomique récessive. Le début néonatal est caractéristique des types 1 et 2 de la maladie, qui résultent respectivement des mutations dans les gènes ATP8B1 et ABCB11. Quatre patients, dont trois sont porteurs de PFIC2 et un ayant un PFIC 1 ont été rapportés dans cette étude. Tous nos patients avaient des manifestations cliniques et biologiques typiques de la maladie. Cependant, nos quatre patients avaient tous une mutation nouvellement décrite dans la littérature. En outre, les trois patients porteurs de PFIC2 avaient la même nouvelle mutation ; ce qui pourrait faciliter le diagnostic génétique chez les patients tunisiens suspects de cette maladie ultérieurement. Par ailleurs, le patient porteur de PFIC1 avait une nouvelle mutation qui se manifeste par un phénotype particulier, à savoir une hypothyroïdie congénitale centrale associée.

Des progrès ont été faits concernant le diagnostic moléculaire de cette pathologie, et ceci particulièrement grâce au séquençage de la nouvelle génération, en effet, celle-ci permet d'optimiser les possibilités d'un diagnostic plus précis, en évitant le recours à d'autres explorations plus invasives ; il en résulte une prise en charge thérapeutique précoce et adéquate, ainsi qu'un conseil génétique approprié.

**Mot clés :** cholestase intrahépatique progressive familiale type 1 et 2, néonatale, mutation, ATP8B1, ABCB11, séquençage de nouvelle génération

**SUMMARY**

Progressive familial intrahepatic is a heterogeneous group of rare autosomal recessive liver disorders. Neonatal onset is characteristic of the PFIC 1 and PFIC 2, which result from mutations in genes respectivelyATP8B1 and ABCB11.

Four Tunisian patients, three of them with PFIC 2 and one with PFIC1, were described. They all had typical clinical and biological features. However, they all had newly reported mutations. The same mutation was found in the patients with PFIC2, which could facilitate the diagnosis in Tunisian patients suspected in the future. The patient diagnosed with PFIC1 had also a newly described mutation, with a probable phenotypic particularity that is congenital hypothyroidism.

Advances are being made to establish a molecular diagnosis in neonatal onset cholestasis. Indeed, next generation sequencing gene panels (NGSGP) potentially decrease the need for invasive procedures in these patients, enable early initiation of treatment and adequate genetic counseling.

**Key words:** progressive familial intrahepatic cholestasis type 1 and 2, neonatal, mutation, ATP8B1, ABCB11, next generation sequencing gene panels

Correspondance
Ines Selmi
Hôpital Mongi Slim La Marsa / Faculté de médecine de Tunis
selmiinesped@yahoo.fr

## INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) is a heterogeneous group of rare genetic autosomal recessive diseases that present with intrahepatic cholestasis, usually in infancy or childhood (1). PFIC1 and PFIC 2 usually appear in newborns (2).

PFIC1 and PFIC2have been mapped respectively to chromosomes 18q12 and 2q24 and with defects in respectively the genes ATP8B1 and *ABCB11* (1, 2, 3). Several mutations have been described in the literature (4, 5).

In Tunisia, there are no published genetic studies of the PFICs with neonatal onset. This is the first genetic study carried out in the Tunisian population except error or omission on our part.

## METHODS

In this study, we reported four cases of PFIC with neonatal onset, which were followed in the pediatric and neonatal department of Mongi Slim Hospital in Tunisia, during the period from January 2014 to December 2019.

This is a retrospective study. Data collection was based on hospitalization records.

The genetic study was carried out thanks to collaboration with the toxicology and genopathy department of the regional university hospital of Lille (France).

## CASE REPORT

– **Case 1:**

Iyed, a 27 days old male infant, first born of consanguineous parents, with no history of liver disease, was admitted in April 2014 with the complaints of jaundice. He was eutrophic at birth. On examination, he had no face dysmorphism. He was icteric. Urine and stools had normal colors. He had hepatomegaly (8 cm) which was firm. He had also a splenomegaly.

Initial biological data were summarized in table I.

Abdominal ultrasonography showed a homogeneous hepatomegaly.

The diagnosis of PFIC either type 1 or 2 was suspected, in face of the low ΄YGT values, and after excluded other differential diagnoses such as disorders of bile acid synthesis or metabolism or panhypopituitarism. The genetic study was not carried out initially, because it is not practicable in Tunisia.

The patient was managed with ursodeoxycholicacid, fat-soluble vitamins and an adequate nutritional support with medium chain triglycerides.

The liver biopsy demonstrated severe active cholestatic and plasmodial cirrhosis, suggesting PFIC2.The biopsy specimen was subjected to immunohistochemistry which showed absent canalicular staining with bile salt export pump (BSEP) antibodies, suggesting a PFIC 2. A first molecular analysis of the *ABCB11* gene did not find a mutation. The diagnosis of PFIC 2 was nevertheless retained on the clinical criteria and the outcomes of the liver immunostaining. The evolution was marked by clinical and biological worsening; he quickly progressed to cirrhosis, portal hypertension and hepatocellular failure and he was candidate for a liver transplant. He had a related donor who was his mother, but unfortunately, he succumbed to his disease before the transplant at the age of 13 months. A complementary genetic study, carried out after his death and which used a larger panel, concluded that there was a mutation in the homozygous state of the gene in our patient*: a transition T>A (NM003742.2:c.1062T>A)* affecting the sequence of exon 10 of the gene. This genotype is compatible with the presence of PFIC2. Both Iyed parents were heterozygous for this gene mutation. At our knowledge, this variant has not been described previously.

– **Case 2:**

Our second patient called Assil is the sister of Iyed (case 1). She was admitted in October 2018. The mother did not receive prenatal diagnosis and did not monitor her pregnancy; she was in denial about the disease. Assil has developed jaundice at the age of 5 days; her mother did not consult until the age of 24 days. On admission, she had bad weight gain, she was icteric. There was gradual abdominal distension due to enlargement of liver size; the left liver was palpable six cm below mid-clavicular line. She had no splenomegaly. Stool was normally colored and the urine was dark.

Laboratory investigations are reported in table I.

Thyroid-stimulating hormone was 2.6 µUI/ml (N: 0.4 - 4), FT4 = 19.2 pmol/l (N: 9 - 25)

Abdominal Ultrasonography showed hyperechoic hepatomegaly.

The diagnosis of PFIC2 was retained in front of a set of clinical, biological and radiological arguments as well as the family history.

Treatment included nutritional support (adequate calories, supplementation of fat-soluble vitamins and medium chain triglycerides), ursodeoxycholic acid and rifampicin.

Currently, the patient is 22 months old, has marked jaundice with intense pruritus grade 3 according to Whitington et al classification (6). She also presents a sever failure to thrive, despite adequate nutritional support by gastric tube at home. She is a candidate for a liver transplant; she has a compatible donor who is her mother.

– **Case 3:**

Zeineb, 7 days old female infant, first born of consanguineous parents, with no family history of liver disease, admitted in another department with the complaints of jaundice and increased abdominal circumference. She was transferred to our department in December 2019, at the age of 17 days for exploration of cholestatic jaundice with neonatal onset.

On initial examination, she was icteric, she had no facial dysmorphism and no associated apparent congenital anomalies. Stool and urine were normally colored, she had hepatomegaly (8 cm) and no splenomegaly; she had a good weight gain.

Liver function tests are summarized in table I. Thyroid-stimulating hormone was 2.2 UI/ml (N: 0.4 - 4), FT4 = 16.3 pmol/l (N: 9 - 25).

Abdominal ultrasonography showed a homogeneous hepatomegaly and absence of dilatation of the bile ducts.

In face of a neonatal cholestasis with normal level of ɣGT, without pale stool, without facial dysmorphia, without liver failure or extra hepatic impairment, the diagnosis of PFIC 2 was strongly suspected.

In our patient, we avoided a variety of explorations, some of which were invasive and we resorted directly to the genetic study. Molecular biology not only confirmed the diagnosis of PFIC 2, but also found the same mutation described in our first patient Iyed; moreover, this genetic study demonstrated a homozygosity for the variant *28 of the *UGTA1* gene, in favor of an associated Gilbert syndrome.

Currently, our patient is 9 months old, she complains of grade 2 pruritus. She was discharged under ursodeoxycholicacid, fat-soluble vitamins and diet enriched with medium chain triglycerides.

– **Case 4:**

Elyes, a boy from a consanguineous marriage, presented at day 4 of life with jaundice who had been treated with phototherapy. As the jaundice persisted, he was admitted at the age of 14 days, to a pediatric department in Djerba. He was icteric, eutrophic, had pale stools and dark urine and hepatomegaly. His biological assessment (Table I) was in favor of PFIC 1 or 2 diagnosis. He was discharged under ursodeoxycholicacid and fat-soluble vitamins (A, D, E, K). Moreover, he had a central congenital hypothyroidism (TSH = 3.03 µU/ml (N: 0.4 - 4) and FT4 = 6.53 pmo/l (N: 9 - 25)). Exploration of the other hypothalamic-pituitary axes was normal. Brain MRI was also normal.

Evolution was marked by the persistence of jaundice and the appearance of an intense and ferocious pruritus (grade 4) associated with a severe failure to thrive. He was hospitalized several times for nutritional care but without improvement.

He was transferred to our service at the age of 17 months for additional support.

On initial examination, he was icteric. He had a severe failure to thrive (weight < - 4DS, height < - 4 DS), he was severely malnourished (upper arm perimeter/cranial perimeter = 0.2, Weight for Height = 45%). He had diffuse scratching lesions. There was abdominal distension due to enlargement of liver size (9 cm); the left liver was palpable 4 cm below mid-clavicular line. He had no splenomegaly.

The biological results are mentioned in the table I.

Abdominal ultrasonography showed a homogeneous hepatomegaly.

He also had a liver biopsy which showed bland canalicular cholestasis, consistent with a PFIC 1.

The diagnosis of PFIC 1 was strongly suspected and

**Table 1.** initial laboratory features in our PFIC 1 and 2 patients

| Case | Type of PFIC | TB/CB (µmol/l) (3 – 17/ <4) | GT (IU/L) (15-125) | ALAT/ASAT (IU/L) (30 – 65) | TP (%) (70-100) | ALP (IU/L) (<400) | Cholesterol (mmol/l) (3.6 – 5.2) | Bile acid (µmol/l) (<10) |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 164.7/95.7 | 63 | 263/816 | 90 | 890 | 3.6 | 299 |
| 2 | 2 | 87/56.9 | 60 | 549/943 | 57 | 836 | 3.97 | 217 |
| 3 | 2 | 76.8/63.9 | 29 | 206/275 | 100 | 459 | 6.09 | 171.7 |
| 4 | 1 | 78/70.1 | 16 | 62/34 | 100 | 594 | 2.05 | 161.5 |

TB: total bilirubin ɣGT:gamma glutamyl transpeptidase TP: prothrombin ratioa CB: conjugated bilirubin ALAT/ASAT: transaminases ALP: alkaline phosphatase

the patient was nutritionally managed; he also received ursodeoxycholicacid, rifampicin and fat-soluble vitamins.

The evolution was not favorable; Elyes complained of a ferocious and disabling pruritus with insomnia, so he had a partial internal biliary diversion (the parents refused the partial external biliary diversion because of the possible stoma complications).

A DNA analysis made in Elyes, showed that it is a homozygous carrier of a variant of particular interest of the *ATP8B1* gene. This variant corresponds to *a transition C>T (NM_005603.4:c.1208C>T)* affecting the sequence of exon 12 of the gene, in line with a PFIC 1. It is a variant described for the first time.

Currently, Elyes is 4 and a half years old, he only rarely consults because he lives in a rural area and has a precarious socio-economic level. He has unfortunately disabling pruritus; he has a delay in psychomotor and emotional development with a depressive syndrome. He does not walk and he does not acquire the language, with bilateral hearing loss in the audiogram. Continuous flow enteral nutrition is in progress but adherence is questionable. He has severe malnutrition, dwarfism and microcephaly. Liver transplantation remains questionable in him.

## DISCUSSION

Progressive familial intrahepatic cholestasis (PFIC) is the prototype of genetic liver diseases manifesting jaundice in early childhood, progressive cirrhosis and failure to thrive (7).

All of our infants were from consanguineous parents, in favor of the autosomal recessive mode of their disease.

All our four patients presented with neonatal onset jaundice, enlarged liver, with or without splenomegaly. Stools were normally colored in 3 of our patients and pale in the fourth. There was failure to thrive in three of our patients, and it was particularly severe in the patient with PFIC1. During the course, all of them had developed an intense pruritus. Only the patient with PFIC 1 had extrahepatic clinical signs, such as hearing loss and growth impairment beyond that attributable to cholestasis. These clinical features join those described in the literature (3, 8, 9, 10). Indeed, the *ATP8B11* gene is expressed in various organs, including liver, pancreas, kidney and small intestine; this may explain other extrahepatic features such as persistent short stature and deafness (2, 11).

The biological data were also similar to those described in other studies. Namely a neonatal cholestasis with low to normal levels of serum ɣGT and elevated serum bile acids. The transaminases levels were very high in PFIC2, and were mildly elevated in patient with PFIC1 (1, 9, 10, 12).

PFIC2 cases have been reported in two families from different regions, however with the same deleterious mutation in the *ABCB11* gene. This could thereby guide the genetic investigation in Tunisian patients suspected of PFIC 2.

Zeineb; our third patient, presents homozygosity for the variant *28of the *UGT1A1* gene, compatible with Gilbert's syndrome. Gilbert syndrome is a common autosomal dominant hereditary condition with incomplete penetrance

(13). To date, no association described in the literature between PFIC2 and Gilbert's disease; but we believe that this syndrome is so common in the general population that it may simply be a fortuitous association.

Concerning the molecular study of our PFIC1patient, the mutation found has never been reported in the literature before. The particularity of this case was the association with a central congenital hypothyroidism, which is not a known clinical manifestation of PFIC1. An article published in 2013, by Emily et al, had reported 2 cases (2 twin patients) of PFIC1 who developed tubular acidosis with congenital hypothyroidism, leading to a wrong diagnosis of Alagille syndrome (14). Indeed, renal tubular acidosis, hypothyroidism and cardiac anomalies have not previously been reported in PFIC1. They explained these atypical manifestations by genetic variability. Considering that *ATP8B1* is known to be expressed in the intestine, pancreas, liver, cholangiocytes and prostate, the relationship between expression of this gene and these extra hepatic features is not clear. It could be the result of other disease-modifying genes closely linked to *ATP8B1* (14). Our PFIC1 patient has a new mutation, which could manifest itself differently on the phenotypic level, associating among other things, a congenital hypothyroidism.

The prognosis of this pathology is poor, despite adequate medical treatment and nutritional care. Liver transplantation is considered as the only curative treatment. However, for PFIC2 patients, the recurrence of the BSEP defect has been reported due to circulating BSEP antibodies (15, 16). In addition, multi-organ manifestations, such as diarrhea and pancreatic insufficiency in PFIC1, may persist or worsen after liver transplantation (7).

Advances made in our ability to establish a molecular diagnosis in infants with genetic diseases, will certainly change the paradigm for evaluation of infants with cholestasis; the use of next generation sequencing gene panels (NGSGP) improved the diagnostic management in these patients (17, 18, 19). On the other hand, molecular biology will allow families to receive specific genetic counseling and risk estimation for future offspring (20). Unfortunately, this genetic counseling was not performed in the first family in our study, which could have prevented the recurrence of this serious pathology in their second child.

## CONCLUSION:

The present study demonstrates that Tunisian PFIC patients have clinical and biological features similar to those reported in the literature. However, they have newly described mutations, which could explain the atypical association with central hypothyroidism in the PFIC1 patient. In the future, recognition of these mutations would facilitate the diagnostic approach in cases of neonatal cholestasis in the Tunisian population, where the rate of consanguinity is high. Genetic studies will also provide prenatal diagnosis.

However, further studies are required to ascertain the genotype and phenotype concordance in PFIC.

## REFERENCES:


1. Rafaella A. Morotti[1], Frederick J. Suchy[2], and Margret S. Magid[1]. Progressive familial intrahepatic cholestasis (PFIC) type 1, 2, and 3: a review of the liver pathology findings. Semin Liver Dis. 2011;31(1):3-10.
2. Anne Davit-Spraul[1], Emmanuel Gonzales[2'3], Christiane Baussan[1], and Emmanuel Jacquemin[2'3]. Progressive Familial Intrahepatic cholestasis. Orphanet J Rare Dis. 2009;4:1.
3. Laura N. Bull, Richard J. Thompson. Progressive Familial Intrahepatic Cholestasis. Clin Liver Dis. 2018;22(4):657-69.
4. Karpen SJ, Kamath BM, Alexander II, et al. Use of a comprehensive 66 gene panel to diagnosis the cause of cholestasis in > 700 individuals. Hepatology. 2017;66(1):655A. [AASLD abstract].
5. Chen HL LH, Wu JF, Wu SH, Chen HL, et al. Panel-based next-generation sequencing for the diagnosis of cholestatic genetic liver diseases: clinical utility and challenges. J Pediatr. 2018;205:153-9.
6. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1998;95:130-6.
7. Huey-Ling Chen, Shang-Hsin Wu, Shu-Hao Hsu, et al. Jaundice revisited: recent advances in the diagnosis and treatment of inherited cholestatic liver diseases. J Biomed Sci. 2018;25:75.
8. W S Lee, P F Chai, L M Looi. Progressive Familial Intrahepatic Cholestasis in Malaysian patients – a report of five cases. Med J Malaysia. 2009;64:216-9.
9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Stapelbroek JM, Peters TA, Van Beurden DH, et al. ATP8B1 is essential for maintaining normal hearing. Proc Natl Acad Sci U S A. 2009;106(24):9709-14.

11. Eva Sticova, Milan Jirsa, and Joanna Pawlowska. New insights in genetic cholestasis: from molecular to clinical implications. J Gastroenterol Hepatol. 2018; 2018:2313675.

12. Emmanuel Jacquemin. Progressive familial intrahepatic cholestasis. Clinics and Research in Hepatology and Gastroenterology. 2012;36:26-35.

13. Andrew Fretzayas, Maria Moustaki, Olga Liapi, Themistocles Karpathios. Gilbert syndrome. Eur J Pediatr. 2012;171:11-5.

14. Emily Copeland, Nisa Renault, Marc Renault, Sarah Dyack, et al. Novel splice-site mutation in ATP8B1 results in atypical progressive familial intrahepatic cholestasis type 1. Journal of Gastroenterology and Hepatology. 2013;28:560-4.

15. Jara P, Hierro L, Martinez-Fernandez P, Alvarez-Doforna R, et al. Recurrence of bile salt export pump deficiency after liver transplantation. N Engl J Med. 2009;361:1359-67.

16. Stindt J, Kluge S, Droge C, Keitel V, et al. Bile salt export pump-reactive antibodies from a polyclonal, multi-inhibitory response in antibody-induced bile salt export pump deficiency. Hepatology. 2016;63:524-37.

17. Amy G. Feldman and Ronald J. Sokol. Neonatal cholestasis: emerging molecular diagnostics and potential novel therapeutics. Nature Reviews Gastroenterology and Hepatology. 2019;16:346-60.

18. Metzker M.L. Sequencing technologies – the next generation. Nat Rev Genetic. 2010;11:31-46.

19. Marco Sciveres, Francesco Cirillo, Emmanuel Jacquemin, Giuseppe Maggiore. Diagnostic protocol of neonatal and infantile cholestasis: can it be improved? J of Pediatr. 2020;216:247.

20. Herbst S M et al. Taking the next step forward diagnosing inherited infantile cholestatic disorders with next generation sequencing. Mol Cell Probes. 2015;29:291-8.