Med. J. Cairo Univ., Vol. 92, No. 3, Accepted 26/5/2024
DOI: 10.22608/MJCU. 647-652, September 2024
www.medicaljournalofcairouniversity.net

Clinical and Genetic Study of Exons (23, 24, 26, 27) of ABCB11 Gene in Egyptian Progressive Familial Intrahepatic Cholestasis 2 Patients

MOHAMMED A. KHEDR, M.D.[1]; TAMER H.A. AMMAR, M.D.[2]; ALIF A. ALLAM, M.D.[1]; ASHRAF Y. ELFERT, M.D.[3]; MONA S. TANTAWY, M.D.[4]; MOHAMED SABBAH MOHAMED, M.Sc.[5] and GIHAN A. SOBHY, M.D.[1]

The Department of Pediatric Hepatology, Gastroenterology, and Nutrition, National Liver Institute[1], Medical Molecular Genetics Department, Institute of Human Genetics and Genome Research[2], Clinical Biochemistry and Molecular Diagnostics Department, National Liver Institute[3], Department of Pathology, National Liver Institute, Menoufia University[4] and Pediatric Department, Shebin Al-Qanater Central Hospital[5]

Abstract

Background: Mutations in the ABCB11 gene result in the uncommon autosomal recessive disease known as progressive familial intrahepatic cholestasis type 2 (PFIC2). Cholestasis with low γ-glutamyltransferase (GGT), hepatosplenomegaly, and severe pruritus are among the clinical symptoms. The exact prevalence is unclear, however the incidence is thought to be between one in 50,000 and one in 100,000 births. These diseases affect both sexes equally and have been reported globally. When a bypass treatment fails or a patient develops increasing liver disease, liver transplantation is necessary and thought to be curative. Even though bile salt excretory pump (BSEP) failure in PFIC2 is a liver-specific issue rather than a systemic disease, a small percentage of allografts have demonstrated indications of recurrent BSEP disease.

Aim of Study: Determine which exons (23, 24, 26, 27) in the ABCB11 gene are mutated in Egyptian PFIC2 patients.

Material and Methods: Ten Egyptian Pfic2 patients were clinically diagnosed, and exons (23, 24, 26, 27) of the ABCB11 gene were then molecularly screened utilizing PCR amplification and singer sequencing of the coding areas.

Results: There were no harmful mutations discovered. Two polymorphisms: A novel c.3056+50A>C and c.477+16G>A were found.

Conclusion: There were no pathogenic mutations found during the screening of exons (23, 24, 26, 27) of the ABCB11 gene in PFIC2, hence it is advised to screen the whole gene and use advanced method of screening find the pathogenic mutations in the affected cases.

Correspondence to: Dr. Mohammed A. Khedr,
The Department of Pediatric Hepatology,
Gastroenterology, and Nutrition, National Liver Institute

Key Words: *Exons 23, 24, 26, 27 – ABCB11 Gene – Familial Intrahepatic Cholestasis 2 Patients.*

Introduction

PROGRESSIVE familial intrahepatic cholestasis (PFIC) is caused by mutations in genes encoding proteins involved in the hepatocellular transport system [1]. There are three primary subtypes of PFIC (PFIC1, PFIC2, and PFIC3) that have been found. It is a heterogeneous collection of rare autosomal recessive liver illnesses of kids **[1]**. PFIC1, or Byler's sickness, is caused by mutations in the ATPase phospholipid transporter 8B1 gene (ATP8B1), which is located on chromosome 18. FIC1 is a transmembrane P-type adenosine triphosphatase that transports phospholipids and is encoded by ATP8B1 **[1,2]**. This "flippase" is responsible for preserving an uneven distribution of phospholipids throughout the hepatocytes' canalicular membrane bilayer, shielding the membrane from hydrophobic bile acids and preserving its structural integrity **[1]**. The bile salt export pump (BSEP), the primary transporter of bile acids from hepatocytes to the canalicular lumen, is encoded by the ATP binding cassette subfamily B member11 gene (ABCB11), which is found on chromosome 2. Mutations in this gene cause PFIC2 **[1,3]**.

Mutations in the gene that codes for multidrug-resistance protein 3 (MDR3/ABCB4), which is found on chromosome 7 and transports phospholipids into the canalicular lumen to neutralize bile salts and prevent damage to biliary epithelia and bile canaliculi, are the cause of PFIC3 **[1,4,5]**.

Cholestasis, jaundice, and pruritus are the primary clinical characteristics of PFIC, and these symptoms usually manifest in infancy or early childhood [1]. In addition to extrahepatic symptoms (PFIC1), PFIC is linked to a number of potentially lethal liver problems, including as portal hypertension, liver failure, cirrhosis, and hepatocellular carcinoma (HCC; PFIC2) [1].

Cholestasis, jaundice, and pruritus are the primary clinical characteristics of PFIC, and these symptoms usually manifest in infancy or early childhood [1]. In addition to extrahepatic symptoms (PFIC1), PFIC is linked to a number of potentially lethal liver problems, including as portal hypertension, liver failure, cirrhosis, and hepatocellular carcinoma (HCC; PFIC2) [1].

Low levels of glutamyl transferase (GGT) are linked to increased serum bile acid concentrations and decreased concentrations of primary bile acid in PFIC1 and PFIC2, while high levels of GGT are linked to PFIC3 [1]. In the past, PFIC was diagnosed using a combination of laboratory, biochemical, and clinical methods; however, in more recent times, genetic testing has emerged as the gold standard. For patients with PFIC, there are no pharmacologic therapy options that are approved that can reduce symptoms or stop the disease from getting worse. Ursodeoxycholic acid (UDCA), bile acid sequestrants, and medications for the symptomatic alleviation of pruritus, such as rifampin (rifampicin) and antihistamines, are examples of off-label therapy [1,6]. However, not every patient responds to these methods, and in most cases, only a partial relief from itching is obtained. Therefore, to reduce the amounts of bile acid in circulation, invasive surgery such as ileal bypass, partial external biliary diversion (PEBD), or partial internal biliary diversion (PIBD) may be required [1,6,7]. In the end, patients with PFIC2 are more likely to require liver transplantation (LT) if they experience liver failure and intractable pruritus [8].

When a permanent stoma is created, external diversions create a conduit between the gallbladder and the abdominal skin. Many of the adverse effects can be avoided by rerouting as much as 50% of the bile flow from the enterohepatic circulation. In addition to alleviating symptoms, external diversion treatments can improve hepatocellular function, halt or even reverse the course of disease, extend the period of time before transplantation, and ultimately prolong the lives of many patients [9,10].

Each patient may experience different outcomes. On the other hand, it has been demonstrated that external diversion procedures are more advantageous early in the course of the disease and less effective if serious liver disease is present. As with any surgery, problems can arise from external diversions, most commonly stoma prolapse. However, there have also been cases of cholangitis documented, thus patients should be advised of the risks and advantages of any such procedure prior to undertaking it. Partial internal biliary drainage procedures are much more recent, and there is less evidence to support their efficacy and safety. To connect the colon and gallbladder, an appendiceal or small intestine conduit must be made. Although long-term evidence supporting this treatment are still missing, patients tend to show alleviation from their pruritis, and theoretical effects on hepatic function and disease progression should be equivalent [9,10].

Material and Methods

Ten PFIC 2 patients were included in the study; they were gathered from Menoufia University's pediatric division of the National Liver Institute between November 2022 and March 2023. The study was approved by the Research Ethical Committee of Menoufia University's National Liver Institute. Every method employed complies with the Helsinki Declaration's 2008 amendment and the ethical standards established by the committee responsible for overseeing human research. The parents or legal guardians of each patient provided their informed consent in order for them to be included in the study.

DNA was extracted from the patients and thei parents' peripheral blood cells using the PAXgene Blood DNA Kit (Qiagen, Germany). By utilizing certain primers developed by ExonPrimer SOFTWARE, The ABCB11 (BSEP) gene's four exons, numbered (23, 24, 26, 27), were amplified.

The initial denaturation step of PCR is carried out at 95°. The 30 cycles begin with 30 seconds of denaturation at 95°, 30 seconds of annealing at 60°, 30 seconds of extension at 72°, and a final 7 minutes of 72° extension. The amplified segment underwent purification and sanger sequencing [11].

The discovered variations were examined according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) guidelines [12].

Results

Ten Egyptian PFIC2 patients six boys and four females from nine different unrelated families were included in the study. Their age ranged from 6 to 120 months, with an average of 42.3±35.824 months. The majority of patients 90% have a positive family history. Every patient has jaundice; in 40% of cases, the onset was sudden, and in 60% of cases, it was subtle. In 90% of cases, jaundice progresses, whereas in 10% of cases, it remains stationary. 10% of cases of pruritus are mild, 40% are moderate, and 50% are severe. In 90% of patients, there was hematemesis and abdominal expansion. Twenty percent of individuals had encephalopathy,

recurring GIT symptoms, and repeated chest infections. In 10% of instances, sensorineural hearing loss was present. Siblings are impacted in half of the cases. Thirty, twenty, and twenty cases had the residence in Sohag, Alex, or Giza, respectively; the remaining ten percent of cases were in Shakira, Damietta, or Kalopia.

Genetic findings include two benign polymorphisms: A novel c.3056+50A>C in intron 23 is found in heterozygous form in Pt2, 3 (Fig. 1). c.3084A>G is a synonymous polymorphism in exon 24 that causes no change in the alanine amino acid number 1028; it was in a heterozygous state in pts. 1, 7, 9, and 10, and in a homozygous state in pt8 (Fig. 2).

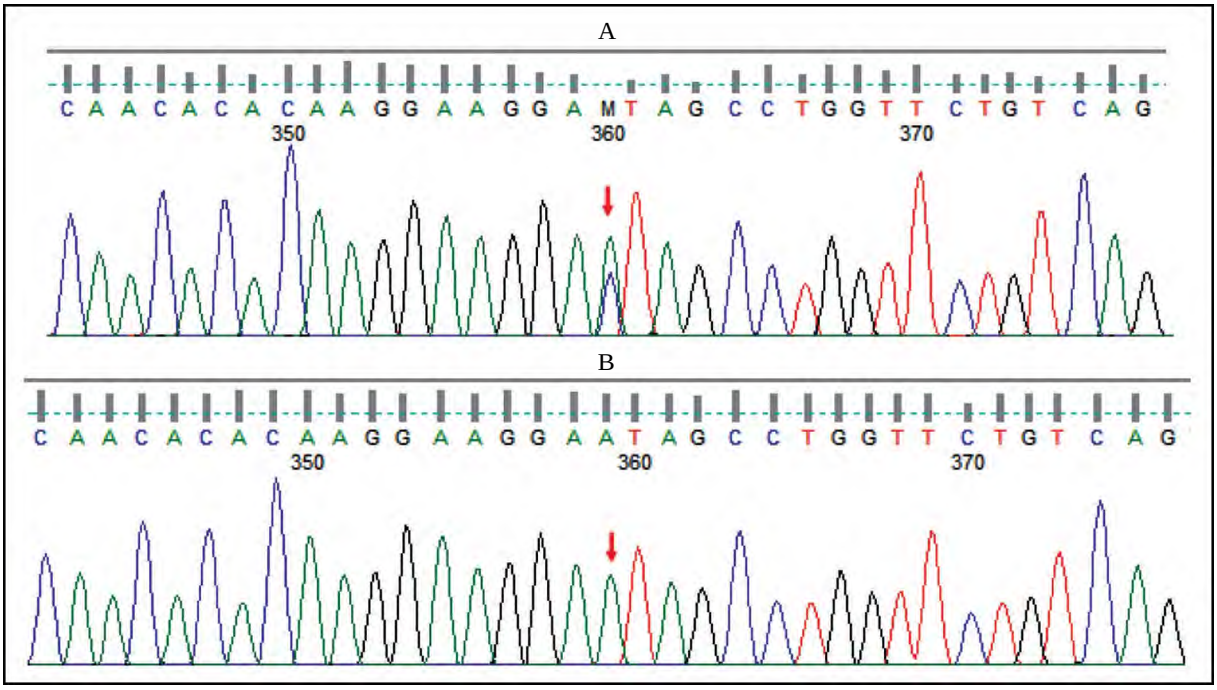

Fig. (1): Portions of the sequencing electropherograms of novel intronic C.3056+50A>C in intron 23, in which A show the heterozygous C.3056+50A>C, and B show wild-type C.3056+50A>C.

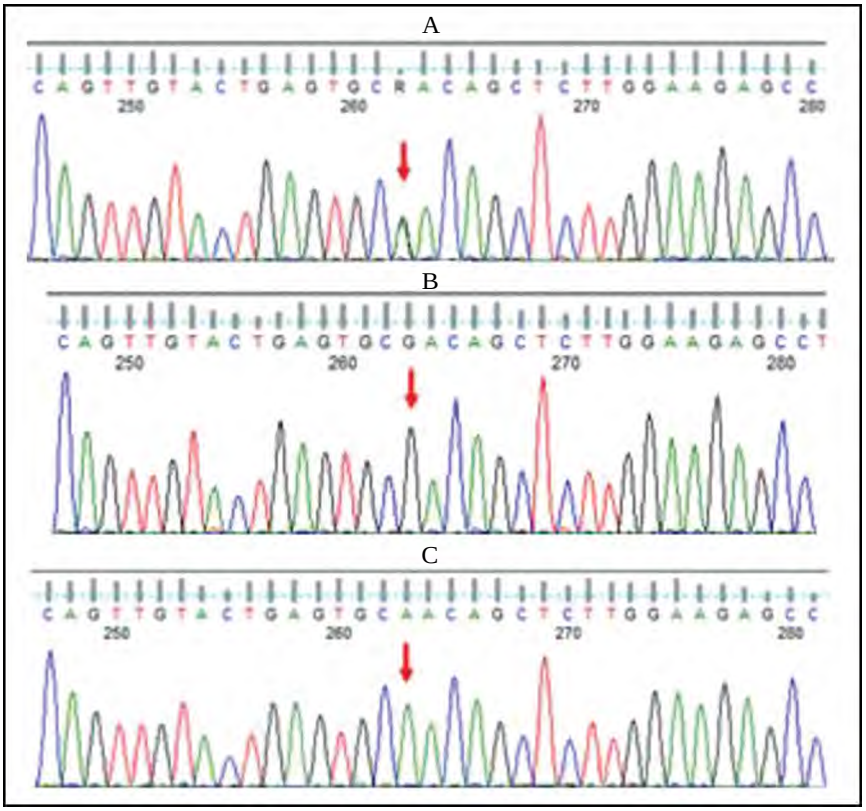

Fig. (2): Portions of the sequencing electropherograms of synonymous c.3084A>G in exon 24, in which A show the heterozygous c.3084A>G, and B show the homozygous c.3084A>G and C show wild-type c.3084A>G.

Discussion

A diverse collection of illnesses known as progressive familial intrahepatic cholestasis (PFIC) are typified by abnormalities in bile secretion and intrahepatic cholestasis that manifests in early childhood or infancy. PFIC 1 (deficiency of FIC1 protein, ATP8B1 gene mutation), PFIC 2 (deficiency of bile salt export pump, mutation of ABCB11 gene), and PFIC 3 (deficiency of multidrug resistance protein-3, mutation of ABCB4 gene) are the most prevalent forms. Through mutational study, novel variations of PFIC, referred to as PFIC 4, 5, and MYO5B related (also known as PFIC 6), have been identified in people with normal gamma-glutamyl transferase cholestasis of unknown cause. Tight junction protein 2 (TJP2) deficit is the cause of PFIC 4, while Farnesoid X receptor deficiency is the cause of PFIC 5. Microvillous inclusion disease (MVID) and isolated cholestasis are both linked to mutations in the MYO5B gene.

The spectrum of presentations in children with TJP2-related cholestasis (PFIC-4) is varied. While some people's diseases are self-limiting, others have progressive liver disease that raises the risk of hepatocellular carcinoma. Therefore, it is advised to have regular screening for hepatocellular carcinoma starting in infancy. Patients with PFIC-5 typically have early-onset coagulopathy, elevated alpha-fetoprotein, and quickly progressing liver disease that eventually necessitates liver transplantation. In patients with MYO5 B-related disorders, solitary cholestasis or cholestasis with intractable diarrhea (MVID) are possible presentations. Since these kids could have worsening cholestasis after intestine transplantation (IT) for MVID, it is best to combine intestinal and liver transplantation, or to use IT in conjunction with biliary diversion. The majority of the PFIC variations may be distinguished by immunohistochemistry, but genetic investigation is necessary for confirmation ***[13]***.

Progressive familial intrahepatic cholestasis 2 is caused by mutations in the ATP-binding cassette subfamily B member 11 gene ABCB11), which encodes the BSEP protein (1321 amino acids) and is the main transporter of bile acids from hepatocytes to the canalicular lumen against a concentration gradient. It is composed of 27 coding exons plus a leading untranslated exon, and it is located on chromosome 2q31.1. Its genomic region is 108 kb. This transporter protein is present at the canalicular membrane of hepatocytes ***[14]***.

Two benign polymorphisms were discovered in this investigation; a novel c.3056+50A>C in intron 23 includes the conversion of adenine into cytosine after 50 bases from the end of exon 23. c.3084A>G in exon 24, includes conversion of adenine to guanine, does not lead to changes in alanine amino acid, so they give the same protein sequence and do not cause disease. c.3084A>G was previously reported many times, it was thought to cause exon skipping. It was reported in 2 patients in ***[15]***, in 8 patients in heterozygous state in ***[16]***.

No deleterious variants were detected in exons (23, 24, 26, 27) in our investigation, based on Insilco prediction algorithms. This could be because the patients were gathered with mutations in other genes related to different types, or because pathogenic mutations were found in exons not explored in ABCB11. The patients may also have mutations in other PFIC types which shares symptoms with PFIC2, such as mutations causing PFIC type 4 in the TJP2 gene, PFIC type 5 in the NR1H4 gene, and PFIC type 6 in the MYO5B gene ***[17]***.

Previously, in earlier studies, pathogenic mutations of ABCB11 in these exons, including nonsense, frame shift, splice site, and missense mutations, were reported. Splice site mutation was reported in intron 24: c.3213_1delG, while 2 nonsense mutations in exons 27: (c.3643C>T, p.Gln1215X), (c.3703C>T, p.Arg1235X) were found. Frame shift deletions were revealed in exon 23 as (c.2906_2917del, p.Lys969_Lys972del), in exon 26 as (c.3438delA p.Val1147X), and (c.3491delT, p.Val1164GlyfsX7) ***[18]***.

Conclusion and limitations:

One of the study's limitations is the work on only 4 exons. A control group does not exist. no harmful mutations in the ABCB11 gene were discovered. We report two polymorphisms: A novel c.3056+50A>C and c.477+16G>A. More research with a greater number of patients is necessary.

References

1- SRIVASTAVA A.: Progressive familial intrahepatic cho–lestasis. J. Clin. Exp. Hepatol., Mar. 4 (1): 25-36, 2014. doi: 10.1016/j.jceh.2013.10.005. Epub 2013 Nov 23.

2- BULL L.N., VAN EIJK M.J., PAWLIKOWSKA L., DEYOUNG J.A., JUIJN J.A., LIAO M., KLOMP L.W., LOMRI N., BERGER R., SCHARSCHMIDT B.F., KNISELY A.S., HOUWEN R.H. and FREIMER N.B.: A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet., Mar. 18 (3): 219-24, 1998. doi: 10.1038/ng0398-219.

3- STRAUTNIEKS S.S., BULL L.N., KNISELY A.S., KOCOSHIS S.A., DAHL N., ARNELL H., SOKAL E., DAHAN K., CHILDS S., LING V., TANNER M.S., KAGALWALLA A.F., NÉMETH A., PAWLOWSKA J., BAKER A., MIELI-VERGANI G., FREIMER N.B., GARDINER R.M. and THOMPSON R.J.: A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet., Nov. 20 (3): 233-8, 1998. doi: 10.1038/3034.

4- DELEUZE J.F., JACQUEMIN E., DUBUISSON C., CRESTEIL D., DUMONT M., ERLINGER S., BERNARD O. and HADCHOUEL M.: Defect of multidrug-resistance 3 gene expression in a subtype of progressive

familial intrahepatic cholestasis. Hepatology, Apr. 23 (4): 904-8, 1996. doi: 10.1002/hep.510230435.

5- DELAUNAY J.L., DURAND-SCHNEIDER AM, DOSSIER C., FALGUIÈRES T., GAUTHEROT J., DAVIT-SPRAUL A., AÏT-SLIMANE T., HOUSSET C., JACQUEMIN E. and MAURICE M.: A functional classification of ABCB4 variations causing progressive familial intrahepatic cholestasis type 3. Hepatology, May 63 (5): 1620-31, 2016. doi: 10.1002/hep.28300. Epub 2015 Dec 23.

6- VAN DER WOERD W.L., HOUWEN R.H. and VAN DE GRAAF S.F.: Current and future therapies for inherited cholestatic liver diseases. World J Gastroenterol., Feb 7; 23 (5): 763-775, 2017. doi: 10.3748/wjg.v23.i5.763.

7- BUSTORFF-SILVA J., SBRAGGIA NETO L., OLÍMPIO H., DE ALCANTARA R.V., MATSUSHIMA E., DE TOMMASO A.M., BRANDÃO M.A. and HESSEL G.: Partial internal biliary diversion through a cholecystojejunocolonic anastomosis--a novel surgical approach for patients with progressive familial intrahepatic cholestasis: A preliminary report. J. Pediatr. Surg., Aug. 42 (8): 1337-40, 2007. doi: 10.1016/j.jpedsurg.2007.03.029.

8- DAVIT-SPRAUL A., FABRE M., BRANCHEREAU S., BAUSSAN C., GONZALES E., STIEGER B., BERNARD O. and JACQUEMIN E.: 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, May 51 (5): 1645-55, 2010. doi: 10.1002/hep.23539.

9- MALI V.P., FUKUDA A., SHIGETA T., UCHIDA H., HIRATA Y., RAHAYATRI T.H., KANAZAWA H., SASAKI K., DE VILLE DE GOYET J. and KASAHARA M.: Total internal biliary diversion during liver transplantation for type 1 progressive familial intrahepatic cholestasis: A novel approach. Pediatr. Transplant, Nov. 20 (7): 981-986, 2016. doi: 10.1111/petr.12782. Epub 2016 Aug 17.

10- BAGHDASARYAN A., FUCHS C.D., ÖSTERREICHER C.H., LEMBERGER U.J., HALILBASIC E., PÅHLMAN I., GRAFFNER H., KRONES E., FICKERT P., WAHLSTRÖM A., STÅHLMAN M., PAUMGARTNER G., MARSCHALL H.U. and TRAUNER M.: Inhibition of intestinal bile acid absorption improves cholestatic liver and bile duct injury in a mouse model of sclerosing cholangitis J Hepatol., Mar. 64 (3): 674-81, 2016. doi: 10.1016/j.jhep.2015.10.024. Epub 2015 Oct 31.

11- AMMAR T.H.A., AL-ETTRIBI G.M.M., ABO HASHISH M.M.A., et al.: Screening of GHSR, GHRHR, GH1 genes in isolated growth hormone deficiency disease in Egyptian patients. Egypt J. Med. Hum. Genet 25: 16, 2024. https://doi.org/10.1186/s43042-024-00480-y.

12- RICHARDS S., AZIZ N., BALE S., BICK D., DAS S., GASTIER-FOSTER J., GRODY W.W., HEGDE M., LYON E., SPECTOR E., VOELKERDING K. and REHM H.L.: ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med., May 17 (5): 405-24, 2015. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5.

13- VINAYAGAMOORTHY V., SRIVASTAVA A. and SARMA M.S.: Newer variants of progressive familial intrahepatic cholestasis. World J Hepatol., Dec 27; 13 (12): 2024-2038, 2021. doi: 10.4254/wjh.v13.i12.2024.

14- VENIGALLA S. and GOURLEY G.R.: Neonatal cholestasis. Semin Perinatol., Oct. 28 (5): 348-55, 2004. doi: 10.1053/j.semperi.2004.09.008.

15- BYRNE J.A., STRAUTNIEKS S.S., IHRKE G., PAGANI F., KNISELY A.S., LINTON K.J., MIELI-VERGANI G. and THOMPSON R.J.: Missense mutations and single nucleotide polymorphisms in ABCB11 impair bile salt export pump processing and function or disrupt pre-messenger RNA splicing. Hepatology, Feb. 49 (2): 553-67, 2009. doi: 10.1002/hep.22683.

16- HU G., HE P., LIU Z., CHEN Q., ZHENG B. and ZHANG Q.: Diagnosis of ABCB11 gene mutations in children with intrahepatic cholestasis using high resolution melting analysis and direct sequencing. Mol. Med. Rep., Sep. 10 (3): 1264-74, 2014. doi: 10.3892/mmr.2014.2349. Epub 2014 Jun 20.

17- ALAM S. and LAL B.B.: Recent updates on progressive familial intrahepatic cholestasis types 1, 2 and 3: Outcome and therapeutic strategies. World J. Hepatol., Jan. 27; 14 (1): 98-118, 2022. doi: 10.4254/wjh.v14.i1.98.

18- STRAUTNIEKS S.S., BYRNE J.A., PAWLIKOWSKA L., CEBECAUEROVÁ D., RAYNER A., DUTTON L., MEIER Y., ANTONIOU A., STIEGER B., ARNELL H., OZÇAY F., AL-HUSSAINI H.F, BASSAS A.F., VERKADE H.J., FISCHLER B., NÉMETH A., KOTALOVÁ R., SHNEIDER B.L., CIELECKA-KUSZYK J., MCCLEAN P., WHITINGTON P.F., SOKAL E., JIRSA M, WALI S.H., JANKOWSKA I., PAWŁOWSKA J., MIELI-VERGANI G., KNISELY A.S., BULL L.N. and THOMPSON R.J.: Severe bile salt export pump deficiency: 82 different ABCB11 mutations in 109 families. Gastroenterology, Apr. 134 (4): 1203-14, 2008. doi: 10.1053/j.gastro.2008.01.038. Epub 2008 Jan 18.

دراسة سريرية ووراثية للإكسونات (23,24,26,27) لجين ABCB11 فى مرضى الركود الصفراوى العائلى المتصاعد داخل الكبد من النوع الثانى المصريين

الخلفية: الطفرات فى الجين ABCB11تؤدى إلى مرض متنحى جسدى نادر يعرف باسم الركود الصفراوى العائلى المتقدم داخل الكبد من النوع 2 (PFIC2) من بين الأعراض السريرية لهذا المرض الركود الصفراوى مع انخفاض إنزيم γ-glutamyltransferase (GGT) ، وتضخم الكبد والطحال، والحكة الشديدة. معدل انتشار هذا المرض الدقيق غير واضح، ولكن يعتقد أن معدل الإصابة يتراوح بين واحد لكل 50,000 وواحد لكل 100,000 ولادة. وتؤثر هذه الأمراض على كلا الجنسين بالتساوى وتم تدوين حالات المرض عالمياً. عندما يفشل العلاج الالتفافى للشريان البابى أو يصاب المريض بأمراض الكبد المتزايدة، فإن زراعة الكبد تصبح ضرورية للعلاج. وهذا المرض كبدى وليس على الرغم من أن فشل مضخة إفراز الملح الصفراوى (BSEP) فى PFIC2 يعد مشكلة خاصة بالكبد وليس مرضاً يصيب جهاز فى الانسان بالكامل فقد ترجع الإصابة بالمرض بعد زرع كبد من متبرع غير متطابق.

الهدف من البحث: التعرف على الطفرات فى الإكسونات (23,24,26,27) فى حين ABCB11 فى مرضى الركود الصفراوى العائلى المتصاعد داخل الكبد من النوع الثانى فى مصر.

المواد والطرق: إجراء تحليل جينى للإكسونات (23,24,26,27) فى الجين لعشرة من المرضى الذين تم تشخيصهم بالإصابة بالركود الصفراوى العائلى المتصاعد داخل الكبد من النوع الثانى من القسم الداخلى والمترددين على العيادة الخارجية بمعهد الكبد القومى بالمنوفية، وقد تم تحليل البيانات البموغرافية والسريرية والمخبرية.

النتائج: لم يتم إكتشاف طفرات مرضية فى المرضى الذين تم خضوعهم للبحث ولكن تم إكتشاف شكلين مفردين حميدين من الاشكال المتعددة للنيوكليتيدات وهما: 16G+477.A>c و 50A+3056.C>c

الإستنتاج: لم يتم اكتشاف أى طفرات مرضية أصناء فحص الإكسونات (23,24,26,27) لجين ABCB11 فى PFIC2 لذا ينصح بفحص الجين بأكمله واستخدام طريقة متقدمة للفحص للعثور على الطفرات المسببة للأمراض فى الحالات المصابة.