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Clinical and Molecular Study of Exons [10-14] of *ABCB11* Gene in PFIC2 Patients in Egypt

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Abstract

Background: Progressive familial intrahepatic cholestasis (PFIC) is a collection of uncommon conditions that are brought on by abnormalities in bile secretion and typically manifest as intrahepatic cholestasis in infancy and youth. These genes are recessive autosomal. It is estimated that the incidence is between one in 50,000 and one in 100,000 births; however, the precise prevalence is unknown. These illnesses have been documented worldwide and impact both sexes equally. These can be roughly classified into three types: PFIC type 1, PFIC type 2, and PFIC type 3. The basis for this classification is the clinical presentation, laboratory results, liver histology, and genetic defect. In PFIC 2, the deficiency is in the ABCB11 gene, which codes for the BSEP protein.

Aim of Study: Identify the mutation in exons [10-14] of the ABCB11 gene in PFIC2 patients in Egypt.

Material and Method: Clinical Diagnosis of 10 Egyptian Pfic2 patients, followed by molecular screening of exons [10-14] of the ABCB11 gene using PCR amplification and sanger sequencing of coding regions.

Results: No pathogenic mutations were found; 5 benign polymorphisms were revealed, including: c.957A>G, c.1083+18A>T, c.1281C>T, c.1331T>C, and c.1638+32T>C.

Conclusion: Screening of exons [10-14] of the ABCB11 gene in PFIC2 revealed no pathogenic mutation, which recommends increasing the number of screening cases and using whole exome sequencing to identify the pathogenic mutations in the affected cases.

Key Words: *ABCB11 – PFIC2 Patients.*

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Introduction

PROGRESSIVE familial intrahepatic cholestasis is a group of uncommon genetic illnesses caused by malfunctioning bile secretion pathways. The illness, which is typically classified into three subtypes (PFIC type 1, PFIC type 2, and PFIC type 3) is generally diagnosed in the early stages of life and frequently manifests as intrahepatic cholestasis signs and symptoms, including pruritis, dark urine, pale stool, loss of appetite, and weariness **[1]**. Individuals with cholestasis exhibit signs and symptoms due to poor bile production and impaired secretion processes caused by mutations within the hepatic system. The first two, PFIC1 and PFIC2, frequently appear in the first few months following birth, while PFIC3 frequently manifests in the early years of life. It has been determined that this disorder is caused by mutations in three genes. In addition to having early life presentations of PFIC1 and PFIC2, they also have a faulty gene, ATP8B1, and ABCB11. Conversely, it is believed that the a forementioned genes are intact in PFIC3, as this variant has abnormalities in ABCB4, a gene that produces the protein multi-drug resistant 3, which is a member of the class of proteins called “flippases” that help translocate a variety of phospholipids from cell membranes into the bile **[2]**. The condition is becoming more common as a result of medical advancements in genetic and molecular testing. The current estimate of its prevalence ranges from 1 in 50,000 to 1 in 100,000 births, while exact numbers are yet unclear. It is estimated that between 10 and 15 percent of all pediatric cholestasis cases are caused by PFIC. Furthermore, this hereditary disorder accounts for about 10% of pediatric liver transplants. It appears that there is no recorded distinc-

tion between males and females. Despite being a disorder that is frequently identified in youngsters, it may not be identified until maturity **[3]**.

PFIC2, which is found on chromosome 2 and is the result of mutations in the ABCB11 gene, encodes a bile salt export pump (BSEP). This protein is in charge of the bile salts export into the bilious fluid, exactly as it sounds. This abnormality has two effects: it keeps the bile from having an appropriate concentration of bile salts, and it makes the bile salts accumulate in the hepatocytes. This causes an over load in the hepatocellular system, which event ually causes the liver's architecture to bed amaged and destroyed. Like we previously described, this variantex hibitsacontinuum of genotypic and phenotypic presentations; never the less, no clear link has been reported. However, research shows that the down stream effects typically indicate the degree of the mutation whether it bean insertion, deletion, missense, or splicing and whether the production of the see xport proteins is completely disrupted or only partially expressed **[4]**.

The majority of individuals who are diagnosed will have cholestasis signs and symptoms. Infants who are clearly jaundiced or have objective signs of hyperbilirubinemia are frequently worked up first. Other findings, meanwhile, might be the first to present, including poor feeding, low weight gain, vomiting, and hepatosplenomegaly. Although less prevalent than other juvenile disorders, this one occasionally manifests as signs or symptoms of fat-soluble vitamin deficiencies, including dry skin, fractures, easy bleeding or bruising, and even nighttime blindness. Again, the presenting symptoms of this underlying illness in adults are typically similar to those previously stated and associated with cholestasis. Other results, though, might also exist. There may be symptoms such as telangiectasias, palmar erythema, gynecomastia, testicular atrophy, variceal hemorrhage, hepatosplenomegaly, and ascites associated with cirrhosis and portal hypertension. Patients may rarely emerge with acute liver failure if certain symptoms go unnoticed. Certain symptoms include an international normalized ratio (INR) of greater than 1.5, elevated levels of alanine aminotransferase (AST) and ALT, encephalopathy, jaundice, and abdominal pain **[1]**.

The majority of problems are associated with portal hypertension's aftereffects. A number of problems can develop, including ascites, hemorrhoids, hepatic encephalopathy, and esophageal and gastric varices leading to large volume hematemesis. Apart from the difficulties mentioned before, end-stage liver disease poses a risk factor for hepatocellular carcinoma. Patients suffering from this disease, especially if it has advanced to cirrhosis, are more vulnerable to developing cancer. It seems that people with PFIC2 are more likely to get cancer than people without cirrhosis.By adulthood, the majority of PFIC patients will have endstage liver disease with substantial fibrosis. Due to the progressive nature of this disorder, there is a considerable risk of morbidity and mortality for patients who choose not to receive a liver transplant **[5]**.

Material and Methods

The study included 10 PFIC 2 patients collected from the pediatric department of the National Liver Institute at Menoufia University from December 2022 to April 2023. The National Liver Institute at Menoufia University's Research Ethical Committee gave the study their blessing. Every technique used complied with the 2008 revision of the Helsinki Declaration and the ethical guidelines set forth by the competent committee for human research. For every patient to be included in the study, the parents or legal guardians gave their informed consent.

The PAXgene Blood DNA Kit (Qiagen, Germany) was used to extract DNA from the peripheral blood lymphocytes of the patients and their parents. Using particular primers created by ExonPrimer SOFTWARE, the exon numbers **[10-14]** of the ABCB11 (BSEP) gene (5 exons) were amplified. Steps of PCR include initial denaturation at 95°. The 30 cycles start with denaturation at 95° for 30 seconds, then annealing at 60° for 30 seconds, and extension at 72° for 30 seconds, followed by a final extension of 72° for 7 minutes. Purification and sanger sequencing of the amplified fragment were done **[6]**. The discovered variations were examined in the 1000 Genomes, Exome Variant Server, and dbSNP141 databases.

Results

The study included ten Egyptian PFIC2 patients (six males and four females) from nine unrelated families. Their average age was 42.3±35.824 months, within the range of 6-120 months. Ninety percent of patients have a positive family history. 50% of cases have siblings affected. The residence was in Sohag, Alex, and Giza in 30, 20, and 20 cases, respectively, while 10% of cases are either in Damietta, Kalopia, or Shakira. All the patients have jaundice; the onset of jaundice was acute in 40% and insidious in 60% of cases. Jaundice is progressive in 90% of cases and stationary in 10% of cases. Pruritis is mild in 10%, moderate in 40%, and severe in 50% of cases. Abdominal enlargement and hematemesis were in 90% of cases. Recurrent chest infections, recurrent GIT symptoms, and encephalopathy were in 20% of cases. Sensorineural hearing loss was in 10% of cases.

Genetic findings include the following benign polymorphisms: Synonymous c.957A>G mutation in exon 10, which does not cause conversion of glycine amino acid number 319 in heterozygous state in PT7 and in homozygous state in pt2, 3,

and 6. c.1083+18A> T and synonymous c.1281C> T in heterozygous state in pt2, 3. Synonymous c.1281C> T does not cause a change in phenyl alanine number 427 (Fig. 1). Benign missense mutation of c.1331T>C in exon 13 is in heterozygous state in pt1 and in homozygous state in pt4–10. The mutation causes the conversion of the valine amino acid number 1444 to alanine. c.1638+32T>C in intron 14 is found in a heterozygous state in pt 1 and in a homozygous state in pt 6–10 (Fig. 2).

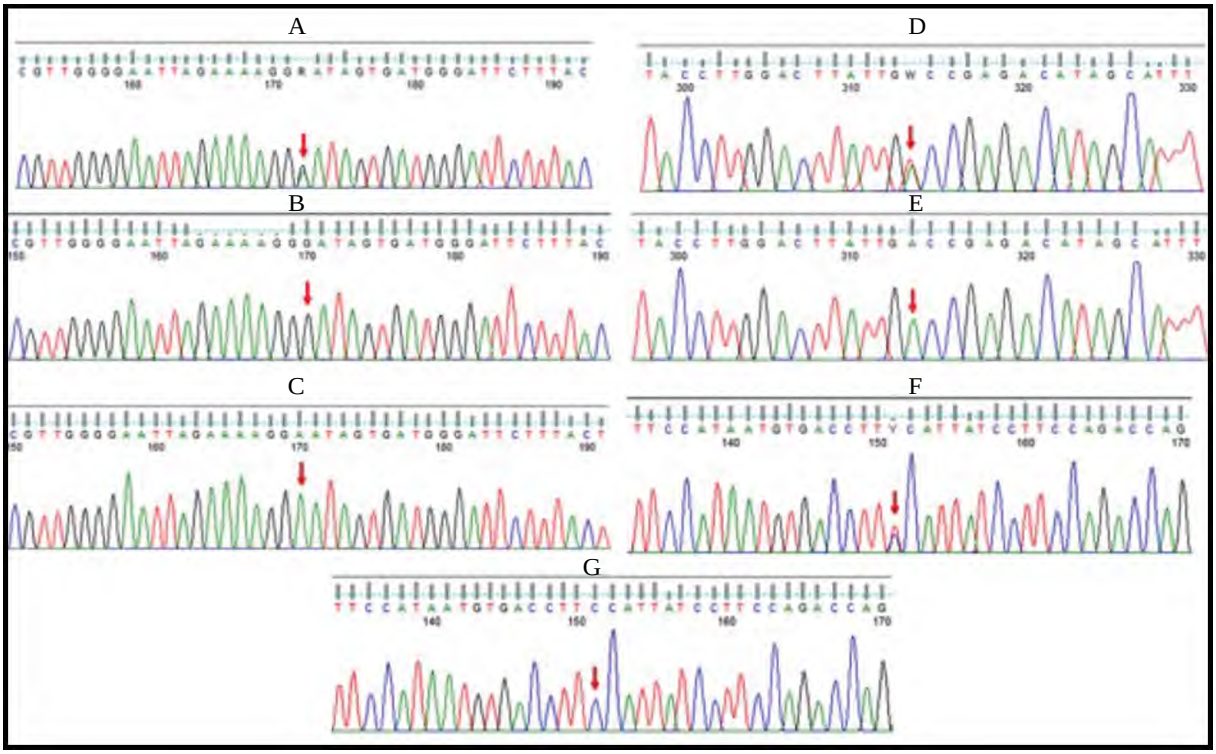

Fig. (1): Portions of the sequencing electropherograms of c.957A> G in exon 10, c.1083+18A>T in intron10 and c.1281C>T in exon 12, in which A shows the heterozygous c.957A>G, B shows homozygous c.957A> G, C show wild type c.957A> G. D. shows the heterozygous c.1083+18A> T, E show wild type c.1083+18A>T, F show the heterozygous c.1281C>T, and G show wild type c.1281C>T.

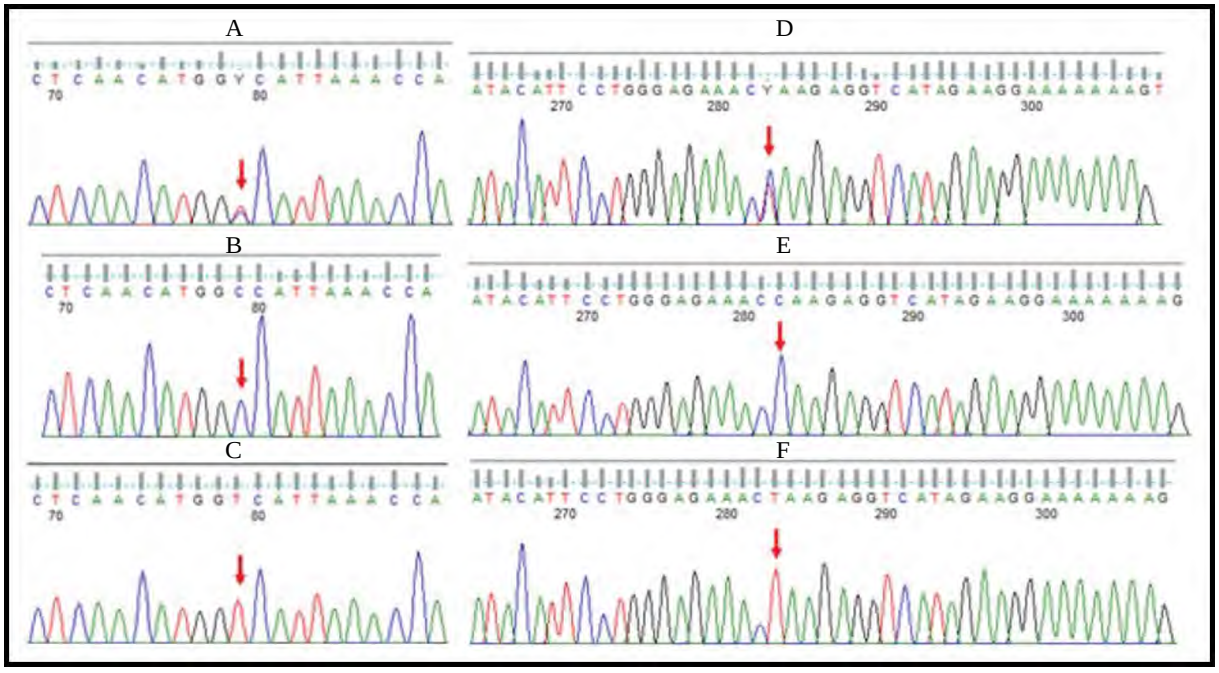

Fig. (2): Portions of the sequencing electropherograms of c.1331T>C in exon 13 and c.1638+32T>C in intron 14, in which A shows the heterozygous c.1331T>C, B shows homozygous c.1331T>C, and C shows wild type c.1331T> C. D shows the heterozygous c.1638+32T> C, E show homozygous c.1638+32T> C F: shows wild type c.1638+32T> C.

Discussion

Intrahepatic cholestasis is the presenting symptom of a heterogeneous collection of rare autosomal recessive illnesses known as progressive familial intrahepatic cholestasis (PFIC), which are linked to bile acid secretion or transport abnormalities. They typically start in childhood and grow throughout maturity, leading to either liver transplantation in infancy or adulthood or end-stage liver disease and death. The disease spectrum has been expanded to include PFIC4 (TJP2 deficiency), PFIC5 (related to NR1H4), and MYO5B-associated cholestasis beyond the previously recognized PFIC1 (Byler disease), PFIC2 (bile salt export pump [BSEP] deficiency), and PFIC3 (MDR3 deficiency) due to sequencing studies that have recently identified new gene mutations associated with previously unexplained cases **[5].**

Mutations in the ATP-binding cassette subfamily B member 11 gene (ABCB11), which encodes the BSEP protein (1321 amino acids) and is the primary transporter of bile acids from hepatocytes to the canalicular lumen against a concentration gradient, result in progressive familial intrahepatic cholestasis 2. It is found on chromosome 2q31.1, spans a 108 kb genomic area, and is made up of 27 coding exons plus a leading untranslated exon. Hepatocytes contain this transporter protein at their canalicular membrane **[7].**

In this study, two synonymous SNPs—c.957A>G in exon 10, and c.1281C>T in exon 12were found. They do not lead to changes in amino acids, so they give the same protein sequence and do not cause disease. Benign missense mutation of c.1331T>C in exon 13 causes the conversion of valine amino acid number 1444 to alanine. c.1083+18A>T in intron 10 and c.1638+32T>C in intron 14 are present after 18 bases and 23 bases from the ends of exons 10 and 14, respectively.

According to Insilco prediction algorithms, no harmful variants were found in exons (10-14) in our analysis; this may be due to either the pathogenic mutations in the exons that are not studied in ABCB11 or the collected patients having mutations in other genes that relate to other types. PFIC, which has the same symptoms as PFIC2, like mutations in the TJP2 gene, which causes PFIC type 4, in the NR1H4 gene, which causes PFIC type 5, and in the MYO5B gene, which causes PFIC type 6 **[8].**

Pathogenic ABCB11 mutations in these exons have previously been described in other investigations, and they include nonsense, frame shift, splice site, and missense mutations. There were reports of frame shift mutations (c. 1583–1584 delTA). (p.Ile528SerfsX21) in exon 14, non-sense mutations as c.1416T>A (p.Tyr472X) in exon 13, c.1558A>T (p.Arg520X) in exon 14, and c.1723C>T p.Arg575X in exon 15. Reported missense mutations involve c.1168G>C (p.Ala390Pro) in exon 11, c.1229G>A (p.Gly410Asp), and c.1238T>G (p.Leu413Trp) in exon 12, c.1388C>T (p.Thr463Ile), c.1396C>A (p.Gln466Lys) and c.1409G>A (p.Arg470Gln) in exon 13, c.1442T>A (p.Val481Glu), c.1445A>G (p.Asp482Gly), c.1460G>C (p.Arg487Pro), c.1468A>G (p.Asn490Asp), c.1535T>C (p.Ile 512Thr) c.1544A>C (p.Asn515Thr), c.1550G>A (p.Arg517His), c.1621A>C (p.Ile541Leu) and c.1622T>C (p.Ile541Thr) in exon 14, c.1643T>A (p.Phe548Tyr), c.1685G>A (p.Gly562Asp), c.1708G>A (p.Ala570Thr) and c.1763C>T (p.Ala588Val) in exon 15, while splice site mutation as c.1435_13_1435–8del in intron 13 was also reported **[9].**

Conclusion and Limitations:

The limitations of the current study include the small sample size of PFIC-2 cases. There is no control group. All the cases are from the same outpatient clinic in Menoufia. No pathogenic mutations were found in the ABCB11 gene in the 10 PFIC2 patients studied. The following polymorphisms were reported: c.957A>G, c.1083+18A>T, c.1281C>T, c.1331T>C, and c.1638+32T>C. Additional studies should be done on a larger number of patients.

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دراسة سريرية وجزيئية للإكسونات (10-14) للجين ABCB11 فى مرضى الركود الصفراوى العائلى المتصاعد داخل الكبد من النوع الثانى فى مصر

يعتبر الركود الصفراوى العائلى المتقدم مجموعة من الأمراض الجينية النادرة التى تؤدى إلى خلل فى تكوين وإفراز الصفراء مما يؤدى إلى تراكمها فى الخلايا والقنوات الكبدية وإحداث الضرر بها .

المرضى الذين يعانون من الركود الصفراوى العائلى المتصاعد من النوع الثانى عادة ما يكون لديهم تضخم فى الكبد ويشير تضخم الطحال الكبير إلى ارتفاع ضغط الدم البابى المرتبط بالتليف المتقدم أو تليف الكبد.

يوجد العديد من أمراض الركود الصفراوى التى تتشابه فى الصورة المرضية السريرية والمخبرية لمرض الركود الصفراوى العائلى المتقدم لذلك يعتبر التحليل الجينى أداة مثالية للتفريق بينهم.

الهدف من البحث: التعرف على الطفرات فى الإكسونات (10-14) فى الجين ABCB11 فى مرضى الركود الصفراوى العائلى المتصاعد داخل الكبد من النوع الثانى فى مصر.

المواد والطرق : إجراء تحليل جينى للإكسونات (10 -14) فى الجين ABCB11 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 الديموغرافية والسريرية والمخبرية.

النتائج : لم يتم إكتشاف طفرات مرضية فى المرضى الذين تم خضوعهم للبحث ولكن تم إكتشاف 5 من الأشكال المفردة الحميدة المتعددة للنيوكليوتيدات والتى تتضمن: c.c+1083.957A>G, c.18A>T, c.1281C>T, and c.1331T>C+1638.32T>C.

الإستنتاج: كشف فحص الإكسونات لم يسفر عن إكتشاف طفرات مرضية والأشكال المفردة الحميدة للنيوكليوتيدات تتواجد فى غير المصابين كما تتواجد بالمصابين للمرض مما يوصى بزيادة عدد حالات الفحص وإستخدام تسلسل الإكسوم الكامل لتحديد الطفرات المسببة للمرض فى الحالات المصابة.