

Molecular Modelling and Evaluation of Hidden Information in ABCB11 Gene Mutations

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ABSTRACT

Background: Cholestatic disorders are divided in the extra and intra-hepatic that created due to the severe liver diseases. ABCB11 encodes the bile salt export pump and this gene is mutated in several forms of intrahepatic cholestasis. So far, some molecular features of this gene was studied.

Objective: Using a developed web server, we identified high number of rare codons in this gene, and four cases were related to BSEP-deficient patients which can be used for drug design.

Material and Methods: By in-silico modelling of ABCB11, some of rare codons in different locations of ATP8b1 gene were identified and evaluated. Using several web servers a number of mutations that converted non-rare codons to rare codon in these patients were identified.

Results: Some of these rare Codons were located at special positions by mutation of which, the new side chains do not seem suitable for protein structure and function. Furthermore, this mutation changed the protein folding rate that may have a critical role in proper folding. Thus, primary change of these codons contributes to BSEP deficiency.

Conclusion: This work is a comprehensive analysis of rare codons of ABCB11 and assessment of a number of these rare codon in protein levels. Rare codons evaluation can enhance our understanding of ABCB11 structural protein of ABCB11, and help us to develop mutation-specific therapies in design of new drugs.

Citation: Zarenezhad M, Dehghani S. M, Ejtehadi F, Fattahi M. R, Mortazavi M, Tabei S. M. B. Evaluation of Hidden Information in ABCB11 Gene Mutations. *J Biomed Phys Eng.* 2019;9(3):303-316. <https://doi.org/10.31661/jbpe.v9i3Jun.680>.

Keywords

ABCB11, Bioinformatics Analysis, Rare Codon, Mutation

Introduction

The bile salt export pump (BSEP, protein product of the ABCB11 gene) is situated in the canalicular membrane of hepatocytes and is responsible for the translocation of bile salts [1]. BSEP belongs to ABC transporter superfamily and has 12-transmembrane span integral membrane proteins. Mutations in ABCB11 are related to a phenotypical spectrum of cholestatic liver diseases. Cholestasis is created due to severe liver diseases where bile cannot flow from the liver to the duodenum [2]. The causes of cholestasis are divided into two groups: those originating outside the liver and those originating within the liver [3]. Some reasons of the cholestasis within the liver include cirrhosis due to viral hepatitis B or C, drugs, acute hepatitis, alcoholic liver dis-

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Received: 1 November 2016
Accepted: 20 December 2016

ease, primary biliary cirrhosis with inflammation and scarring of the bile ducts [4]. At present, specific gene defects have been identified for PFIC2 which are caused by the deficient in gene product required for bile formation and canalicular export [2, 5]. Previously, the molecular evaluation of these PFICs including exon characterization [6], locus mapping [7], sequencing [8] and gene mutations have been studied.

The rare codons that are introduced as genetic hidden information are shown to have a critical role in protein activity and folding, and can help in problem solving of diseases and drug design [9, 10]. Codon-usage analysis can also contribute to understanding the interaction between RNA viruses and the immune response of the hosts [10]. Several mutations of ABCB11 associated with BSEP deficiency have previously been introduced [1, 11]. However, there is no study about rare codons of ABCB11 gene, and in this study we evaluated the situation of these rare codons and variations in the structure of ABCB11. For this, by submission of PFIC3 gene in the I-TASSER server, a three dimensional model of BSEP protein was created [12]. In addition, rare codons of ABCB11 gene were detected using the Sherlocc program [13], LaTcOm (<http://structure.biol.ucy.ac.cy/latcom.html>) [14], ATGme [15] and RaCC server (<http://nihserver.mbi.ucla.edu/RACC/>). By PyMOL [16] and SPDBV software [17], the characteristics of these rare codons were studied in the 3D model of BSEP protein. In the following, these variations and their relationships with rare codon, were evaluated. In this study, a large number of genetic mutations of ABCB11 gene were evaluated for their relations with rare codons and PFIC2 disease. Furthermore, some interesting results demonstrate that some of these mutations have a destructive effect on the structure of BSEP protein and result in PFIC2 disease. These findings help the elucidation of hidden information of this gene. The overall results of this study are thought to be useful in

the design of new efficient drugs.

Material and Methods

Rare Codons Analysis

For bioinformatics analysis of ABCB11 gene, the nucleotide sequences and features of this gene were retrieved from <http://www.ncbi.nlm.nih.gov/genome/>. By use of nucleotide sequence of ABCB11 gene, rare codons of this gene were detected using the following servers. Rare codon calculator (RaCC) (<http://nihserver.mbi.ucla.edu/RACC/>) detected problematic residues as arginine (AGG, AGA, CGA), leucine (CTA), isoleucine (ATA) and proline (CCC). ATGme [15] detected rare codon in three steps: (i) Input of the ATP8b1 sequence; (ii) Input of the codon usage table of Homo sapiens [gbpri]: 93487 CDS's (iii) Detection of rare codons. LaTcOm [14] reported three algorithms are implemented for the detection of rare codon clusters: i) % minimax algorithm, ii) sliding window approach and iii) a linear-time algorithm named MSS. Then, the RCC positions were visualized within the submitted sequences. Sherlocc's program [13] detected rare codon clusters by retrieving the nucleotide sequence of proteins in each Pfam protein family alignments. By these servers, some rare codons were identified in the nucleotide sequences of ABCB11 gene.

Study of Rare Codons in the Structure of ABCB11

To evaluate the position of these new identified mutations and rare codon in the structure of BSEP protein, a 3D structure of BSEP was created in the I-TASSER web server [12] based on multiple-threading alignments by LOMETS [18]. Models with the best "Confidence Score" and Z-score were chosen by I-TASSER server. The best model with suitable Z-score and confidence was visualized using PyMOL [16] and Swiss PDB viewer [17]. With ExPasy's Prot Param (<http://us.expasy.org/tools/protparam.html>) server, the total number of positive and

negative residues, physico-chemical parameters, molecular weight and other features of this model were computed. Hydrogen bonds were also detected by PIC web server [19] and WHAT IF web server [20]. Finally, the situation and relationships of these mutations and rare codons were evaluated in the structure of BSEP.

Results

Preparation of Molecular Modelling Structure of ABCB11

For understanding the protein structure and function, preparation of 3D structure of protein is a vital process in biology [21]. Our studies show that heretofore has not been determined as the crystal structure of BSEP, and it is obligatory to provide the 3D model from this protein. For this, by submitting the sequence of ATP8B1 in I-TSSAR Web Server, 3D models of these proteins were obtained. The I-TSSAR Web Server generated five models and best model showed -1.82 values of overall C-score, 0.49±0.15 value of TM-Score and Exp. RMSD was 14.1±3.9 (Figure 1).

In the following, the physiochemical proper-

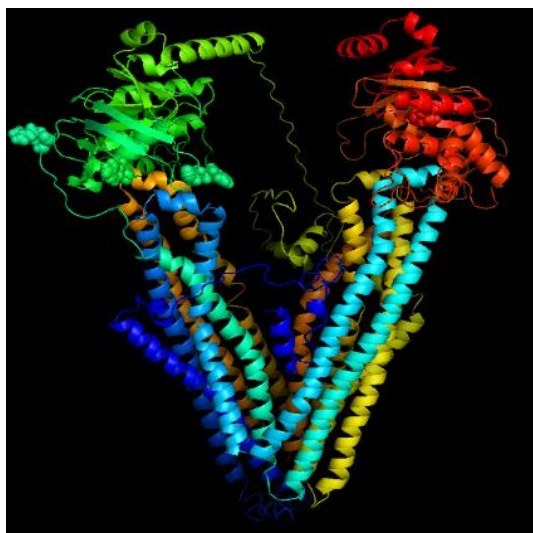


Figure 1: The ribbon diagram of BSEP protein modelled in I-TSSAR Web Servers.

ties of ATP8B1 protein model were calculated in ProtParam tool (Table 1) [22].

Detection of Rare Codon Clusters

Using UniProt database (<http://www.uniprot.org/>), the Pfam accession number of BSEP protein was identified as PF00664 (ABC_membrane. 2 hits) and PF00005 (ABC_tran. 2 hits). Pfam is a comprehensive collection of protein domains and families [23]. These Pfam was analyzed in the Sherloc program [13] and results show that any rare codon cluster was identified in these Pfam accession number of ABCB11 genes. Next, the nucleotide sequence of ABCB11 gene was analyzed in ATGme server [15]. Using the codon usage table of Homo sapiens [gbpri]: 93487 CDS's (40662582 codons) (<http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=9606>), this gene was analyzed, and the rare and highly rare codons were shown and highlighted in orange and red, respectively (Figure 2). Moreover, GC and AT contents of this gene were GC%: 45.44, AT%:54.56, calculated by this server.

In the following, RaCC server was used. By introduction of problematic residue codons as

Table 1: In silico physico-chemical features of BSEP protein obtained from ProtParam tool.

Parameters	ATP8b1
Theoretical pI	6.17
Molecular weight	146407.18
Sequence length	1321
Extinction coefficients (M-1 cm-1 at 260 nm)*	129370- 128120
Asp + Glu	143
Arg + Lys	135
Instability index	33.29
Grand average of hydropathicity	0.011
Aliphatic index	92.60

*First number is based on the assumption that both cysteine residues form cystine and the second number that both cysteines are reduced.

ATG TCT GAC TCA **GTA** ATT CTT **CGA** AGT **ATA** AAG AAA TTT GGA GAG GAG AAT GAT GGT TTT GAG TCA GAT AAA TCA TAT AAT
 AAT GAT AAG AAA TCA AGG **TTA** CAA GAT GAG AAG AAA GGT GAT GGC GTT AGA GTT GGC TTC TTT CAA TTG TTT CGG TTT TCT
 TCA TCA ACT GAC ATT TGG CTG ATG TTT GTG GGA AGT TTG TGT GCA TTT CTC CAT GGA **ATA** GCC CAG CCA GGC GTG **CTA** CTC
 ATT TTT GGC ACA ATG ACA GAT GTT TTT ATT GAC TAC GAC GTT GAG **TTA** CAA GAA CTC CAG ATT CCA GGA AAA GCA TGT GTG
 AAT AAC ACC ATT **GTA** TGG ACT AAC AGT TCC CTC AAC CAG AAC ATG ACA AAT GGA ACA **CGT** TGT GGG TTG CTG AAC ATC GAG
 AGC GAA ATG ATC AAA TTT GCC AGT TAC TAT GCT GGA ATT GCT GTC GCA **GTA** CTT ATC ACA GGA TAT ATT CAA **ATA** TGC TTT
 TGG GTC ATT GCC GCA GCT **CGT** CAG **ATA** CAG AAA ATG AGA AAA TTT TAC TTT AGG AGA **ATA** ATG AGA ATG GAA **ATA** GGC TGG
 TTT GAC TGC AAT TCA GTG GGG GAG CTG AAT ACA AGA TTC TCT GAT GAT ATT AAT AAA **ATC** AAT GAT GCC **ATA** GCT GAC CAA
 ATG GCC CTT TTC ATT CAG CGC ATG ACC **TCG** ACC ATC TGT GGT TTC CTG TTG GGA TTT TTC AGG GGT TGG AAA CTG ACC TTG
 GTT ATT ATT TCT GTC AGC CCT CTC ATT GGG ATT GGA GCA GCC ACC ATT GGT CTG AGT GTG TCC AAG TTT **ACG** GAC TAT GAG
 CTG AAG GCC TAT GCC AAA GCA GGG GTG GTG GCT GAT GAA GTC ATT TCA TCA ATG AGA ACA GTG GCT CTT **TCG** TTT GGT GGT GAG
 AAA AGA GAG GTT GAA AGG TAT GAG AAA AAT CTT GTG TTC GCC CAG **CGT** TGG GGA ATT AGA AAA GGA **ATA** GTG ATG GGA TTC
 TTT ACT GGA TTC GTG TGG TGT CAC ATC TTT TTG TGT TAT GCA CTG GCC TTC TGG TAC GGC TCC ACA CTT GTC CTG GAT GAA
 GGA AAT TAT ACA CCA GGA ACC CTT GTC CAG ATT TTC CPT AGT GTC **ATA** GGA GCT **TTA** AAT CTT GGC AAT GCT TCT
 TGT TTG GAA GCC TTT GCA ACT GGA **CGT** GCA GCA GCC ACC AGC ATT TTT GAG ACA **ATA** GAC AGG AAA CCC ATC ATT GAC TGC
 ATG TCA GAA GAT GGT TAC AAG TTG GAT **CGA** ATC AAG GGT GAA ATT GAA TTC CAT AAT GTG ACC TTC CAT TAT CCT TCC AGA
 CCA GAG GTG AAG ATT **CTA** AAT GAC CTC AAC ATG CTC ATT AAA CCA GGG GAA ATG ACA GCT CTG **GTA** CCA CCC AGT GGA GCT
 GGA AAA AGT ACA GCA CTG CAA CTC ATT CAG **CGA** TTC TAT GAC CCC TGT GAA GGA ATG GTG ACC GTG GAT GGC CAT GAC ATT
 GGC TCT CTT AAC ATT CAG TGG CTT AGA GAT CAG ATT GGG **ATA** GTG GAG CAA GAG CCA GTT CTG TTC TCT ACC ACC ATT GCA
 GAA AAT ATT CGC TAT GGC AGA GAA GAT GCA ACA ATG GAA GAC **ATA** GTC CAA GCT GCC AAG GAG GCT AAT GCC TAC ACA TTC
 ATC ATG GAC CTG CCA CAG CAA TTT GAC ACC CTT GTT GGA GAA GGA GGA GGC CAG ATG AGT GGT GGC CAG AAA CAA AGG **GTA**
 GCT ATC GCC AGA GCC CTC ATC **CGA** AAT CCC AAG ATT CTG CTT TTG GAC ATG GCC ACC TCA GCT CTG GAC AAT GAG AGT GAA
 GCC ATG GTG CAA GAA GTG CTG AGT AAG ATT CAG CAT GGG CAC ACA ATC ATT TCA GTT GCT CAT CGC TTG TCT **ACG** GTC AGA
 GCT GCA GAT ACC ATC ATT GGT TTT GAA CAT GGC ACT GCA GTG GAA AGA GGG ACC CAT GAA GAA **TTA** CTG GAA AGG AAA GGT
 GTT TAC TTC ACT **CTA** GTG ACT TTG CAA AGC CAG GGA AAT CAA GCT CTT AAT GAA GAG GAC **ATA** AAG GAT GCA ACT GAA GAT
 GAC ATG CTT **CGC** AGG ACC TTT AGC AGA GGG AGC TAC CAG GAT **TTA** AGG GCT TCC ATC CGG CAA CGC TCC AAG TCT CAG
 CTT TCT TAC CTG GTG CAC GAA CCT CCA **TTA** GCT GTT **GTA** GAT CAT AAG TCT ACC TAT GAA GAA GAT AGA AAG GAC AAG GAC
 ATT CCT GTG CAG GAA GAA GTT GAA CCT GCC CCA GTT AGG AGG ATT CTG AAA TTC AGT GCT CCA GAA TGG CCC TAC ATG CTG
GTA GGG TCT GTG GGT GGA GCT GTG AAC GGG ACA CTC ACC CCC TTG TAT GCC TTT **TTA** TTC AGC CAG AAT CTT GGG ACT TTT
 TCA ATT CCT GAT AAA GAG GAA CAA AGG TCA CAG ATC AAT GGT GTG TGC **CTA** CTT TTT **GTA** GCA ATG GGC TGT **GTA** TCT CTT
 TTC ACC CAA TTT **CTA** CAG GGA TAT GCC TTT GCT AAA TCT GGG GAG CTC **CTA** ACA AAA AGG **CTA** **CGT** AAA TTT GGT TTC AGG
 GCA ATG CTG GGG CAA GAT ATT GCC TGG TTT GAT GAC CTC AGA AAT AGC CCT GGA GCA TTG ACA ACA AGA CTT GCT ACA GAT
 GCT TCC CAA GTT CAA GGG GCT GCC GGC TCT CAG ATC GGG ATG **ATA** GTC AAT TCC TTC ACT AAC GTC ACT GTG GCC ATG ATC
 ATT GCC TTC TCC TTT AGC TGG AAG CTG AGC CTG GTC ATC TTG TGC TTC TTC CCC TTC TTG GCT **TTA** TCA GGA GCC ACA CAG
 ACC AGG ATG TTG ACA GGA TTT GCC TCT **CGA** GAT AAG CAG GCC CTC GAG ATT ACA AAT GAA GAG ATT CCA GGC CTC AGT
 AAC ATC CGC ACT GTT GCT GGA ATT GGA AAG GAG AGG CGG TTC ATT GAA GCA CTT GAG ACT GAG CTG GAG AAG CCC TTC AAG
 ACA GCC ATT CAG AAA GCC AAT ATT TAC GGA TTT TGC TTT GCC TTT GCC CAG TGC ATC ATG TTT ATT **CGC** AAT TCT GCT TCC
 TAC AGA TAT GGA GGT TAC **TTA** ATC TTC AAT GAG GGG CTC CAT TTC AGC TAT GTG TTC AGG GTG ATC TCT GCA GTT **GTA** CTG
 AGT GCA ACA GCT CTT GGA AGA GCC TTC TCT TAC ACC CCA AGT TAT GCA AAA GCT AAA **ATA** TCA GCT GCA CGC TTT TTT CAA
 CTG CTG GAC **CGA** CAA CCC CCA ATC AGT **GTA** TAC AAT ACT GCA GGT GAA AAA TGG GAC AAC TTC CAG GGG AAG ATT GAT TTT
 GTT GAT TGT AAA TTT ACA TAT CCT TCT **CGA** CCT GAC **TCG** CAA TTT CTG AAT GGT CTC TCA GTG **TCG** ATT AGT CCA GGG CAG
 ACA CTG **CGC** TTT GTT GGG AGC AGT GGA TGT GGC AAA AGC ACT AGC ATT CAG CTG TTG GAA **CGT** TTC TAT GAT CCT GAT CAA
 GGG AAG GTG ATG **ATA** GAT GGT CAT GAC AGC AAA AAA **GTA** AAT GTC CAG TTC CTC CGC TCA AAC ATT GGA ATT GTT TCC CAG
 GAA CCA GTG TTG TTT GCC TGT AGC **ATA** ATG GAC AAT ATC AAG TAT GGA GAC AAC ACC AAA GAA ATT CCC ATG GAA AGA GTC
ATA GCA GCT GCA AAA GAG GCT CAG CTG CAT GAT TTT GTG ATG TCA CTC CCA GAG AAA TAT GAA ACT AAT GGT TCC CAG
 GGG TCT CAA CTC TCT AGA GGG GAG AAA CAA CGC ATT GCT ATT GCT CGG GCC ATT **GTA** **CGA** GAT CCT AAA ATC TTG **CTA** **CTA**
 GAT GAA GCC ACT TCT GCC **TTA** GAC ACA GAA AGT GAA AAG **ACG** GTG CAG GTT GCT **CTA** GAC AAA GCC AGA GAG GGT CGG ACC
 TGC ATT GTC ATT GCC CAT CGC TTG TCC AAC ATC CAG AAC **CGC** GAT ATC ATT GCT GTC ATG GCA CAG GGG GTG GTG ATT GAA
 AAG GGG ACC CAT GAA GAA CTG ATG GCC CAA AAA GGA GCC TAC TAC AAA **CTA** GTC ACC ACT GGA TCC CCC ATC AGT **TGA**

Figure 2: Schematic representation of the rare (orange) and highly rare (red) codons in ABCB-11 gene.

Pro, Ile, Arg and Leu, this result was refined. This analysis shows that ABCB11 gene has 48 rare codons for Arg, 18 rare codons for Ile, 11 single rare codons for Leu and 11 rare codons for Pro (Figure 3). This analysis also showed that ABCB11 gene has two tandem double repeats of rare Arg codon.

Later, by LaTcOm web tool rare codon clusters of this gene were detected [14]. In LaTcOm, three algorithms of MSS, sliding window and % MINMAX were employed. The reference codon usage table was used from CUTG database [24] these algorithms. Figure 4 shows the location of RCCs in this gene using

these algorithms (Figure 4; A, B and C).

These results demonstrate that MSS detected 6 clusters, Minmax detected 15 and sliding-window detected 10 clusters. It is important to note that the cluster length selected for MSS algorithms was 21 codons and for Minmax and sliding-window algorithms were 25 codons. The characteristics and position of these RCCs in the ABCB11 gene were calculated (data not shown).

Evaluation of some Mutation Associated with Rare Codons

Some mutations in ABCB11 gene which

atg tct gac tca gta att ctt **CGA** agt **ATA** aag aaa ttt gga gag gag aat gat ggt ttt gag tca gat aaa tca tat aat
aat gat aag aaa tca **AGG** tta caa gat gag aag aaa ggt gat ggc gtt **AGA** gtt ggc ttc ttt caa ttg ttt cgg ttt tct
tca tca act gac att tgg ctg atg ttt gtg gga agt ttg tgt gca ttt ctc cat gga **ATA** gcc cag cca ggc gtg **CTA** ctc
att ttt ggc aca atg aca gat gtt ttt att gac tac gac gtt gag tta caa gaa ctc cag att cca gga aaa gca tgt gtg
aat aac acc att gta tgg act aac agt tcc ctc aac cag aac atg aca aat gga aca cgt tgt ggg ttg ctg aac atc gag
agc gaa atg atc aaa ttt gcc agt tac tat gct gga att gct gtc gca gta ctt atc aca gga tat att caa **ATA** tgc ttt
tgg gtc att gcc gca gct cgt cag **ATA** cag aaa atg **AGA** aaa ttt tac ttt **AGG AGA ATA** atg **AGA** atg gaa **ATA** ggg tgg
ttt gac tgc aat tca gtg ggg gag ctg aat aca **AGA** ttc tct gat gat att aat aaa atc aat gat gcc **ATA** gct gac caa
atg gcc ctt ttc att cag cgc atg acc tgc acc atc tgt ggt ttc ctg ttg gga ttt ttc **AGG** ggt tgg aaa ctg acc ttg
ggt att att tct gtc acc cct ctc att gga gcc acc att ggt agt agt ggt tcc aag ttt acg gac tat gag
ctg aag gcc tat gcc aaa gca ggg gtg gtg gct gat gaa gtc att tca tca atg **AGA** aca gtg gct gct ttt ggt ggt gag
aaa **AGA** gag gtt gaa **AGG** tat gag aaa aat ctt gtg ttc gcc cag cgt tgg gga att **AGA** aaa gga **ATA** gtg atg gga ttc
ttt act ggc **AGA** tto gtg tgg tgt ctc atc ttt ttg tgt tat gca ctg gcc ttc tgg tac ggc tcc aca ctt gtc ctg gat gaa
gga gaa tat aca cca gga acc ctt gtc cag att ttc ctc agt gtc **ATA** gta gga gct tta aat ctt ggc aat gcc tct cct
tgt ttg gaa gcc ttt gca act gga cgt gca gca gcc acc agc att ttt gag aca **ATA** gac **AGG** aaa **CCC** atc att gac tgc
atg tca gaa gat ggt tac aag ttg gat **CGA** atc aag gct gca att gaa ttt ctc cat aat gtg acc ttc cat tat cct tcc **AGA**
cca gag gtg aag att **CTA** aat gac ctc aac atg gtc att aaa cca ggg gaa atg aca gct ctg gta gga **CCC** agt gga gct
gga aaa agt aca gca ctg caa ctc att cag **CGA** ttc tat gac **CCC** tgt gaa gga atg gtg acc gtg gat ggc cat gac att
cgc tct ctt aac att cag tgg ctt **AGA** gat cag att ggg **ATA** gtg gag caa gag cca gtt ctg ttc gat acc acc att gca
gaa aat att cgc tat ggc **AGA** gaa gat gca aca atg gaa gac **ATA** gtc caa gct gcc aag gag gcc aat gcc tac aac ttc
atc atg gac ctg cca cag caa ttt gac acc ctt gtt gga gaa gga ggc cag atg agt ggt ggc cag aaa caa **AGG** gta
gct atc gcc **AGA** gcc ctc atc **CGA** aat **CCC** aag att ctg ctt ttg gag atg gcc acc tca gct ctg gac aat gag agt gaa
gcc atg gtg caa gaa gtg ctg agt aag att cag cat ggg cac aca atc att tca gtt gct cat cgc ttg tct acg gtc **AGA**
gct gca gat acc atc att ggt ttt gaa cat ggc act gca gtg gaa **AGA** ggg acc cat gaa gaa tta ctg gaa **AGG** aaa ggt
gtt tac ttc act **CTA** gtg act ttg caa agc cag gca aat caa gct ctt aat gaa gag gac **ATA** aag gat gca act gaa gat
gac atg ctt cgc **AGG** acc ttt agc **AGA** ggg agc tac cag gat agt tta **AGG** gct tcc atc cgg caa cgc tcc aag tct cag
ctt tct tac ctg gtg cac gaa cct cca tta gct gtt gta gat cat aag tct acc tat gaa gaa gat **AGA** aag gac aag gac
att cct gtg cag gaa gaa gtt gaa cct gcc caa gtt **AGG AGG** att ctg aaa ttc agt gct cca gaa tgg **CCC** tac atg ctg
gta ggg tct gtg ggt gca gct gtg aac ggg aca gtc aca **CCC** ttg tat gcc ttt tta ttc agc cag att ctt ggg act ttt
tca att cct gat aaa gag gaa caa **AGG** tca cag atc aat ggt gtg tgc **CTA** ctt ttt gta gca atg gcc tgt gta tct ctt
ttc acc caa ttt **CTA** cag gaa tat gcc ttt gct aaa tct ggg gag ctc **CTA** aca aaa **AGG** **CTA** cgt aaa ttt ggt ttc **AGG**
gca atg ctg ggg caa gat att gcc tgg ttt gat gac ctc **AGA** aat agc cct gga gca ttg aca aca **AGA** ctt gct aca gat
gct tcc caa gtt caa ggg gct gcc ggc tct cag atc ggg atg **ATA** gtc aat tcc ttc act aac gtc act gtg gcc atg atc
att gcc ttc tcc ttt agc tgg aag ctg agc ctg gtc atc ttg tgc ttc ttc **CCC** ttc ttg gct tta tca gga gcc aca cag
acc **AGG** atg ttg aca gga ttt gcc tct **CGA** gat aag cag gcc ctg gag atg gtg gga cag att aca aat gaa gcc ctc agt
aac atc cgc act gtt gct gga att gga aag gag **AGG** cgg ttc att gaa gca ctt gag act gag atg gag aag **CCC** ttc aag
aca gcc att cag aaa gcc aat att tac gga ttc tgc ttt gcc ttt gcc cag tgc atc atg ttt atc gcg aat tct gct tcc
tac **AGA** tat gga ggt tac tta atc tcc aat gag ggg ctc cat ttc agc tat gtg ttc **AGG** gtg atc tct gca gtt gta ctg
agt gca aca gct ctt gga **AGA** gcc ttc tct tac acc aca agt tat gca aaa gct aaa **ATA** tca gct gca cgc ttt ttt caa
ctg ctg gac **CGA** caa **CCC** cca atc agt gta tac aat cca ggt gaa aaa tgg gac aac ttc cag ggg aag att gat ttt
ggt gat tgt aaa ttt aca tat cct tct **CGA** cct gac tgc caa gtt ctg aat ggt ctc tca gtg tgc att agt cca ggg cag
aca ctg gog ttt gtt ggg agc agt gga tgt ggc aaa agc act agc att cag ctg ttg gaa cgt ttc tat gat cct gat caa
ggg aag gtg atg **ATA** gat ggt cat gac agc aaa aaa gta aat gct cag ttc ctc cgc tca aac att gga att gtt tcc cag
gaa cca gtg ttg ttt gcc tgt agc **ATA** atg gac aat atc aag tat gga gac aac acc aaa gaa att **CCC** atg gaa **AGA** gtc
ATA gca gct gca aaa cag gct cag ctg cat gat ttt gtc atg tca ctc cca gag aaa tat gaa act aac gtt ggg tcc cag
ggg tct caa ctc tct **AGA** ggg gag aaa caa cgc att gct att gct cgg gcc att gta **CGA** gat cct aaa atc ttg **CTA** **CTA**
gat gaa gcc act tct gcc tta gac aca gaa agt gaa aag acg gtg cag gtt gct **CTA** gac aaa gcc **AGA** gag ggt cgg acc
tgc att gtc att gcc cat cgc ttg tcc acc atc cag aac cgc gat atc att gct gtc atg gca cag ggg gtg atg gaa
aag ggg acc cat gaa gaa ctg atg gcc caa aaa gga gcc tac tac aaa **CTA** gtc acc act gga tcc **CCC** atc agt tga

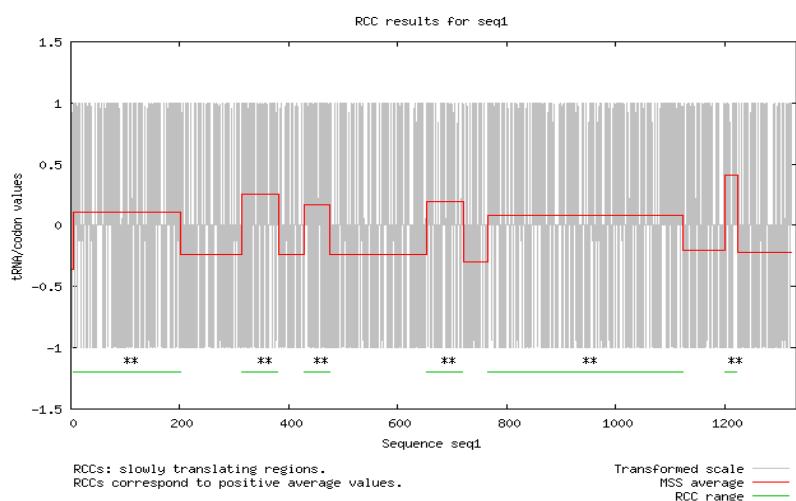
Figure 3: Representation the rare codon of Arg, Leu, Ile, and Pro in the ABCB11 gene. These residues display in red, blue, green, orange, and red, respectively.

are responsible for PFIC2 disease were presented previously [1]. In Figure 5, a large number of these mutations were highlighted in red color. As seen, these mutations were scattered throughout the nucleotide sequence of ABCB11 gene. In the following, those rare codons which were associated with mutations in ABCB11 and responsible for PFIC2 disease were focused. In this figure, we show some of these mutations in relation to rare codons.

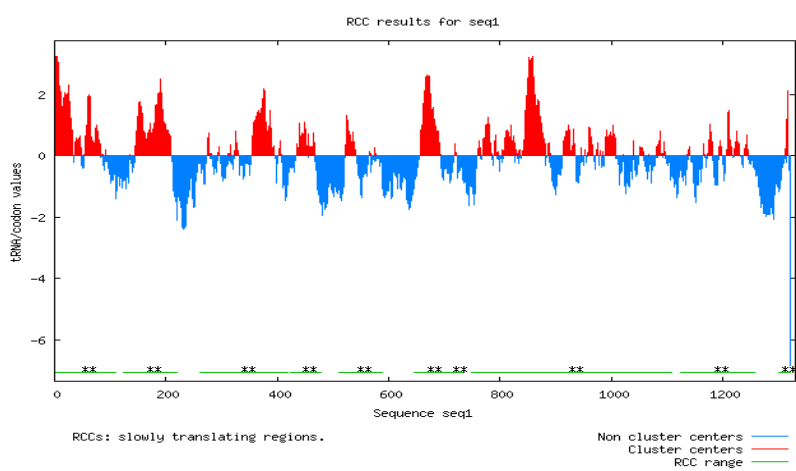
Some of these mutations overlap rare codons and some of these mutations overlap the change of codons resulted in the production of new rare codons. After their comparison, these

overlapping rare codons and new rare codons were structurally studied precisely. These rare codons identified in this study are located in different locations of BSEP protein structure (Table 2).

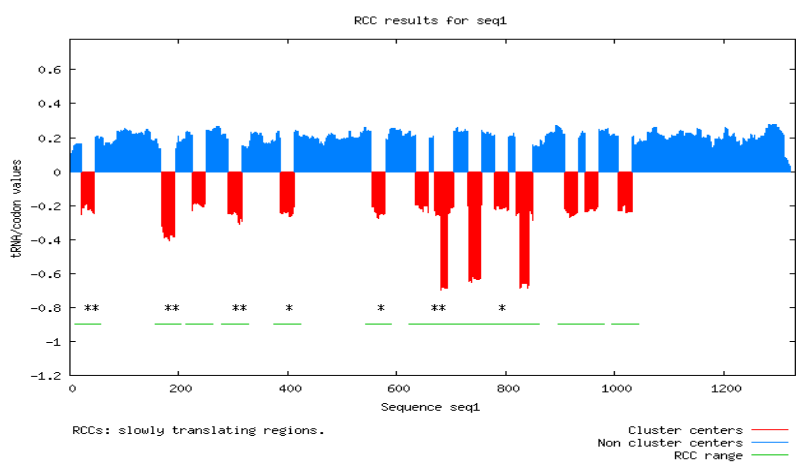
Analyzing the molecular model of BSEP showed that Arg⁴¹⁵ residue (a residue with rare codon), forms a hydrogen bond with Gln⁴¹⁴ (Figure 6). But, with mutation of Arg⁴¹⁵ residue to Gln, this hydrogen bond was disrupted. The significance of this change is that this mutation caused BRIC disorder in these patients [25] (Figure 6). Furthermore, analyzing the 3D model of ATP8B1 structure in PIC server



A



B



C

Figure 4: The representation of RCCs location in ABCB11 gene using MSS algorithm (A), minmax algorithm (B), and sliding window method (C).

atg tct gac tca gta att ctt CGA agt ATA aag aaa ttt gga gag gag aat gat ggt ttt gag tca gat aaa tca tat aat
 aat gat aag aaa tca AGG tta caa gat gag aag aaa ggt gat ggc gtt AGA gtt ggc ttc ttt caa ttc ttt cgg ttt tct
 tca tca act gac att tgg ctg atg ttt gtg gga agt ttt gtt gca ttt ctc cat gga ATA gcc cag cca ggc gtg CTA ctc
 att ttt ggc aca atg aca gat gtt ttt att gac tac gac gtt gag tta caa gaa ctc cag att cca gga aaa gca tgt gtg
 aat aac acc att gta tgg act aac agt tcc ctc aac cag aac atg aca aat gga aca cgt tgt ggg ttg ctg aac atc gag
 agc gag atg atc aaa ttt gcc agt tac tat gct gga att gct gtc gca gta ctt atc aca gga tat att caa ATA tgc ttt
 tgg gtc att gcc gca gct cgt cag ATA cag aaa atg AGA aaa ttt tac ttt AGG AGA ATA atg AGA atg gag ATA ggg tgg
 ttt gac tgc aat tca gtg ggg gag ttc aat aca AGA ttc tct gat gat att aat aaa atc aat gat gcc ATA gct gac caa
 atg gcc ctt ttc att cag cgc atg acc tcg acc atc tgt ggt ttc ctg ttg gga ttt ttc AGG tgc tgg aaa ctg acc ttg
 gtt att att tct gtc agc cct ctc att ggg att gga gca gcc acc att tgc ctg agt gtg tcc aag ttt acg gac tat gag
 ctg aag gcc tat gcc aaa gca ggg gtg gtg gct gat gaa ttc att tca tca atg AGA aca gtg gct gct ttt ggt ggt gag
 aaa AGA gag gtt gaa AGG tat gag aaa aat ctt gtg ttc gcc cag tgc tgg gga att AGA aaa gag ATA gtg atg gga ttc
 ttt att ttt gga ttc ggt tgt ctc atc ttt ttg tct gca ctg gcc ttc tgg tac ggc tcc aca ctt gac atc ctg gat gaa
 gga gaa tat aca cca gga acc ctt gtc cag att ttc ctc agt gtc ATA gta gga gct tta aat ctt gcc aat gcc tct cct
 tgt ttg gaa gcc ttt gca act gga cgt gca gca gcc acc agc att ttt gag aca ATA gac AGG aaa ttc atc att gac tgc
 atg tca gaa gat ggt tac aag tgc gat CGA atc aag ggt gaa att gaa ttc cat aat gtg acc ttc cat tat cct gcc AGA
 cca gag gtg aag att CTA aat gac ttc aac atg ctt att aaa cca ggg gaa atg aca gct ctg gta gga ttc agt gga gct
 gga gag atg aca ctg gaa ctc att cag CGA ttc tat gac ttt tgt gaa gga atg gtg acc tgc tgc gat gcc cat att
 cgc tct ctt aac att cag tgg ctt AGA gat cag att ggg ATA gtg gag caa gag cca gtt ctg ttc tct acc acc att gca
 gaa aat att cgc tat gcc AGA gaa gat gca aca atg gaa gac ATA gtc caa gct gcc aag gag gcc aat gcc tac aac ttc
 ttc atg gac ctg cca cag caa ttt gac acc ctt gtt gga gaa gga gga gcc cag atg ggt ggc cag aaa caa AGG gta
 gct atc gcc AGA gcc ctc atc CGA aat ttt aag att ctg ctt ttg gac atg gcc acc tca gct ctg gac aat gag agt gaa
 gcc atg gtc caa gaa gtg ctg agt aag att cag cat ggg cac aca atc att tca gtt gct cat cgc ttg tct acg gtc AGA
 gtt gca gat acc att ttt ggt ttt gaa cat gcc act ggc act gca atg gaa gaa AGA ggg acc cat gaa gaa tta ctg gaa AGG
 gtt tac ttc act CTA gtg act ttg caa agc cag gga aat caa gct ctt aat gaa gag gac ATA aag gat gca acc gaa gat
 gac tgc ctt cgc AGG acc ttt agc AGA ggg agc tac cag gat agt tta AGG gct tcc atc cgg caa cgc tcc aag tct cag
 ctt tct tac ctg gtg cac gag tct cca tta gct gtt gta gat cat aag tct acc tat gaa gaa gat AGA aag gac aag gac
 att cct gtg cag gaa gaa gtt gaa cct gcc cca gtt AGG AGG att ctg aaa ttc agt gct cca gaa tgg ttc tac atg ctg
 gta ggg tct gtg ggt gca gct gtg aac ggg aca gtc aca ttt ttg tat gcc ttt tta ttc agc cag att ctt ggg act ttt
 tca att cct gat aag gaa caa AGG tca cag atc aat ggt gtt tgc CTA ctt ttt gta gca atg gcc ttt gta tct ctt
 ttc acc caa ttt CTA cag gga ttc gcc ttt gct aaa tct ggg gag ctc CTA aca aaa AGG CTA cgt aaa ttt ggt ttc AGG
 gca atg ctg ggg caa gat att gcc tgg ttt gat gac ctc AGA aat agc cct gga gca ttg aca aca AGA ctt gct aca gat
 gct tcc caa gtt caa ggg gct gcc tct cag atc ggg atg ATA gtc aat tcc ttc act aac gtc act gtc gcc atg atc
 att gcc ttc tcc ttt agc tgg aag ctg agc ctg gtc atc ttg tgc ttc ttc ttc ttg gct tta tca gga gcc aca cag
 acc AGG atg ttg aca gga ttt gcc tct CGA gat aag cag gcc ctt gtg gag atg gtg gga cag att aca aat gaa gcc ctc agt
 aac atc cgc act gtt ggt gga att gga aag gag AGG cgt ttt gat gca cct gag act gag act gag aag ttc aag
 aca gcc att cag aaa gcc aat att tac gga ttc tgc ttt gcc ttt gcc cag tgc atc atg ttt att gcg aat tct gct tcc
 tac AGA tat gga ggt tac tta atc tcc aat gag ggc ctc cat ttc agc tat gtg ttc AGG gtg atc tct gca gtt gta ctg
 agt gca aca gct ctt gga AGA gcc ttc tct tac acc cca agt tat gca aaa gct aaa ATA tca gct gca cgc ttt ttt caa
 ctg ctg gac CGA caa ttc cca atc agt gta tac aat act gca ggt gaa aaa tgg gac aac ttc cag ggg aag att gat ttt
 gtt gat tgt aaa ttt aca tat cct tct CGA cct gac tcg caa gtt ctg aat ggt ctc tca gtg tgc att agt cca ggg cag
 aca gat cgc ttt gtt ggg agc agt gga tgt ggc aaa agc act agc att cag ctg ttg gaa cgt ttc tat gat cct gat caa
 ggg aag gtg atg ATA gat ggt cat gac agc aaa aaa gta aat gtc cag ttc ctc cgc tca aac att gga att gtt tcc cag
 gaa cca gtg ttg ttt gcc tgt agc ATA atg gac aat atc aag tat gga gac aac acc aaa gaa att ttc atg gag AGA gtc
 ATA gca gct gca aaa gac gct cag ttc cat gat ttt gtc atg tca ctc cca gag aaa tat gaa act aac gtt ggg tcc cag
 ggg tct caa ctc tct AGA ggg gag aaa caa cgc att gct att gct cgc gcc att gta CGA gat cct aaa atc ttg CTA ttc
 gat gaa gcc act tct gcc tta gac aca gaa agt gaa aag acg gtg cag gtt gct CTA gac aaa gcc AGA gag ggt cgg acc
 tgc att gtc att gcc cat cgc tgc tcc acc atc cag aac gcg gat atc att gct gtc atg gca cag ggt gtg atg gaa
 aag cgc acc cat gaa gaa ctg atg gcc caa aaa gga gcc tac tac aaa CTA gtc acc act gga tcc ttc atc agt tga

Figure 5: Schematic representation of the codon usage of ABCB11 gene and position of rare codons and mutations, highlighted in yellow and red, respectively.

showed the interaction of these residues with other residues.

Another missense mutation (1295 G>C) was also detected resulting in PFIC (Figure 7) [26]. In this mutation, the codon sequence of Arg⁴³² (AGA as rare codon) changed to Thr⁴³² (ACA). These original and substituted residues constitute hydrogen bonds with Ser⁴³¹ as shown in Figure 7B. The significance of this change is that Arg⁴³² is located in the interior space of BSEP protein channel, and this mutation caused BRIC disorder [26] (Figure 7).

Analyzing the ATP8B13D model showed that Arg⁴⁷⁰ residue (a residue with rare codon),

forms a hydrogen bond with Leu⁴⁶⁷, Ile⁴⁶⁸, Ala⁴⁷², Asp⁴⁷³, Asp⁴⁸⁵ and Gln⁴⁶⁶ (Figure 8). But, with mutation of Arg⁴⁷⁰ residue to Gln, some of these hydrogen bonds were disrupted. This mutation also caused BRIC disorder in patients with this mutation in the ABCB11 gene [27] (Figure 8).

Another mutation (3724 C>A) was also detected that resulted to PFIC (Figure 9A) [27]. In this mutation, the codon sequence of Leu¹²⁴² (CTA as rare codon) changed to Ile¹²⁴² (ATA). These original and substituted residues constitute the hydrogen bonds with Ser⁴³¹ shown in Figure 9B Ile¹²⁷³.

Table 2: The 1-4 numbers are the position of rare codon in ABCB11 gene that mutated to the non-rare codon and 5-12 numbers are the position of non-rare codons that mutated to the rare codon in the PFIC2 patients and analyzed.

Number	Exon	Nucleotide Change	Predicted Protein Effect	Location in Protein	Reference
1	12	c.1244G>A	R415Q	NBF	[25]
2	12	c.1295G>C	R432T	NBF	[26]
3	13	c.1409G>A	R470Q	Adj WA	[27]
4	27	c.3724C>A	L1242I	WB	[27]
5	11	c.1168G>C	A390P	NBF	[27]
6	15	c.1779T>A	S593R	NBF1	[28]
7	13	c.1388C>T	T463I	WA	[27]
8	18	c.2130T>C	P710P	IC3	[29]
9	20-21	c.2576C>G	T859R	IC4	[27]
10	22	c.2776G>C	A926P	IC5	[30]
11	23	c.2944G>A	G982R	TM11	[31]
12	25	c.3346G>C	G1116R	WA	

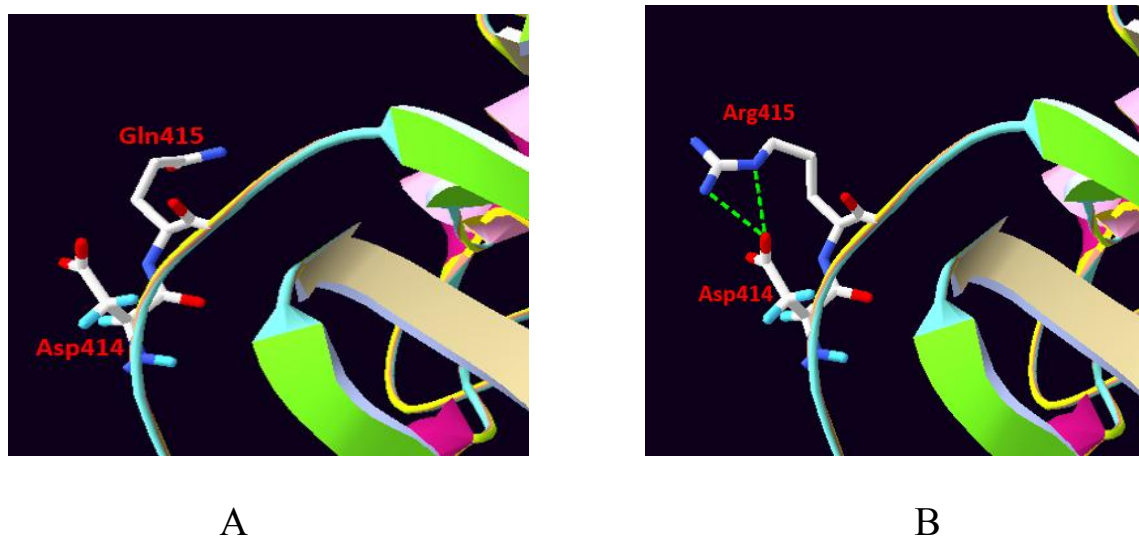


Figure 6: A) the ribbon diagram of BSEP protein, with location of Arg⁴¹⁵ residue (rare codon). The Arg⁴¹⁵ residue forms the hydrogen bond with Gln⁴¹⁴ B) mutation Arg⁴¹⁵ to Gln⁴¹⁵.

Discussion

Three types of PFIC which are referred to autosomal-recessive liver disorders are related to mutations in hepatocellular transport-system genes [28-33]. Mutations in ABCB11 gene have a variety in phenotype of autosomal recessive cholestasis liver diseases [1].

Liver disease in BSEP deficiency attributes to intrahepatic accumulation of toxic bile salts and failed the secretion of toxic bile salts [29]. Our comprehensive evaluation of these ABCB11 mutations show that all of these mutations that are resulted in cholestasis liver diseases are scattered throughout the gene and

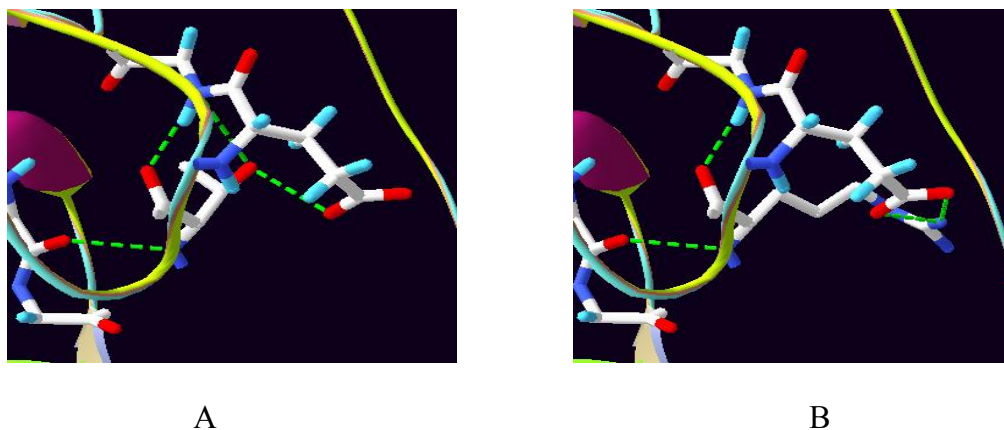


Figure 7: A) the ribbon representation of BSEP protein, with location of Arg⁴³² residue (rare codon). B) Mutation of Arg⁴³² to Thr⁴³².

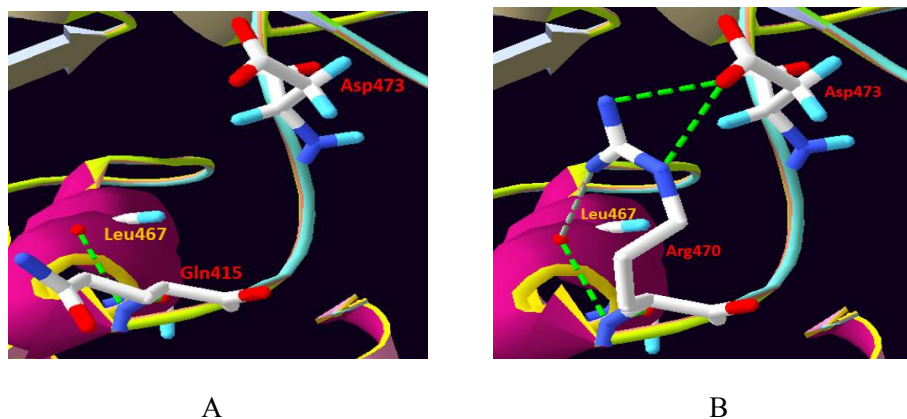


Figure 8: A) the ribbon diagram of BSEP protein, with location of Arg⁴⁷⁰ residue (rare codon). The Arg⁴⁷⁰ residue forms the hydrogen bond with Asp⁴⁷³ B) mutation Arg⁴⁷⁰ to Gln⁴⁷⁰.

are not concentrated in special regions of this gene. This tells that the protein structure and function of BSEP has a very high sensitivity to the mutations and structural changes. This shows that any mutation in this structure can have destructive effects on the structure and function of BSEP. Re-evaluation of this mutation can provide a new approach in the study of these patients and design of new drugs. In this regard, considering the hidden information as “rare” codons that are infrequently used by cells and the specific roles of these rare codons in the proper folding of proteins

is critical.

We have previously conducted the identification of detection of rare codons and molecular modelling of some proteins in our lab and have a good experience in these techniques [34-39]. For better evaluation of ABCB11 gene in this study, the detecting and studying of rare codons were conducted. In the following, the relation of some ABCB11 mutation with rare codons was studied. For the detection of rare codons, the following web server was used. For Pfam detection, the UniProt database identified two Pfams for ABCB11 as PF00664 (ABC_mem-

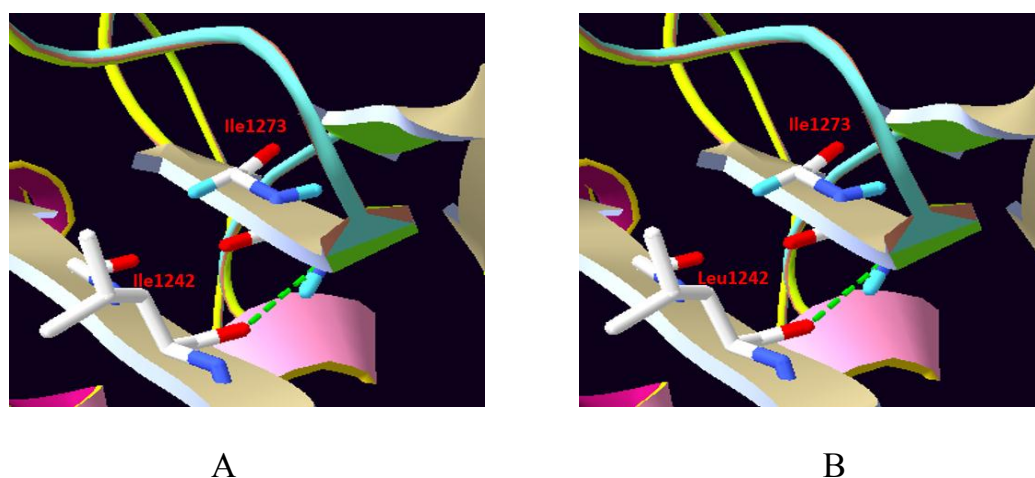


Figure 9: A) The ribbon diagram of BSEP protein, with location of Leu¹²⁴² residue (rare codon). B) Mutation of Leu¹²⁴² to Ile¹²⁴².

brane. 2 hits) and PF00005 (ABC_tran. 2 hits). These Pfams were analyzed in the Sherlock program that identified no rare codon clusters in the ABCB11 gene. In the following, this sequence nucleotide of this gene was analyzed in the ATGme web server that detected the 69 rare codon and 10 highly rare codons that may have a critical role in proper folding of protein chain. In addition, this gene was analyzed in the RaCC server detecting 48 rare codons for Arg, 18 rare codons for Ile, 11 rare codon for Leu and 11 rare codons for Pro. Finally, using LaTcOm web tool, the RCCs of this gene were detected. Results showed a large number of RCCs in the ABCB11 gene in these three algorithms.

The overall evaluation of rare codons of ABCB11 gene showed a large number of rare codons and rare codon clusters. This connotes that the protein structure of ABCB11 has important hidden features that need to guarantee the proper folding of this protein. For this reason, a large number of rare codons slowly had done the overall folding rate of this pump so that the final protein has a correct structure and function. However, these results show a large number of the rare codons of Arg and with large number of non-covalent hydrogen

bonds play a special role in the correct folding of ABCB11. Finally, we focus on some rare codons related to PFIC2. In this regard, 3D molecular modelling of ABCB11 was conducted in I-TSSAR Web Server.

The precise analysis reveals that four rare codons were mutated in PFIC2 disease. These four rare codons are distributed in different regions in the structure of BSEP protein (Figure 10).

Arg⁸⁵⁹ (rare codon). E) The ribbon diagram of BSEP protein, with location of Gly⁹⁸² residue. F) Mutation Gly⁹⁸² to Arg⁹⁸² (rare codon). G) The ribbon diagram of BSEP protein, with location of Gly¹¹¹⁶ residue. H) Mutation Gly¹¹¹⁶ to Arg¹¹¹⁶ (rare codon).

Structural analysis demonstrates that these rare codons form some hydrogen bonds with other residues disrupted with mutation in PFIC2 patients. This mutation with the disruption of these hydrogen bonds or change in the protein folding rate affects the protein folding that may disrupt the proper structure and function of ABCB11. It shows the critical role of these residues in the process of protein folding. However, other hypotheses should be considered too.

Table 2 shows new rare codons which are

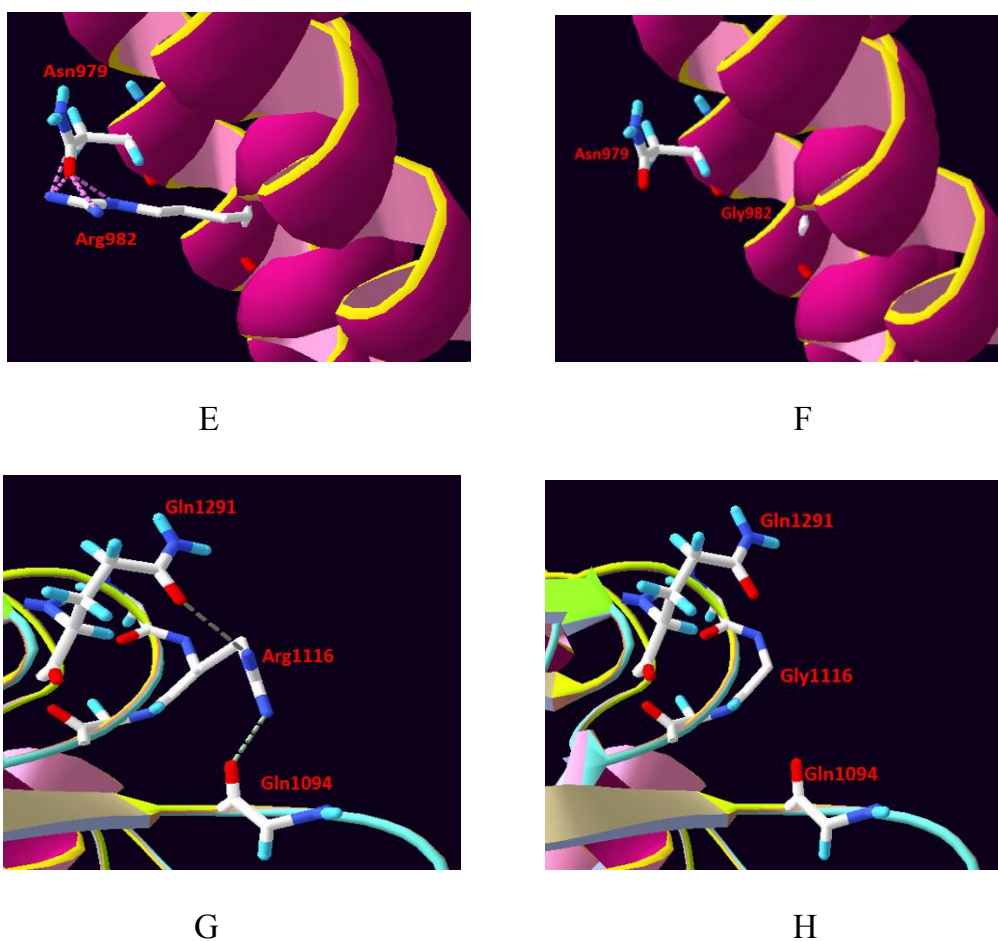


Figure 10: A) The ribbon diagram of BSEP protein, with location of Ser⁵³⁹ residue. B) mutation Ser⁵³⁹ to Arg⁵³⁹ (rare codon). C) The ribbon diagram of BSEP protein, with location of Thr⁸⁵⁹ residue. D) mutation Thr⁸⁵⁹ to Arg⁸⁵⁹ (rare codon). E) The ribbon diagram of BSEP protein, with location of Gly⁹⁸² residue. F) Mutation Gly⁹⁸² to Arg⁹⁸² (rare codon). G) The ribbon diagram of BSEP protein, with location of Gly¹¹¹⁶ residue. H) Mutation Gly¹¹¹⁶ to Arg¹¹¹⁶ (rare codon).

caused by some mutations of PFIC2 patients. In these patients, non-rare codons were converted to rare codons that interfere with suitable protein folding rate. These mutations change the hydrogen bond network affecting the structure and function of BSEP protein. On the other hand, these mutations changed the protein folding rate interfering with correct protein folding. Besides this, these new residues have a different side chain in comparison with original residues that may create the structural repulsion interfering with proper folding and functional activity of BSEP. Final-

ly, these mutations either by change of folding rate or by change of hydrogen interaction have a negative effect on the BSEP and result in PFIC2 disease.

Conclusion

Meanwhile, some in-vivo and in-silico evidence as molecular docking and evaluating these mutations is needed for our theoretical study confirmation. Our data showed that rare codon positions might have an essential role in folding and activity of BSEP. This study may also provide new insights into drug de-

sign for the treatment of PFIC2, in the future.

Acknowledgment

The study was performed as a PhD thesis in Shiraz University of Medical Sciences with grant No. of 93-7346.

Data Sharing Statement: All data were extracted from database of clinics and hospitals affiliated to Shiraz University of Medical Sciences.

Conflict of Interest

There is no conflict of interest for this study.

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