Clinica Chimica Acta 495 (2019) 598–605

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

A homozygous *ABCB4* mutation causing an LPAC syndrome evolves into cholangiocarcinoma

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ARTICLE INFO

Keywords:
LPAC syndrome
ABCB4 mutation
QPCR
Cholangiocarcinoma
UDCA therapy

ABSTRACT

Low phospholipid-associated cholelithiasis (LPAC) is characterized by the association of *ABCB4* mutations and low biliary phospholipid concentration with symptomatic and recurring cholelithiasis. In the present study, we reported a case of a 63-year-old woman, who presented a biliary pain beginning at the age of 30, recurrent after cholecystectomy, along with "comet-tail shadows" revealed by ultrasonography thus, fulfilling the diagnosis of LPAC. This disease evolved into a cholangiocarcinoma. To understand the molecular basis of this phenotype, we performed the *ABCB4* gene sequencing, followed by *in silico* analysis and Q-RT-PCR assay. The results displayed a homozygous missense sequence variation (c.140G > A, p.Arg47Gln), predicted as pathogenic according to MutPred. Accordingly, this gave rise to a decreased hepatic *ABCB4* mRNA level and structural alterations of the mutated protein. Eventually, we reported, here, the first description of an *ABCB4* missense mutation (p.Arg47Gln) at homozygous state in a Tunisian LPAC syndrome. An elucidation of its functional consequences was performed. Besides, this case suggests that the delayed diagnosis of LPAC syndrome and the lack of UDCA treatment may contribute in the development of complications, such as cholangiocarcinoma.

1. Introduction

Low Phospholipid-Associated Cholelithiasis (LPAC syndrome – OMIM #600803) is a cholestatic disorder that affects the biliary function of the liver among young adults. Clinically, the presence of intrahepatic cholesterol stones, the recurrence of biliary symptoms after cholecystectomy and the onset of first symptoms before the age of 40 represent the three criteria characterizing LPAC. The presence of at least two of these criteria, establishes the diagnosis, which is consolidated by a personal history of gravidic cholestasis and/or a family history of cholelithiasis[1]. This disease has recently been described in the European population and considered as a peculiar form of intrahepatic cholelithiasis. It is caused by genetic defects in the *ABCB4* gene, encoding the MDR3 protein which is the transporter of the phosphatidylcholine (PC) during bile secretion. The transported PC is crucial for the solubilization of the cholesterol and the neutralization of bile acids [2]. Indeed, the lack of PC in the bile leads to the production of lithogenic bile, with a detergent effect, responsible for the crystallization of cholesterol as stones and an excessive secretion of the gamma-glutamyltransferase (GGT) by the damaged cholangiocytes [3].

In 2013, 79 disease-causing mutations were shown in a large interesting study, comprising 156 LPAC patients. All truncating and 74% of missense variants were detected at heterozygous state, while 26% of the missense ones were homozygous or compound heterozygous [4]. The functional characterization pertaining to some of these mutations indicated that they altered the MDR3 protein within different degrees; however, a residual activity of MDR3 is always maintained in LPAC patients [5–7]. In addition to the LPAC syndrome, it has been shown that the MDR3 deficiency in adults is associated with a wide spectrum of hepatobiliary disorders with variable severity [8]. This variable phenotypic expression confirmed the implication of further factors, ong with the genetic component, in the development of these *ABCB4*-related diseases, as previously suggested [9,10].

For the treatment of LPAC patients, UDCA is systematically used. This therapy allows prompt relief of symptoms, taking into account its several beneficial effects [11]. In fact, a favorable outcome has been

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https://doi.org/10.1016/j.cca.2019.06.007
Received 4 March 2019; Received in revised form 16 May 2019; Accepted 6 June 2019
Available online 07 June 2019

displayed by the majority of LPAC patients [1,4]. Furthermore, a reduction in the number of stones has been observed in one cohort of adult patients, occurring *ABCB4* mutations after 3 years or more of UDCA treatment [12]. However, it is worth mentioning that this medical therapy should be initiated earlier in order to avoid the occurrence of the symptoms or the manifestation of potential complications related to the MDR3 deficiency [13].

In this study, we examined a Tunisian patient, developing clinical and biochemical features consistent with LPAC. A sequencing analysis of *ABCB4* gene was performed to support this diagnosis. A clinical follow-up was also maintained to assess the evolution of the disease.

2. Materials and methods

2.1. Patient

In this report, we were interested in a Tunisian case of an adult woman (60-year-old at first medical care) suffering from a cholelithiasis. Two additional healthy members from the family's patient were tested. All recruited individuals have signed an Informed consent, according to the hospital ethics committee (Habib Bourguiba-Sfax, Tunisia) before starting the study.

The studied patient was clinically diagnosed with LPAC syndrome, according to the three well-characterized criteria identified by Rosmorduc et al. [1]. This disease has been evolved towards a cholangiocarcinoma treated by a left liver lobectomy. A brief description of the patient's medical history is provided below.

In 2015, the 60-years-old patient was referred to the General Surgery Department at the university hospital Habib Bourguiba (Sfax-Tunisia) for a mild acute biliary pancreatitis (lipase at 922 UI/L, Balthazar grade B) and treated by a cholecystectomy. She reported similar episodes of hepatic colic since the age of 30, and remained without treatment. Regarding the family medical history, we note that her brother and sister were also subject to cholecystectomy at the age of 39 and 45, respectively.

In 2017, the patient was admitted again for a new onset of mild acute pancreatitis (lipase at 1090 UI/L, Balthazar grade B). As part of the etiologic assessment, a CT scan and magnetic resonance cholangiopancreatography exhibited a slight dilatation of the left intrahepatic bile ducts with micro-lithiasis, without the dilatation of extrahepatic bile ducts or any tumor thickening. Abdominal ultrasound confirmed the left intrahepatic biliary dilatation with no tumor thickening and revealed typical “comet-tail shadows” (or intrahepatic hyperechogenic foci) in all the liver. The shadows were motionless, as opposed to pneumobilia (Fig. 1A). Biochemistry displayed a biological cholestasis (Bilirubin 42.5 µmol/l, GGT 475 UI/L, ALP 330 UI/L, ASAT 322 UI/L, ALAT 458 UI/L). In addition, there was neither alteration of the general state nor elevation of the tumor markers (CEA 1.3 ng/ml, CA19–9 < 1 U/ml).

Based on the recurrence of acute pancreatitis after cholecystectomy, the onset of biliary pains before the age of 40, and the hepatolithiasis manifested by the ‘comet tail’ shadows images revealed by ultrasonography, the LPAC diagnosis was performed. According to this diagnosis, an UDCA therapy was prescribed (ursodeoxycholic acid with a dose of 600 mg QD), however, no follow-up has been recorded since then.

In 2018, the patient experienced a third episode of acute pancreatitis manifested by abdominal pain associated with vomiting and with sign of severity (lipase at 2597 UI/L, Balthazar grade C). MRI revealed, through T2-weighted signal acquisition and 3D MRCP, irregular and dilated left bile ducts, containing macroscopic signal voids corresponding to lithiasis (Fig. 1B). T1-weighted signal acquisition with fat saturation after gadolinium chelate injection obtained at the portal vein showed a left intrahepatic mass of 3 cm in long axis with irregular contrast enhancement, thus giving rise to an intrahepatic cholangiocarcioma (Fig. 1C). Additional explorations indicated that there was neither alteration of the general state nor elevation of the tumor markers (ACE 1.6 ng/ml, CA19–9 < 1 U/ml). Besides, a biological cholestasis (Bilirubin 32.5 µmol/l, ALP 560 U/l, ASAT 294 U/l ALAT 455 U/l) was noticed.

Taken into account the noncompliance of the prescribed treatment and the appearance of a tumor progressing hugely in favor of a left intrahepatic cholangiocarcinoma, it was decided to perform a surgical resection despite the absence of alteration pertaining to the general condition and the absence of elevation of the tumor markers. Hence, the patient had a left liver lobectomy. Macroscopic anatomopathological exam confirmed the presence of an intra-hepatic lithiasis upstream the tumor process (Fig. 1D). Further, the microscopic exam concluded into the existence of differentiated Cholangiocarcinoma, a 3 cm intraductal growth at its widest point was developed on a high grade biliary intra epithelial neoplasia (bil IN3), with neither cirrhosis nor vascular invasion nor perineural tumor invasion (Fig. 1E). Thus, we report herein a very rare case of degenerated LPAC syndrome. The patient has remained asymptomatic with no tumor relapse, for a period of 1 year up to now.

2.2. Molecular investigations

2.2.1. Sequence screening of ABCB4 gene

Total genomic DNA was isolated from blood leukocytes of the studied patient and of her family's members by the standard phenol-chloroform method [14]. The entire coding regions and flanking intronic parts were amplified in a thermal cycle (Applied Biosystems 2720). Direct sequencing of PCR products was performed with the ABI Prism BigDye Terminator Cycle sequencing. Through Blast, results were compared with reference sequences (NM_018849 and NP_061337) looking for putative variants. When identified, the variant was also searched in the family's members' blood DNA samples by sequencing the appropriate exon.

2.2.2. Quantitative analysis of hepatic ABCB4 gene expression

Total hepatic RNA was extracted from the liver's patient and control samples, using the Trizol™ agent as described previously [15]. An RT reaction was then performed, using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher) following the manufacturer's instructions. The generated hepatic cDNA was the subject of a quantitative PCR experiment (Applied Biosystems StepOne™), using *Power* SYBR Green PCR Master Mix (Applied Biosystems). The threshold cycles (Ct) were determined for the target (*ABCB4* and *NR1H4*) and reference (*B2M*) genes in both patient and control samples. Subsequently, the relative gene expression levels were calculated using the formula:

$$R = \frac{\mathrm{E\ (target)}^{\Delta \mathrm{CT\ target\ (control-patient)}}}{\mathrm{E\ (ref)}^{\Delta \mathrm{CT\ ref(control-patient)}}}$$

where E represents Efficiency. This formula provides a more accurate result since it takes into account the difference in terms of efficiencies pertaining to the two PCR (of target and reference genes) [16].

2.3. Bioinformatic tools

Various *in silico* analyses have been elaborated to characterize the identified variant(s).

2.3.1. Degree of conservation of the altered sites

We used the Clustal Omega program to generate the multiple alignment of the peptide sequences, corresponding to the *ABCB4* gene of different species and to the ABCB family's genes, separately. The aligned sequences were retrieved from the NCBI database.

2.3.2. Prediction of pathogenicity

We evaluated the impact of the found variant(s) on MDR3 protein

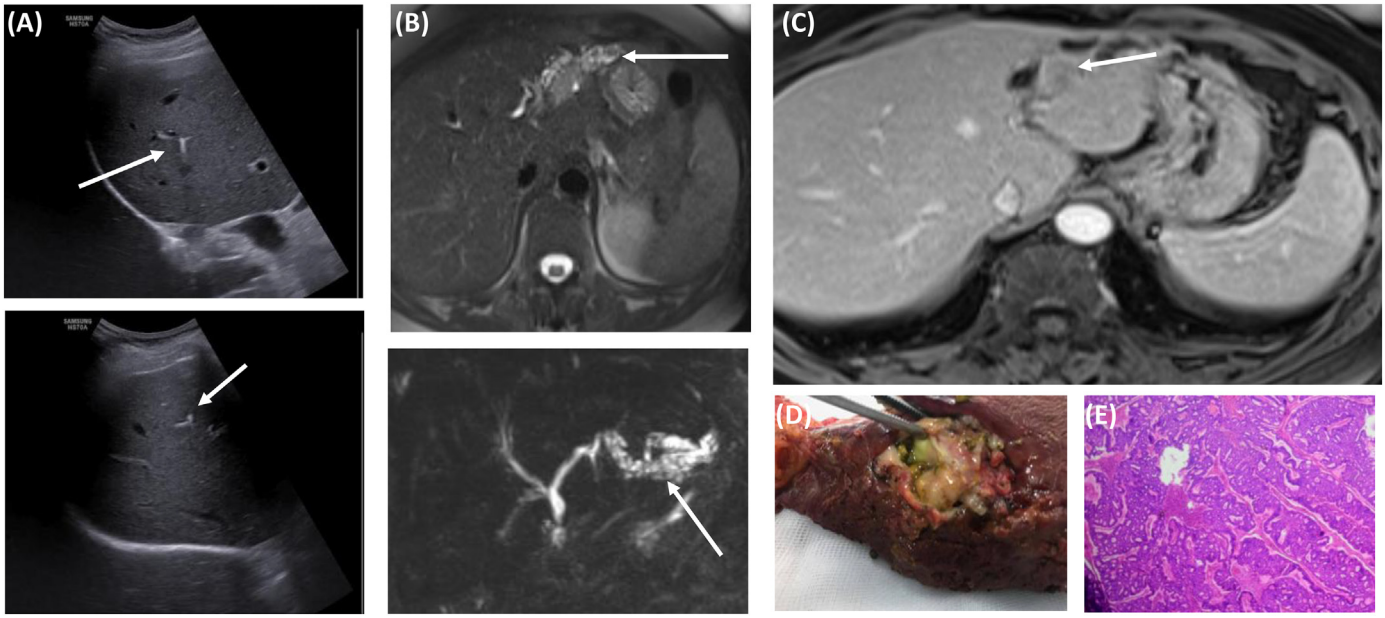

Fig. 1. Various explorations of the studied patient. (A) An abdominal ultrasound shows typical comet-tail shadows (or intrahepatic hyperechogenic foci) (White arrows). The comet tail is not mobile, as opposed to pneumobilia. (B) T2-weighted acquisition and 3D MRCP show irregular and dilated left bile ducts containing macroscopic signal voids corresponding to stones (White arrows). (C) T1-weighted acquisition with fat saturation after gadolinium chelate injection obtained at portal phase shows a left intrahepatic mass with irregular contrast enhancement suggestive of intrahepatic cholangiocarcioma (White arrow). (D) Photo of the specimen of left intrahepatic mass removed by the hepatectomy suggesting an intrahepatic cholangiocarcinoma. (E) Liver biopsy confirmed the cholangiocarcinoma formation.

by MutPred tool. Its high accuracy (> 90%), in terms of predicting the pathogenicity of *ABCB4* variants previously proved, was the basis of our choice [7].

2.3.3. Structural analysis of the identified variant(s)

Firstly, we assessed the effect of the tested variant(s) on the stability of MDR3 protein by I.Mutant 2.0 and iPTREE-STAB programs [17,18]. Structural defects were highlighted by Swiss-PDB viewer V4.1 through a comparison of wild type (WT) and mutated models, generated by RaptorX [19,20]. Putative alterations of aggregation-prone and amyloid-forming regions in the protein sequence as well as chaperone-binding sites were also evaluated through the SNPeffect4.0 program [21].

2.3.4. Prediction of stability changes of mRNA

We predicted stability changes caused by the nucleotide substitution (s) on the mRNA stability through Mfold and KineFold, two thermodynamic-based methods [22,23]. Changes in the minimum free energy ($\Delta\Delta G = \Delta G_{mut} - \Delta G_{wt}$) of mRNA fragments of five sizes (25, 81, 131, 211, 279 nt), harboring either the WT or the mutated allele in the middle, were calculated. The choice of two predictive programs and fragments of variables sizes aimed to enhance the accuracy of our results.

2.3.5. Prediction of the phosphorylation profile

This prediction was performed using the NetPhos3.1 server. A score of 0.5 was selected as a threshold. Threonine, Serine and Tyrosine residues with a score ≥0.5 were considered phosphorylated [24].

3. Results

In the current study, we described a Tunisian patient with clinical and biochemical features consistent with LPAC syndrome. Referring to this diagnosis, added to the familial history of lithiasis, a molecular screening of *ABCB4* was carried out to identify the disease-causing mutation.

The mutational sequencing of the 28 coding exons pertaining to the *ABCB4* gene and parts of its flanking boundaries revealed, in the studied patient, the presence of a homozygous c.140G > A (p.Arg47Gln) transition in exon 4 of *ABCB4* gene. This substitution was present at heterozygous state in the patient's mother and was absent in the unaffected brother and in controls (Fig. 2A). It affected a highly-conserved R47 residue throughout different species and also in 7/11 of proteins belonging to the ABCB family (Fig. 2B).

Results supplied by MutPred showed that the identified variation possessed a probability of 61% (> 50%) to be deleterious. Besides, it was predicted to be associated with a loss of MoRF binding with a significant *p*-value ($P = .0236$).

3.1. Additional analysis of the effects of the c.140G > A (p.Arg47Gln) mutation

3.1.1. Impact on the ABCB4 mRNA

To evaluate the impact of the c.140G > A on the *ABCB4* mRNA stability, we elaborated a computational analysis, including Mfold and KineFold programs. Positive ΔΔG values were supplied by both programs and independently to the size of the analyzed fragment, supporting the destabilization effect of the G to A substitution at the position 140 (Fig. 3A). Subsequently, we investigated the consequence of the predicted decrease of stability on the hepatic mRNA level of *ABCB4* gene through a real-time quantitative PCR assay. Ct values were generated from both normal and patient hepatic samples for both *B2M* and *ABCB4* genes, allowing the estimation of the mRNA fold expression. Results showed a 1.4-fold reduction in the abundance of the *ABCB4* mRNA harboring the mutation, with respect to the control (Fig. 3B).

3.1.2. Impact on the MDR3 structure and stability

We explored the putative consequences of the amino acid substitution Arg47Gln on the MDR3 stability and structure. The supplied results given by I-Mutant2.0 and IPTREE-STAB were concordant (ΔΔG = -1.12, and − 1.76, respectively), in favor of a decrease in the thermal stability of the mutated protein compared to the WT one. In

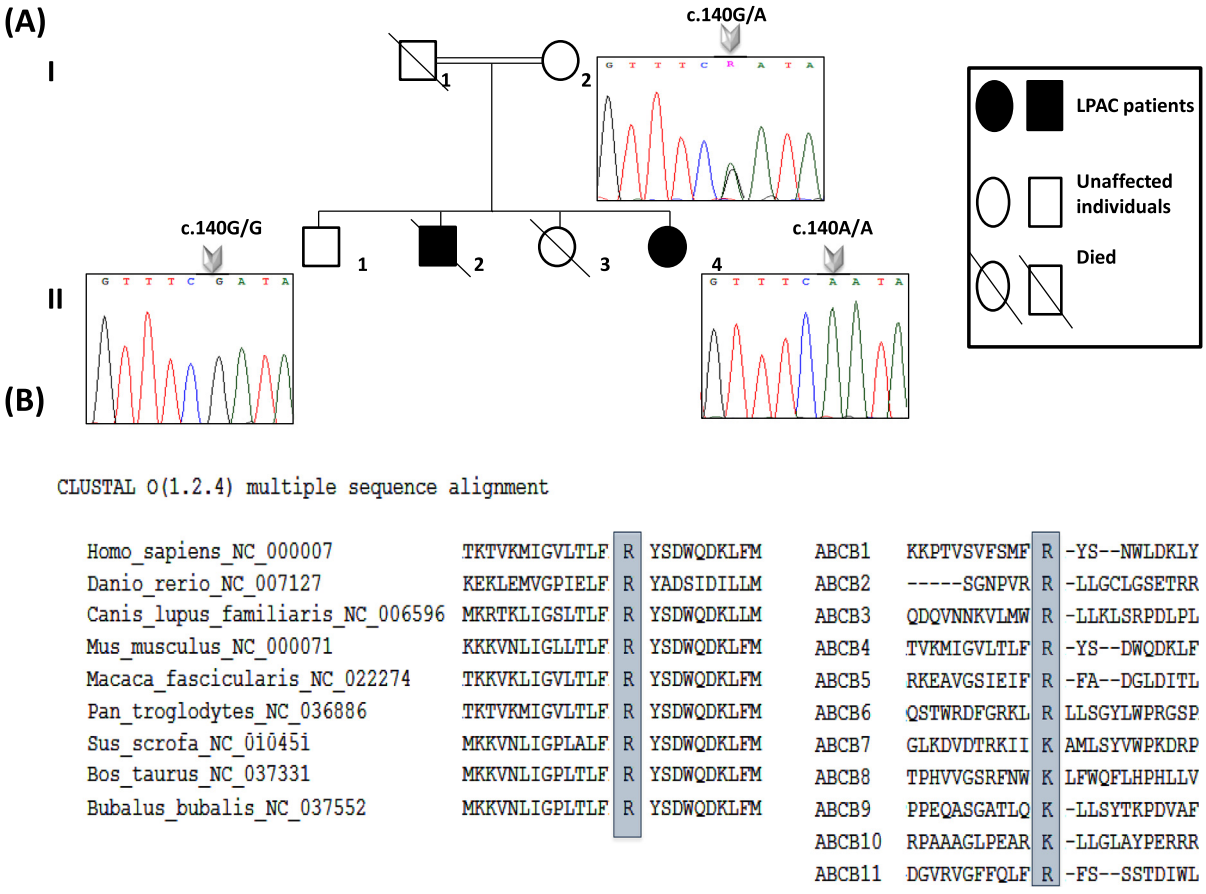

Fig. 2. (A) Pedigree of the studied family with LPAC syndrome presenting the mode of inheritance of the described substitution c.140G > A. Generations are indicated on the left in Roman numerals. Individuals of each generation are numerated (B) Multiple alignment of *ABCB4* sequences of different species and Human *ABCB* sequences.

addition, the analysis of the Arg47Gln by SNPeffect 4.0 showed that the aggregation tendency of the mutated protein is more important than the WT protein (dTANGO = 261 > 50). This increase in the aggregation tendency, added to the predicted destabilization effect of the Arg47Gln, prompted us to elaborate a modeling study in order to assess and visualize the eventual structural rearrangements behind these consequences. The superposition of both p.47Q and p.47R models showed that the root mean square deviation between the two structures was significant (7.87 Å > 3), reflecting a remarkable difference in the overall architecture of both proteins. Likewise, the comparison of protein regions, encompassing amino acids Val42-Gln52 harboring Arg47 or Gln47 in the middle, demonstrated that the p.Arg47Gln modified the number of hydrogen bunds as well as the amino-acids involved in these connections. These changes may disrupt the MDR3 protein conformation (Fig. 4B).

All these analyses at both RNA and protein levels, in addition to the overall pathogenicity predicted by MutPred, consolidated the damaging effect of the c.140G > A (p.Arg47Gln) mutation.

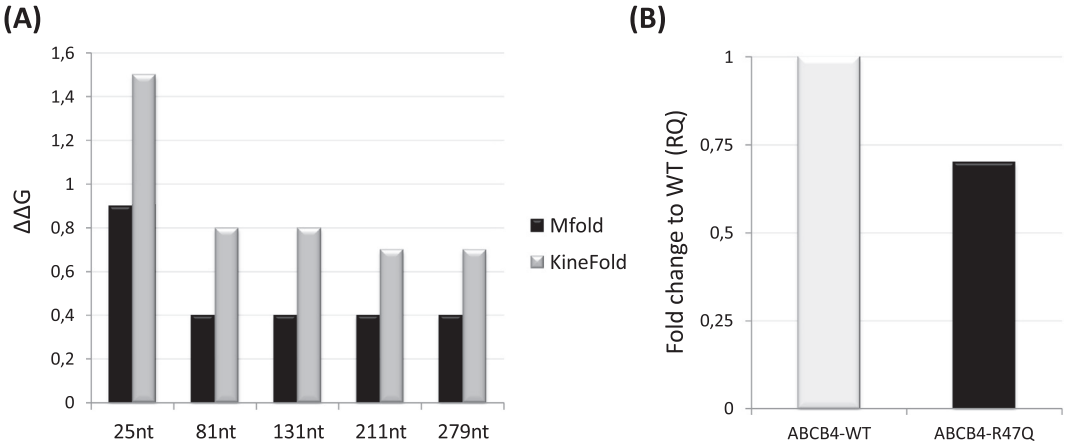

Fig. 3. Estimation of the *ABCB4*-mRNA abundance by *in silico* and quantitative analysis (A) Predictive results supplied by Mfold and KineFold through ΔΔG calculation (= ΔG variant – ΔG WT) (B) Hepatic *ABCB4* mRNA expression level as determined by qPCR.

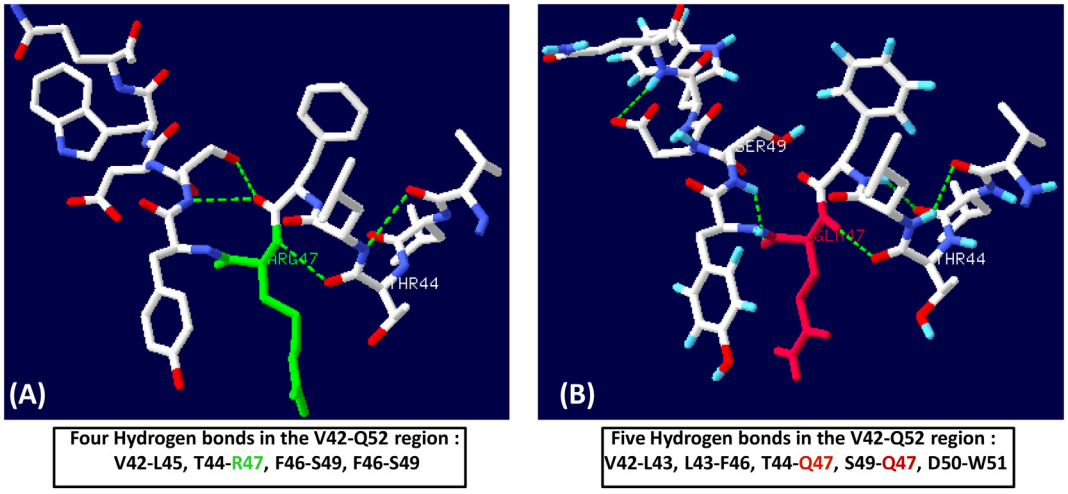

Fig. 4. Three-dimensional structure of the MDR3 Val42-Gln52 peptide harboring WT(A) and Mutated residue (B) colored with green and red, respectively. Hydrogen bonds were indicated in green dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

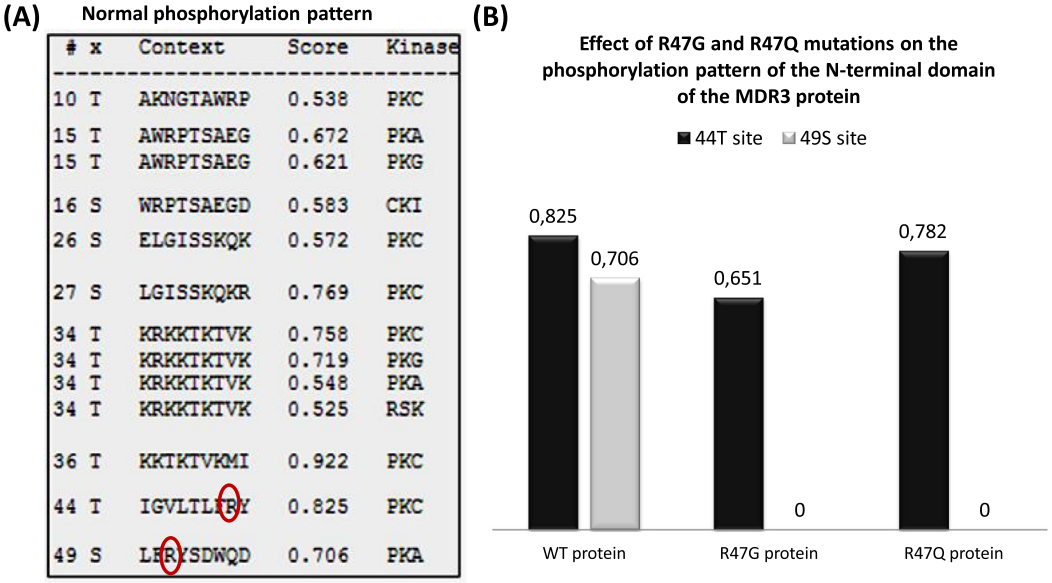

Fig. 5. *In silico* analysis of the N-terminal domain of MDR3 protein by NetPhos sever (A) and comparative study of the effect of Arg47Gly and Arg47Gln mutations on the WT phosphorylation profile (B).

4. Discussion

In the present study, we explored a Tunisian patient who manifested typical clinical and biochemical features of LPAC syndrome. The mutational screening of the *ABCB4* gene confirmed this phenotype and revealed a missense mutation c.140G > A (p.Arg47Gln) at homozygous state. We showed that this mutation led to an impairment of the hepatic *ABCB4* mRNA abundance and disrupted the MDR3 protein structure. Clinically, we revealed an evolution of the LPAC syndrome to a cholangiocarcinoma (CCa), 3 years after the diagnosis.

Herein, we described the p.Arg47Gln mutation for the first time in a homozygous state in the LPAC syndrome. Previously, it has already been described three times at compound heterozygous state in association with Gly319Glu, Arg406Gln and Thr82Asn, respectively, within patients developing hepatobiliary abnomalies [12,25,26]. The altered amino-acid is a highly-conserved amino-acid, belonging to the MDR3 N-terminal domain. In this domain, two heterozygous mutations, p.Thr34Met and p.Arg47Gly, have been previously described in patients suffering from LPAC syndrome and from recurrent cholangitis, respectively [12]. These two mutations disrupt the transport activity of MDR3 protein through the alteration of the phosphorylation process as shown by Gautherot, based on *in vitro* molecular assay [6]. This finding prompted us to perform an *in silico* study to assess the impact of the Arg47Gln on this post-translational modification, in comparison with p.Arg47Gly, for which the effect on the phosphorylation has been elucidated. Results supplied by NetPhos3.1 revealed that both mutations disrupt the normal phosphorylation pattern, suggesting that the Arg47Gln could impair the MDR3 activity by altering the phosphorylation as confirmed for the Arg47Gly mutation (Fig. 5). Additionally, we indicated that Arg47Gln altered the abundance of the hepatic mutated mRNA. Hence, we suggested that this alteration, added to the predicted destabilization effect on the MDR3 protein, may reduce the amount of the MDR3 protein and therefore its secretion activity. Indeed, the faint staining of the mutated Arg47Gln protein compared to the WT, showed in the western blotting, consolidated our hypothesis [26]. In addition to the impairment of the MDR3 activity, the disruption of its structure could accentuate the damaging effect caused by Arg47Gln.

The p.Arg47Gln mutation has been revealed at heterozygous state or in compound heterozygous with other mutations in four cases

Table 1
Comparative analysis of the clinical data and the disease evolution between the studied family and cases carrying the R47Q mutation reported in the bibliography.

Studied case	Age of onset	*ABCB4* gene mutation	Clinical data	Additional data	Treatment outcome and Evolution	Reference
The case under study	60 years	R47Q homozygous	LPAC syndrome at presentation	Ultrasound examination: intrahepatic hyperchoic foci	2 years after the LPAC diagnosis and without treatment, development of an intrahepatic cholangiocarcinoma	Our study
The patient's mother	–	R47Q heterozygous	Free of symptoms until the age of 78 years	–	–	
Case 1	44 years	R47Q/G319E Compound heterozygous	Complete LPAC syndrome at presentation Marked cholestasis during the two pregnancies	–	Free of symptoms under UDCA treatment during 4 years	[25]
Case 2 (case 1's sister)	37 years	R47Q heterozygous	LPAC syndrome	–	Free of symptoms under UDCA treatment during 4 years	
Case 3	24 years	R47Q/R406Q Compound heterozygous	Recurrent cholangitis	- Cholangiography: Large intrahepatic bile duct spindle-shaped dilatation - Histology: Mild portal inflammation	Improvement of liver biochemical tests and decrease of liver damage after 2 years of UDCA treatment then increase of cholangitis episodes and a partial hepatectomy has been proposed.	[10]
Case 4	23 years	R47Q/T82N Compound heterozygous	PFIC3 disease	- Histology: Severe fibrosis and incomplete cirrhosis - Immunohistochemical study: a reduced MDR3 expression	Resolution of clinical symptoms and disappearance of fibrosis after 9 years of UDCA treatment	[26]

Table 2
Characteristics of the reported cases presenting an association MDR3 deficiency- cholangiocarcinoma.

Case	Age at diagnosis	Clinical presentation	Outcome after the hepatectomy[a]	ABCB4 heterozygous mutation	MDR3 Familial history		Ref
1	54 years	- A Recurrent cholangitis associated with a CCa	Not available	c.1005 + 5G > A	–		[32]
2	53 years	- A perihilar CCa - Signs of cholangitis and ductopenia in the non-tumoral liver tissues	Under UDCA therapy, 13 months after the diagnosis, recurrence of the CCa and death of the patient	c.1469 T > C (p.I490T)	Patient's daughter	- Age at the onset: 14 years - Medical history: Cholecystectomy for symptomatic gallstone, Manifestation of jaundice and itching which resolved without treatment - Current state: At the age of 23 years, the patient is asymptomatic	[33]
3	55 years	- A Large perihilar CCa - Inflammatory biliary ducts and ductopenia in the non-tumoral liver tissues	- 10 months after the surgery: recurrence of the CCa - 31 months after the surgery: Death of the patient	c.2932T > C (p.S978P)	Patient's sister	- Age at the onset: 20 years - Medical history: Cholecystectomy for symptomatic gallstone, Development of an ICP, Detection of a common bile duct stenosis and intrahepatic gallstones - Current state: At the age of 38 years, the patient suffered from pruritus and signs of portal hypertension with elevated liver enzymes, typical symptoms of MDR3 deficiency	

CCa: Cholangiocarcinoma.
[a] The three affected patients were the subject of a hepatectomy for the ablation of the CCa.

reported previously in the literature and experienced different clinical phenotypes (Table 1). This variability has already been described in adults carrying *ABCB4* mutations [2,27–29]. In the explored cases with Arg47Gln, both the motif of presentation and the age of onset were different. At the molecular level, we supposed that the cumulative effect of both mutations in each patient determined the extent of the disruption the bile composition and influenced the severity of the phenotype as well as the age at onset. Interestingly, the heterozygous state of the Arg47Gln mutation was associated with the development of the LPAC syndrome for the case 2 (Table 1), while it had no effect on the studied patient's mother. Thus, as highlighted elsewhere, the MDR3 deficiency in adult subjects constituted only a genetic susceptibility factor for the development of hepatobiliary diseases [30]. For example, it has been shown that in the PBC (Primary Biliary Cholangitis) and ICC (Idiopathic Chronic Cholestasis) phenotypes, patients who had a defect in *ABCB4* gene manifested the disease earlier, compared to those without *ABCB4* mutations [9]. The implication of additional factors (Other liver disease, infection, diet, *etc.*), in addition to the genetic background, has been highlighted recently since the identification of heterozygous *ABCB4* mutations in pediatric subjects suffering from chronic cholestasis, commonly related to homozygous *ABCB4* mutations [31]. The hormonal status can also be considered as a modifier factor. The high prevalence of LPAC syndrome in female patients compared to male ones consolidated its implication [4].

Despite the implication of various factors in the development of hepatobiliary diseases, the *ABCB4* genotyping should be performed for all adult patients manifesting biliary symptoms. When the *ABCB4* mutation has been identified, the UDCA treatment should be initiated at once to ensure a regression of biliary symptoms and ovoid the development of putative complications. The CCa (Cholangiocarcinoma), considered as one of these complications, was discovered 2 years after the genetic testing in our studied patient. This manifestation is described in our study for the fourth time, after three cases described in 2012 and harboring the heterozygous c.1005 + 5G > A, c.1469 T > C (p.Ile490Thr) and c.2932T > C (p.Ser978Pro) *ABCB4* mutations, separately [2,3].These mutations were associated with the CCa after the age of 50 within the three patients, who were asymptomatic before this age. Interestingly, another phenotypic expression of the exonic mutations (p.Ile490Thr and p.Ser978Pro) was observed at the age < 20 years in the family's members of the patients with CCa (Table 2).This observation showed that the development of the CCa by patients with MDR3 deficiency is not related to the type of the carried mutation and its severity. It could be considered that the absence of the UDCA treatment for our patient or even its late administration (Table 2, case 2) enhanced the development of the liver disease to a CCa. This suggestion is consolidated by the study of Poupon which revealed that numerous adult patients harboring *ABCB4* mutations and under UDCA therapy have become asymptomatic [12]. Further, a long-term UDCA therapy allowed the disappearance of a fibrosis for a patient with Arg47Gln/Thr82Asn mutations in *ABCB4* [26]. Besides, various studies elucidated the existence of other genetic and environmental factors favoring the CCa appearance, in addition to the MDR3 deficiency. In fact, different polymorphisms in genes encoding for proteins responsible for the DNA repair, metabolism of carcinogens, inflammation or also biliary transporters have been considered as susceptibility factors to CCa [34–38]. In addition, diabetes, obesity, alcohol use, tobacco smoking, exposure to the thorotrast or genotoxins added to the chronic hepatitis B and C virus infection have been described as well-established risk factors for CCa development [39–42].

5. Conclusion

In conclusion, we reported here a Tunisian patient suffering from the LPAC syndrome with the homozygous c.140G > A mutation in *ABCB4* gene, leading to the substitution Arg47Gln in the N-terminal domain of MDR3 protein. Further, we elucidated the molecular mechanism behind this pathogenic character by both Q-PCR analysis and structural modeling, supported by data from the literature. Additionally, the clinical investigation allowed the description of the fourth case of CCa in patients with *ABCB4* mutations. Interestingly, we suggest that the delayed diagnosis of LPAC syndrome as well as the lack of the UDCA therapy represented two putative factors which play a role in the CCa development in patients suffering from MDR3 deficiency.

Funding

This work was supported by the Ministry of Higher Education and Scientific Research in Tunisia.

Declaration of Competing Interest

None.

Acknowledgments

We would like to thank the patient's family for their participation.

We are also grateful to the staff of both Anatomo-Pathology and the general surgery Departments in the Habib-Bourguiba hospital (Tunisia) as well as the medical biology and pathology department of the St Antoine hospital (Paris, France) for their collaboration.

We owe special thanks to Mr. Wassim Hariz, founder and former head of the English Unit and Editorial Board at the Sfax Faculty of sciences, for having proofread this paper.

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