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Phenotypic variability in Tunisian PFIC3 patients harboring a complex genotype with a differential clinical outcome of UDCA treatment

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ABSTRACT

Introduction: Progressive familial intrahepatic cholestasis type 3 (PFIC3) is a chronic autosomal recessive disorder characterized by a wide spectrum of clinical severity generally related to the degree of pathogenicity of the causal sequence variation in *ABCB*4 gene.

Patients and methods: The present study reports the molecular investigation by Next Generation Sequencing (NGS) of five related patients with PFIC3 disease followed by bioinformatic analysis. A biochemical follow-up is also performed to assess the response of the ursodeoxycholic acid treatment.

Results: The molecular results revealed complex genotype in homozygous state in all patients including a pathogenic c.1436C > T (P479L) variation in the *ABCB4* gene and two well-known polymorphisms, the V444A in *ABCB11* gene and the D19H in the *ABCG8* gene. Although the presence of the same genetic background, all patients present the disease at different ages and clinical signs with a variable degree of clinical severity at diagnosis. Additionally, a differential outcome to the treatment has been pointed out.

Conclusion: Our results provide evidence regarding the putative intervention of modifier factors in the phenotypic variability reported for the first time in the PFIC3 disease and highlight the importance of an early administration of the UDCA as a good solution to ovoid the disease progression.

1. Introduction

Progressive familial intrahepatic cholestasis type 3 (PFIC3; OMIM # 602347) is an autosomal recessive chronic disorder characterized by the manifestation of a jaundice associated with a moderate pruritus in addition to hepatomegaly and splenomegaly with an age of onset ranging from infancy to childhood (20 years) [1]. It is caused by alterations in the MDR3 protein; a hepatocellular transporter of phosphatidylcholine (PC) from hepatocytes into the bile [2]. This biliary PC is incorporated into bile salts micelles to form mixed micelles, thereby neutralizing the toxicity of bile salts and ensuring the solubilization of cholesterol [3].The MDR3 deficiency in PFIC3 is responsible for an absence or a low level of PC in bile, thus promoting the detergent activity of bile salts and enhancing the risk of cholesterol crystallization. These consequences could explain the development of a ductular proliferation and the high GGT serum activity, two prominent features of PFIC3 disease [4].

PFIC3 disease is mainly caused by sequence variations in *ABCB4* gene located on chromosome 7p21 and encoding the MDR3 protein [5]. Interestingly, a mild MDR3 deficiency caused by heterozygous variations in this gene could predispose to a spectrum of liver diseases with less severity as compared to the PFIC3 [6].

Up to now, several mutations have been described either at homozygous or compound heterozygous states mostly in the European population [7] [8].However, the African population is poorly explored with only two described sequence variations in *ABCB4* gene in the North of Africa including the p.R595X in exon 15 and the c.937_992 in/del in exon 9 as well as several polymorphisms [9] [10].

The Ursodeoxycholic Acid (UDCA) was proposed as the initial treatment of the PFIC3 disease which modulates the biliary bile composition, stimulates the hepatobiliary secretion and protects against the apoptosis [11]. Nevertheless, the improvement of clinical and biochemical states was observed in only 60% of UDCA treated PFIC3 patients while the rest of patients were classified as partial or non-responders and required liver transplantation [12].

In the present study, molecular analysis through the Next

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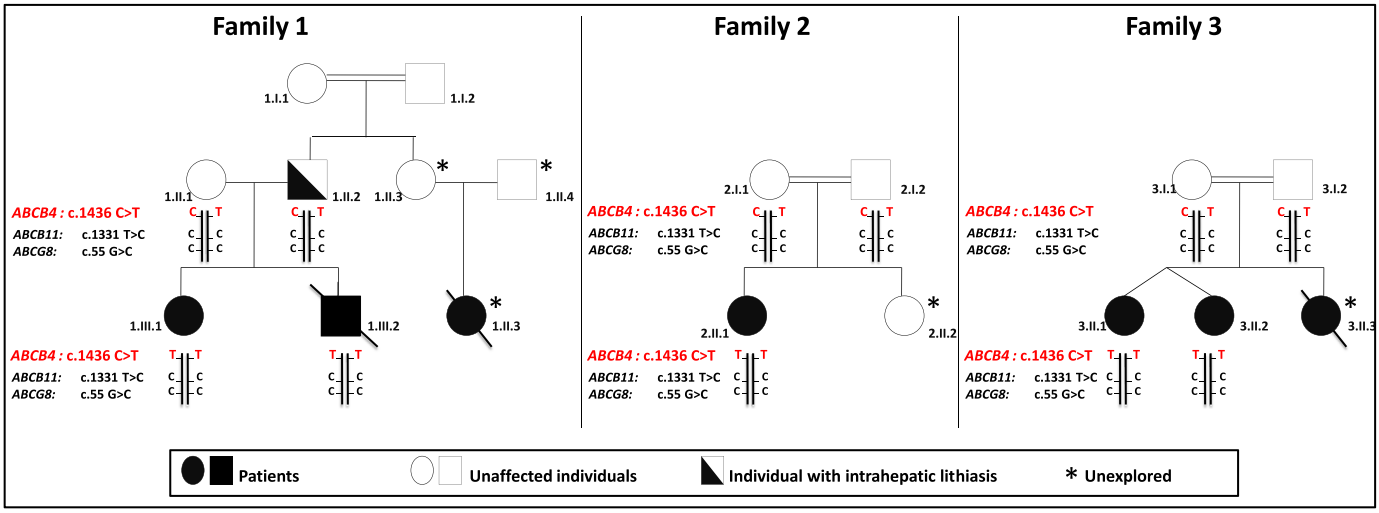

Fig. 1. Pedigree of the three related sub families with PFIC3 disease. Asterisks indicate the patients who died before the current study. The numbers under the individuals represent identification numbers for each individual.

Generation Sequencing (NGS) and a biochemical follow-up during the UDCA treatment were performed in a large consanguineous Tunisian family with PFIC3·The molecular results revealed the P479L variant in the *ABCB4* gene associated to the V444A and D19H variants in *BSEP* and *ABCG8* genes respectively in all patients at homozygous state. Besides, a differential outcome to UDCA therapy and an intra-familial phenotypic variability was described for the first time within the PFIC3 disease.

2. Patients and methods

2.1. Patients

The study enrolled five Tunisian patients from three sub families belonging to a large consanguineous Tunisian family with PFIC3 disease (Fig. 1). All the affected children were followed in the pediatric service of the Taher Sfar Hospital in Mahdiya-Tunisia. The clinical mode of presentation and the biochemical features at first examination were in favor of a PFIC3 disease. The absence of other known causes of cholestatic disease verified by serological tests and liver ultrasonography in addition to the detection of ductular proliferation in the majority of patients' livers confirmed this diagnosis. Clinical presentation, biochemical data and histological features of the liver biopsy were summarized in Table 1.

In family 1, Siblings 1.III.1 and 1.III.2, who were asymptomatic until the age of 7 years, then developed jaundice and splenomegaly as first symptoms. Nevertheless, a totally conserved liver function (PT = 100%) was observed in the patient 1.III.1 while her brother manifested a middle liver failure (PT = 55%). Their father (1.II.2) had intrahepatic cholelithiasis, whereas no hepatic abnormalities were noticed in their mother.

In family 2, an early manifestation of clinical symptoms of PFIC3 disease has been developed by the patient 1.II.1 with a correct liver function (PT = 97%).

Family 3 included three affected siblings. Both patients 3.II.1 and 3.II.2 presented the same clinical features at the age of 1.5 years associated with normal values of GGT but were retained as PFIC3patients based on the family history of this disease. Their sister, 3.II.3 manifested first symptoms later but with a more severe liver disease which progressed rapidly to the total liver failure and death.

2.2. Methods

The clinical follow-up was assessed by regular measurements of GGT and alkaline phosphatase (ALP) known as cholestasis markers and the Prothrombin rate (PT),used as an indicator of the liver function.

For the molecular characterization, all affected subjects and their parents underwent a genomic DNA isolation from peripheral blood leukocytes using the previously described phenol-chloroform protocol [13].

2.2.1. Molecular exploration of the patient III.1 of family 1 byNext-generation-sequencing (NGS)

DNA sample from 1.III.1 was processed for NGS. NGS 60-Kbcapture-based enrichment was performed on genomic DNA. One microgram of DNA was fragmented with a Bioruptor (Diagenode, Seraing, Belgium). After the DNA 3′ end repair, adenylation, and ligation with the NEXTflex™ DNA barcodes (Bioo Scientific, TX, Austin, USA) for 48 samples, a double size selection (250-450b) was performed using a KAPA Library Preparation Kit for Illumina platforms (KAPA Biosystems, London, UK) resulting in 48 patients' libraries.

24 libraries were multiplexed into a single micro-tube before hybridization to the custom60-Kb capture probes “BiligeneVB” (Roche NimbleGen). The 60-Kb panel includes 154 exons and an exon-intron junction of at least 100 b. Hybridization was performed overnight at 47 °C. Two successive captures ran. After successive stringent washing on pure capture beads (Roche NimbleGen), captures were quantified using the Qubit-2 fluorimeter and qualified with the BioanalyserMultiNA before being paired-end sequenced (2x150b) on the MiSeq® sequencer with a Micro Flow cell 300 V2. After 19 h of sequencing, the FASTQ files were uploaded on Sophia DDM® platform (v3.1.5). Quality and variant analyses were performed automatically by the algorithms of SOPHiA. Systematically, the variants of interest identified were confirmed using Sanger methodology performed on the ABI 3500DX/XL sequencer. Each identified variant by DDM Sophia was analyzed by various *in silico predictive* programs included in the used system.

2.2.2. Additional in silico assessment of the identified variants

The evolutionary conservation of the altered amino-acid sites was performed by ConSurf server. In the output, a score of conservation ranging from 1 (variable) to 9 (very conserved) was attributed to each position based on an established alignment assured by this server [14]. Additionally, the deleterious effect was assessed through three *in silico*