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The clinical course of interstitial lung disease in an adult patient with an ABCA3 homozygous complex allele under hydroxychloroquine and a review of the literature

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Abstract. The gene mutations responsible for ABCA3 protein deficiency are involved in respiratory distress of the newborn and much more rarely in adult interstitial lung diseases (ILD). An adult patient homozygous for a complex allele encompassing the p.Ala1027Pro likely pathogenic mutation and the p.Gly974Asp variation was followed for a late-onset and fibrotic ILD. The evolution was marked by progressive clinical and functional degradation despite corticosteroid pulses. The patient, who was first registered on the list for lung transplantation, was improved quickly and persistently for at least 6.5 years with hydroxychloroquine treatment, allowing removal from the transplant list.

Key words: ILD, ABCA3, Hydroxychloroquine

Introduction

The ATP Binding Cassette subfamily A member 3 (ABCA3) is a transmembrane transporter protein member of the ATP-Binding Cassette family that allows the transport of phospholipids and hydrophobic surfactant proteins (SP)-B and SP-C into the lamellar bodies(1). The gene mutations responsible for ABCA3 protein deficiency are usually expressed in an autosomal recessive manner and are involved in respiratory distress of the newborn and more rarely in adult interstitial lung diseases (ILD)(2,3).

To our knowledge, the efficacy of hydroxychloroquine has never been described in the treatment of adult interstitial lung disease (ILD) linked to ABCA3 deficiency.

Case Report

A 35-year-old female never-smoker was followed for a fibrotic ILD.

She was born at term, weighing 1.7 kg. Her main medical history was scoliosis, operated on at the age of 15 by right thoracotomy. She presented no respiratory distress or respiratory event from childhood until she was 35 years old. There was no environmental or occupational exposure. She presented no argument for a Connective Tissue Disease.

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Respiratory symptoms began progressively at age 35 with exertion dyspnea (stage 1 mMRC). She had bilateral crackles and a BMI of 17 kg/m2. Chest x-ray (shown in Fig. 1a) and thoracic CT (shown in Fig. 1c) showed an ILD with a predominance of mosaic ground glass opacities associated with apical consolidations (suggestive of pleuropulmonary fibroelastosis) and rare honeycomb changes. Forced vital capacity (FVC) was at 1.38 L (47% of predicted values, shown in Fig. 1e), Total Lung capacity 2.42 liters (56%) and DLCo 3.16 mL/min/mmHg (15%). The patient did not undergo a lung biopsy due to respiratory failure. After the patient's consent and at the age of 40, a molecular genetics analysis identified a homozygous complex allele carrying the p.Ala1027Pro (c.3079G>C) likely pathogenic mutation, and the p.Gly974Asp (c.2921_2922delinsAC) variation of unknown significance. This was found to result from 10.5-Mb uniparental disomy of chromosome 16. Both variations were carried in the heterozygous state by the asymptomatic mother (shown in Fig. 2), demonstrating the maternal origin of the disomy found in the patient.

The evolution was marked by a clinical (dyspnea stage 4 mMRC) and functional progressive degradation (Fig. 1e). At the age of 40 (60 months after ILD diagnosis), she received six 3-day monthly intravenous corticosteroid pulses at a dose of 1 g/day and oral corticosteroids at a dose of 1 mg/kg/d, allowing a clinical and functional (FVC) transient improvement (shown in Fig. 1e). Despite this treatment, the patient presented 4 exacerbations requiring repeated hospitalizations and no improvement in radiological alveolar opacities in 6 months, which led to registering her on a lung transplantation list. Considering the low benefit of the corticosteroid pulses and to limit the complications of daily systemic corticosteroid therapy given the lung transplant project, it was stopped after 9 months. A hydroxychloroquine treatment was then started as monotherapy at a dose of 400 mg per day. Clinical and functional improvements were observed within the first 3 months of treatment (72 months after ILD diagnosis). Chest X-ray (shown in Fig. 1b) and thoracic CT (shown in Fig. 1d) showed a drastic decrease in the interstitial opacities. After 6.5 years of hydroxychloroquine treatment, the patient improved dyspnea (3 mMRC stage) and FVC at 1.32 L (45% of predicted values and a gain of 200 ml compared to the time of the introduction of hydroxychloroquine, shown in Fig. 1e). The patient presented no exacerbation, and she was removed from the transplantation list. No hydroxychloroquine side effect was noticed.

Discussion

To our knowledge, the present report is the first case of hydroxychloroquine's efficacy in treating ILD in an adult patient with two mutated *ABCA3* alleles. Hydroxychloroquine resulted in the postponement of the lung transplant project.

Marked variability of clinical and radiological patterns is described in ABCA3 deficiency, ranging from fatal neonatal respiratory distress syndrome to ILD with slowly progressive lung fibrosis (4). The pathophysiological mechanisms explaining the occurrence of ILD in patients with homozygous *ABCA3* mutations are insufficiently known. The various mutations of *ABCA3* result in different functional effects on type 2 epithelial cells' homeostasis(5,6). It has been shown that some *ABCA3* mutations affect the traffic or the folding of ABCA3 and lead to partial or complete retention of mutant ABCA3 in the endoplasmic reticulum (ER) compartment inducing an ER stress, type II AEC apoptosis, and autophagy(5,6). Other mutations can lead to a functional defect of ABCA3 but with no localization abnormalities and have no effect on intracellular stress and apoptotic signaling(5). In the homozygous allele we identified, the p.Gly974Asp and p.Ala1027Pro missense variations target amino acids from the second extracellular domain of the transporter (fourth extracellular loop). Both variations are not described in the gnomAD general population database, and the corresponding amino acids are not fully conserved in vertebrates. The p.Gly974Asp variation is not prone to alter splicing. It has been classified as a variant of unknown significance. By contrast, the p.Ala1027Pro change is prone to disrupt the secondary structure of the protein as it introduces a proline residue within a beta-sheet (shown in Fig. 3). In addition, this mutation creates an acceptor splice site in exon 22 that could lead to a proportion of irrelevant transcripts. The new splice site lies 11bp downstream of the mutation; it is weaker than the usual site of intron 21 (MaxEntScan score: 6.17 vs. 9.92), and the SpliceAI tool predicts that this alternative acceptor site has a 38% probability of being used for splicing. The p.Ala1027Pro mutation was previously identified in two pediatric patients in trans of two distinct

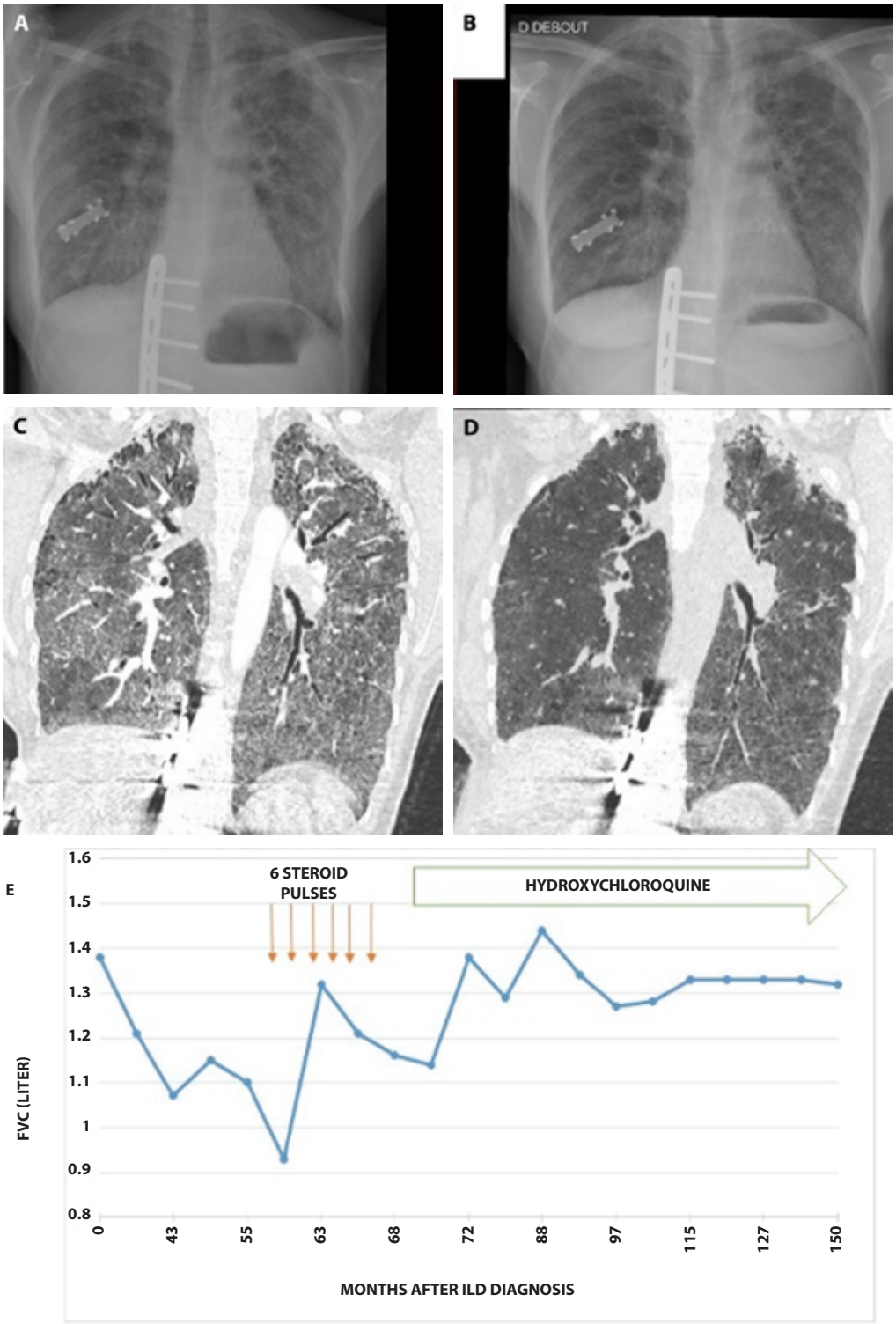

Figure 1. Comparison of thoracic radiographs between the 60th (Fig. 1a) and 72nd month after ILD diagnosis (Fig. 1b). Comparison of thoracic CT in parenchymal window between the 58th (Fig. 1c) and 72th month after ILD diagnosis (Fig. 1d). Evolution before and after initiation of treatment by hydroxychloroquine of FVC (liter, Y axis) in the patient with two *ABCA3* mutated alleles during the 150 months after the ILD diagnosis (months, X axis) (Fig. 1e).

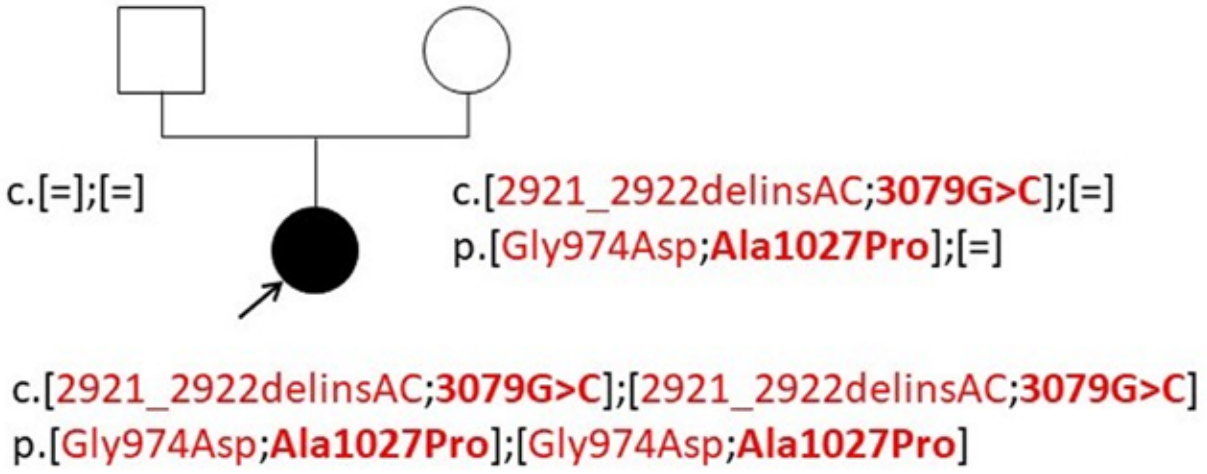

Figure 2. The pedigree of the family illustrates the homozygosity of the proband (arrow) for a complex *ABCA3* allele inherited from her asymptomatic heterozygous mother. A partial 10.5Mb maternal isodisomy of chromosome 16 in the proband led to her bi-allelic genotype for the mutation that is not carried by the father.

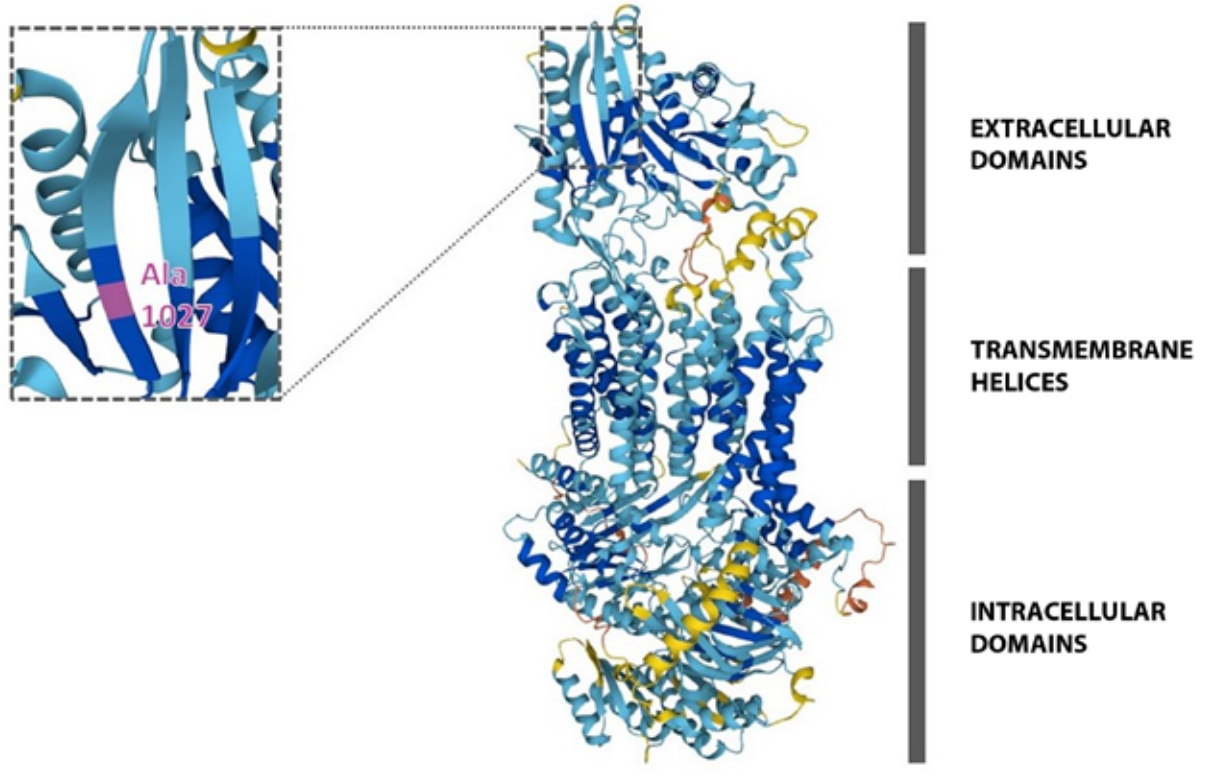

Figure 3. ABCA3 transporter protein scheme. The replacement of alanine 1027, located within a beta-sheet (inset), by a proline, is predicted to disrupt the secondary structure of the protein. Protein structure is predicted by AlphaFold (AF-Q99758-F1). Dark blue and light blue chains respectively show very high confidence and confident levels of structure prediction.

ABCA3 missense mutations, but with no functional tests ((7) and unpublished data from M.L.).

The onset of the disease occurred in adulthood in our patient, as already shown in several others (7–10) (table 1). ILD due to ABCA3 mutations shows variable expressivity that mainly consists of different ages at diagnosis and prognosis, depending on the severity of the mutation(s). Children who carry two severe

Table 1. Cases of patients with bi-allelic ABCA3 pathogenic who have been diagnosed in infancy and survived beyond puberty, or who have been diagnosed when adults

Age at presentation (years)	Follow-up age (years)	Allele 1	Allele 2	Reference
Birth	**21**	**p.E292V**	**p.N1076K**	(16)
33	**36**	**p.R709W**	**p.I1193M**	(7)
41	**43**	**p.E292V**	**p.Ser1028Valfs*103**	(8)
52 (two brothers)	**57**	**p.G964D**	**p.G964D**	(9)
52	**?**	**p.G964D**	**p.G964D**	(9)
3	**29**	**p.G964S**	**p. R1482W**	(4)
18	**25**	**p.R280C**	**p.E292V**	(10)
61	**61**	**p.E292V**	**p.R1484P**	(10)
77	**79**	**p.G559R**	**p.T1582S**	(10)
Birth	**39**	**p.R43C**	**p.F1203del**	(15)
Birth	**adult**	**p.R43C**	**p.F1203del**	(15)

mutations (i.e., leading to a premature stop codon) die shortly after birth or within the first year. On the other hand, those who carry a milder mutation in one or both allele(s) can survive later in life, some showing the first signs of the disease in adults. Subjects with identical genotypes show similar disease severity. One of these milder mutations is p.E292V which has been described in four adult patients (table 1). It has indeed been shown to be a hypomorphic mutation with a functional deficiency demonstrated *in vitro*(11)), leading to less severe phenotypes when present in one of the alleles of the patients.

There is virtually no specific treatment for patients with ILD due to *ABCA3* mutations. Several pediatric studies reported improvement of the ground glass opacities with the use of corticosteroids and azithromycin, but with transient efficacy(12). Rare cases of a child with *ABCA3* mutations treated with hydroxychloroquine for a prolonged period had a stable respiratory function(3,4). Hydroxychloroquine may have interesting immunomodulatory properties that could explain the benefit observed in our study. It inhibits autophagy as it raises the lysosomal pH, which leads to inhibition of both fusion of the autophagosome with lysosome(13) and inhibition of chemotaxis, phagocytosis, and antigen presentation(14).

Conclusion

To conclude, this is the first case of adulthood ILD with probable *ABCA3* mutation where we showed the effectiveness of hydroxychloroquine. This needs to be confirmed in studies involving a larger number of patients.

Statement of Ethics: In accordance with French law, informed consent and the agreement of an Ethical Review Board are not essential for a retrospective study of data collection corresponding to current practice. The patient has given consent for the molecular study. Written informed consent was obtained from the patient to publish this case report and any accompanying images.

Author Contributions: All authors undertook the study. SMA, MF, ML and NN accessed and verified the data. All authors participated in the development and finalization of the manuscript. All authors had full access to all the data in the study. SMA, ML and MF had final responsibility for the decision to submit for publication.

Data Availability Statement: We will make anonymized individual participant data available to the scientific community with as few restrictions as feasible while retaining exclusive use until the publication of major outcomes. Data requests from qualified researchers should be submitted to SMA (s.marchandadam@univ-tours.fr) for consideration.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Links

gnomAD	https://gnomad.broadinstitute.org/
MaxEntScan	http://hollywood.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
SpliceAI	https://github.com/Illumina/SpliceAI

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