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Liver disease related to alpha1-antitrypsin deficiency in French children: The DEFI-ALPHA cohort

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Abstract

Background & Aims: To identify prognostic factors for liver disease in children with alpha-1 antitrypsin deficiency, irrespective of phenotype, using the DEFI-ALPHA cohort.

Methods: Retrospective, then prospective from 2010, multicentre study including children known to have alpha-1 antitrypsin blood concentration below 0.8 g/L, born in France since 1989. Clinical and biological data were collected. Liver disease was

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Abbreviations: AAT, alpha1-antitrypsin; ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase; IUGR, intrauterine growth retardation; PHT, portal hypertension; UDCA, ursodeoxycholic acid.

Clinical Trial Registry: ClinicalTrials.gov: Polygen Defi-Alpha: Genetic Polymorphisms Study in Children With Alpha-1 Antitrypsin Deficiency, included in the DEFI-ALPHA Cohort. NCT01862211.

classified as "severe" (portal hypertension, liver failure, liver transplantation or death); "moderate" (persistent abnormal liver biology without portal hypertension); and "mild/none" (normal or almost normal liver biology and native liver). Prognostic factors for severe liver disease were evaluated using a Cox semiparametric model.

Results: In January 2017, 153 patients from 19 centres had been included; genotypes were PIZZ in 81.9%, PISZ in 8.1%, other in 10.0%. Mean ± SD follow-up was 4.7 ± 2.1 years. Half of patients had moderate liver disease. Twenty-eight children (18.3%) had severe liver disease (mean age 2.5 years, range: 0-11.6): diagnosis of alpha-1 antitrypsin deficiency was made before two months of age in 65.4%, genotypes were PIZZ in 25 (89.3%), PISZ in 2, $PIM_{like}Z$ in 1, 15 children underwent liver transplantation, 1 child died at 3 years of age. Neonatal cholestasis was significantly associated with severe liver disease ($P = 0.007$).

Conclusion: Alpha-1 antitrypsin-deficient patients presenting with neonatal cholestasis were likely to develop severe liver disease. Some patients with non-homozygous ZZ genotype can develop severe liver disease, such as PISZ and M variants, when associated with predisposing factors. Further genetic studies will help to identify other factors involved in the development of liver complications.

KEYWORDS

cirrhosis, liver enzyme, liver transplantation, metabolic liver disease

1 | INTRODUCTION

Alpha1-antitrypsin (AAT) deficiency is a genetic disease that predisposes to lung and liver damage. Prevalence is approximately 1/2500 in Europe and 1/6000 in France.[1,2] In subjects with the wild-type MM genotype, blood AAT concentration is approximately 1.5 g/L. Severely deficient AAT concentrations are mostly observed in ZZ homozygous patients[3,4] in whom this deficiency leads to emphysema; AAT is a protease inhibitor protecting the lungs against elastase secreted by neutrophils during inflammation. Some mutations prevent normal protein folding that leads to liver damage owing to aggregation of misfolded AAT proteins within the endoplasmic reticulum of hepatocytes.[4] The severity of the liver disease ranges from transient neonatal cholestasis to cirrhosis and liver transplantation (LT) in childhood.[6] An association between the severity of liver disease and phenotype has been reported, the most severe being with homozygous ZZ patients.[7] However, not every patient with the PIZZ genotype develops severe liver disease, probably due to genetic modifiers. Conversely, other genotypes could be responsible for liver disease.[8]

Adult and paediatric cohorts have been studied for factors responsible for liver disease,[9,10] including two large paediatric cohorts. Sveger identified patients through an extensive neonatal screening programme,[9] and Teckman et al[15] investigated the factors associated with liver disease in ZZ and SZ patients. We reported herein an interim analysis of the French paediatric cohort DEFI-ALPHA (DEFIciency ALPHA1-antitrypsin),[16] a longitudinal study that includes patients with AAT deficiency, irrespective of phenotype, which aimed to identify prognostic factors for liver disease.

Key points

- Two thirds of children known to have AAT deficiency in France have some degree of liver disease; it was severe with portal hypertension in 18% of this French cohort.
- Neonatal cholestasis should lead to investigate AAT deficiency because of its association with severe liver disease.
- Liver disease can also be found in patients with genotypes other than PIZZ, especially when associated with other predisposing factors.

2 | PATIENTS AND METHODS

2.1 | Patients

All patients born after 1989 and known to have an AAT blood concentration below 0.8 g/L, irrespective of AAT phenotype, could be included during his/her follow-up in one of the 28 participating French paediatric hepatology units. Children diagnosed for AAT deficiency came to medical attention through evocative anamnesis or clinical arguments. The only exclusion criterion was refusal to participate. Inclusion began in October 2010 (initially retrospectively, then prospectively) and is still ongoing. Patients included up to December 2016 were considered in the present report.

TABLE 1 Characteristics of the studied population

	n = 153
Age at diagnosis, years (SD)	1.8 (3)
Before 2 mo, n (%)[a]	74 (52.5)
Before 1 y, n (%)[a]	92 (65.2)
Missing data	12
Age on December 2016, years	13.2 (6.4)
Gender, n (%)	
Girl	55 (35.9)
Boy	98 (64.1)
ALT or GGT ≥ 2N, n (%)[a]	99 (73.3)
Missing data	18
UDCA therapy, n (%)[a]	94 (69.1)
Missing data	17
IUGR, n (%)[a]	22 (20.0)
Missing data	43
Breastfeeding, n (%)[a]	33 (45.8)
Missing data	81
Mode of diagnosis, n (%)[a]	
Neonatal cholestasis	74 (52.5)
Abnormal liver function	34 (24.1)
Familial screening	24 (17.0)
Other	9 (6.4)
Missing data	12
AAT level, g/L (SD)	0.4 (0.2)
Genotypes, n (%)[a]	
PIZZ	122 (81.9)
PISZ	12 (8.1)
PIMZ	7 (4.7)
PISS	3 (2.0)
PIFZ	1
PIZ/D256V	1
$PIM_{like}Z$	1
$PIMM_{malton}$	1
$PIM_{malton}Z$	1
Missing	4

AAT, alpha1-antitrypsin; ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase; IUGR, intrauterine growth retardation; UDCA, ursodeoxycholic acid.
[a]Among those with available data.

2.2 | Data collection

Clinical, biological and morphological data were collected using case report forms, at inclusion and at each scheduled visit. They were anonymously computerised in accordance with the data protection laws in France. Medsharing SA (Fontenay-Sous-Bois, France) carried out the data management.

The recorded data were as follows: gender, presence of intrauterine growth retardation (IUGR), neonatal breastfeeding, age and context of diagnosis, genotype, ursodeoxycholic acid (UDCA) therapy, history of familial liver disease, clinical, biological and imaging data and death. Liver biopsy was not systematically performed and not reported in this study.

Patients were categorised according to the estimated grade of liver disease in December 2016. Severe liver disease was defined by portal hypertension (PHT), evidenced by platelets below 150 000/mm^3 on two occasions, or Doppler ultrasound (portosystemic derivations, decreased or reverse portal flow, increased small omentum/aorta ratio), or the presence of oesophageal varices, liver failure (hepatic encephalopathy, or prothrombin time or factor V less than 50%), LT or liver-related death. Moderate liver disease was defined by persistent abnormal liver enzymes (alanine aminotransferase—ALT or gamma-glutamyltranspeptidase—GGT, more than twice the upper limit of normal) without PHT. Mild or no liver disease was defined by the absence of PHT or liver failure, and mildly abnormal or normal liver enzymes (ALT and GGT less than twice the upper limit of normal).

2.3 | Ethics and regulatory aspects

The study received approval from the local institutional review board (*Comité de protection des personnes*, 2 October 2008) and the national data protection committee (DR-2010-328, 29 October 2010). Written informed consent was obtained from parents or adult patients.

2.4 | Statistical analysis

Qualitative variables were expressed as number (n) and percentage, quantitative variables as mean ± standard deviation (SD). The normality of distribution was verified with the Kolmogorov-Smirnov test, graphically checked with a histogram. Categorical variables were compared with the chi-square or Fisher's exact test as appropriate; quantitative variables were compared between groups with the Student t test, Wilcoxon non-parametric test or Kruskal-Wallis test as appropriate.

Severe liver disease-free survival curves were obtained using a Kaplan-Meier model and compared between the groups using the log-rank test. Severe liver disease-free survival was defined as the time from AAT deficiency diagnosis to severe liver disease, or last known status.

Prognostic factors for severe liver disease-free survival were evaluated using a Cox semiparametric model, after verification of the proportional hazard hypothesis, first as a univariate analysis, then as multivariate analysis, including significant factors from the univariate analysis and the relevant adjustment variables such as age and sex. Logistic regression was carried out in order to determine risk factors for moderate liver disease, first as a univariate analysis, then as multivariate analysis, including significant factors from the univariate analysis and the relevant adjustment variables such as age and sex.

The statistical tests were bilateral, and the level of significance was set to 5% ($P < 0.05$). Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC, USA).

TABLE 2 Clinical forms of the liver disease

	Severe[a](n = 28)	Moderate[b](n = 75)	Mild or none[c] (n = 39)
Gender, n (%)			
Girl	7 (25.0)	29 (38.7)	15 (38.5)
Boy	21 (75.0)	46 (61.3)	24 (61.5)
Mean age at diagnosis, years	1.2 (2.5)	0.9 (1.9)	3.9 (4.2)
<2 mo, n (%)[d]	17 (65.4)	47 (62.3)	8 (22.2)
<1 y, n (%)[d]	21 (80.8)	58 (77.3)	10 (27.8)
Missing data	2	0	3
Neonatal cholestasis, n (%)[d]	21 (80.8)	42 (56.0)	8 (23.5)
Missing data	2	0	2
AAT, g/L	0.4 (0.2)	0.4 (0.1)	0.5 (0.2)
UDCA, %[d]	24 (96.0)	56 (78.9)	13 (35.1)
Missing data	3	4	2
Genotype, n (%)[d]			
PIZZ	25 (89.3)	69 (93.2)	20 (55.6)
PISZ	2 (7.1)	4 (5.4)	5 (13.9)
PIMZ	0	0	6 (16.7)
PISS	0	0	3 (8.3)
$PIM_{like}Z$	1 (3.6)	0	0
$PIMM_{malton}$	0	1 (1.4)	0
$PIM_{malton}Z$	0	0	1 (2.8)
PIZ/D256V	0	0	1 (2.8)
Missing data	0	1	3
AAT within genotypes (g/L)			
PIZZ	0.4 (0.2)	0.3 (0.1)	0.3 (0.1)
PISZ	0.7 (0.2)	0.6 (0.1)	0.7 (0.1)
PIMZ			0.6 (0.1)

AAT, alpha1-antitrypsin; UDCA, ursodeoxycholic acid.
Values are means (SD), unless indicated as percentages.
[a]Severe liver disease: portal hypertension, liver failure, liver transplantation or death.
[b]Moderate liver disease: significant perturbation of liver biology (ALT and/or GGT > 2N) without criteria of severe liver disease.
[c]Mild or no liver disease: no criteria of severe liver disease, ALT and GGT < 2N.
[d]Among those with available data.

3 | RESULTS

3.1 | Demographic data

Between October 2010 and December 2016, 153 patients were included (Table 1) by 19 of the 28 centres. The mean ± SD duration of follow-up from inclusion to December 2016 was 4.7 ± 2.1 years.

3.2 | Liver disease

According to the definitions used herein, 28 patients (18.3%) had severe liver disease (Table 2); all had PHT, which was diagnosed at a mean 2.6 years of age (range: 0-11.6). One patient who also had Joubert syndrome died at the age of 3 years from ascites infection. None had liver failure before transplantation. Fifteen patients underwent a LT (53.6%), 11 boys and four girls, at a mean ± SD age of 6.8 ± 4.7 years. The indications for LT were severe PHT or worsening of cholestasis. The genotype of the transplanted patients was PIZZ in 13 children, PISZ in 1 and $PIM_{like}Z$ in 1. The child with PHT and $PIM_{like}Z$ was also diagnosed with cystic fibrosis. Twelve children (42.9%) had PHT but did not yet need LT. The mean ± SD age at diagnosis of PHT was 4.1 ± 3.8 years. The genotype was PIZZ in 11 cases (91.7%) and PISZ in 1 case. The patient with PISZ also had a type III glycogen storage disease.

Moderate liver disease was found in 75 patients (49.0%, Table 2). The mode of diagnosis was a neonatal cholestasis in 42 patients (56.0%), abnormal concentration of liver enzymes in 22 (29.3%) and familial screening in 10 patients (13.3%). One of these patients with $PIMM_{malton}$ genotype was 3 years of age at last follow-up

TABLE 3 Comparison of liver parameters (ALT and GGT) by age and gender

ALT	ALAT < 2N N = 73	ALAT ≥ 2N N = 53	*P*-value
Gender, n (%)			
Girl	27 (25.0)	16 (38.7)	0.43
Boy	46 (75.0)	37 (61.3)	
Age at diagnosis			
0-2 mo, n (%)[a]	41 (56.9)	23 (43.4)	0.19
2 mo - 4 y, n (%)[a]	19 (26.4)	22 (41.5)	
>4 y, n (%)[a]	12 (16.7)	98 (15.1)	
Missing data	1	0	
GGT	**GGT < 2N N = 41**	**GGT > 2N N = 73**	***P*-value**
Gender, n (%)			
Girl	13 (31.7)	25 (34.3)	0.78
Boy	28 (68.3)	48 (65.7)	
Age at diagnosis			
0-2 mo, n (%)[a]	7 (17.5)	57 (78.1)	<0.0001
2 mo-4 y, n (%)[a]	18 (45.0)	15 (20.6)	
>4 y, n (%)[a]	15 (37.5)	1 (1.4)	
Missing data	1	0	

ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase.
[a]Among those with available data.

and had moderately elevated liver enzymes but an abnormal liver elastography.

The last group of 39 patients had a mild or no liver disease (25.5%, Table 2).

In 10 of the 153 patients (6.5%), included through a familial screening from an index case, no liver function tests were available at the time of diagnosis, and they were not classified in any of the three groups. None of them had severe liver disease at last follow-up.

Liver parameters (ALT, GGT) were not significantly different according to gender. ALT was not significantly different according to age, whereas GGT was greater when the diagnosis was made before the age of 2 months (Table 3). Both median ALT and GGT were significantly higher with the severity of the disease; $P < 0.0001$ (Figure 1). Median ALT was 118 UI/L (range: 39-1340) in severe liver disease, 74 UI/L (range 14-284) in moderate liver disease and 54 UI/L (range 11-78) in mild/no liver disease. Median GGT was 477 UI/L (range: 29-1380) in severe liver disease, 247 UI/L (range 11-2085) in moderate liver disease and 18 UI/L (range 9-74) in mild/no liver disease.

3.3 | Mode of diagnosis

The context of diagnosis was recorded for 141 patients (Table 4). A total of 74 (52.5%, 49 boys) presented with neonatal cholestasis, and among these, 68 of 73 (one missing data for genotype) (93.2%) had the PIZZ genotype, 21 of 68 (30.9%) progressed to severe liver disease, 12 (57.1% of the severe) were transplanted, and 41/68 (60.3%) had moderate liver disease (6 missing data for severity of liver disease). Workup for abnormal liver tests led to the diagnosis in 34 patients (24.1%). Among these, 25 (73.5%) had the PIZZ genotype and 7 (20.6%) the PISZ genotype, 2 (5.9%) developed severe liver disease and 22 (64.7%) had moderate liver disease at last follow-up. Twenty-four asymptomatic patients (17.0%) were identified through screening from the families of 21 index cases. Among these, 18 of 23 (78.3%) had the PIZZ genotype and 2 of 23 (8.7%) the PISZ genotype (1 missing data for genotype), 1 of 21 (4.8%) progressed to severe liver disease, and 10 of 21 (47.6%) had moderate liver disease (3 missing data for severity of liver disease). The other modes of diagnosis were hepatomegaly (n = 2), abdominal pain (n = 1), respiratory symptom (n = 4), fortuitously (n = 2) or not determined (n = 12).

3.4 | Role of genotype

3.4.1 | PIZZ and PISZ genotypes

The genotype was recorded for 149 patients. A total of 122 (81.9%) had the PIZZ genotype (77 boys). Among these, 68 of 112 (60.7%) were diagnosed during the workup for neonatal cholestasis, 25 of 112 (22.3%) with abnormal liver enzymes, and 18 of 112 (16.1%) through familial screening; in one case (0.9%), the presenting symptom was hepatomegaly, and the context of diagnosis was unknown for the remaining 10 patients. The mean ± SD AAT concentration of these patients was 0.4 ± 0.1 g/L. Severe liver disease developed in 25 of 110 patients (22.7%), and 13 were transplanted. Moderate liver disease was found in 69 of 110 patients (62.7%), and a mild or no liver disease in 16 of 110 (14.5%); in 12 patients, no liver tests were available at diagnosis, but they did not have criteria of severe liver disease at last follow-up.

The PISZ genotype was found in 12 patients (8.1%, 9 boys), and the diagnosis was made at a mean ± SD age of 5.0 ± 4.4 years. A total of 2 of 11 (18.2%) had severe liver disease (one was transplanted), 4 of 11 (36.4%) had moderate liver disease, and 5 of 11 (45.5%) had mild or no liver disease; no liver tests were available in one patient. The mean ± SD AAT concentration was 0.6 ± 0.1 g/L. One child (8.3%) had a neonatal cholestasis, 7 (58.3%) were diagnosed with abnormal liver enzymes, 2 (16.7%) through familial screening, and 2 (16.7%) because of hepatosplenomegaly.

3.4.2 | Other genotypes

One patient had $M_{like}Z$ genotype and was also diagnosed for cystic fibrosis. This patient had developed severe liver disease and had already undergone LT. Two patients carried the M_{malton} variant. One was heterozygous (MM_{malton}) and developed moderate liver disease from 2 years of age. The other one also carried the Z allele ($M_{malton}Z$) and presented with neonatal cholestasis, and subsequently improved with normal concentration of liver enzymes

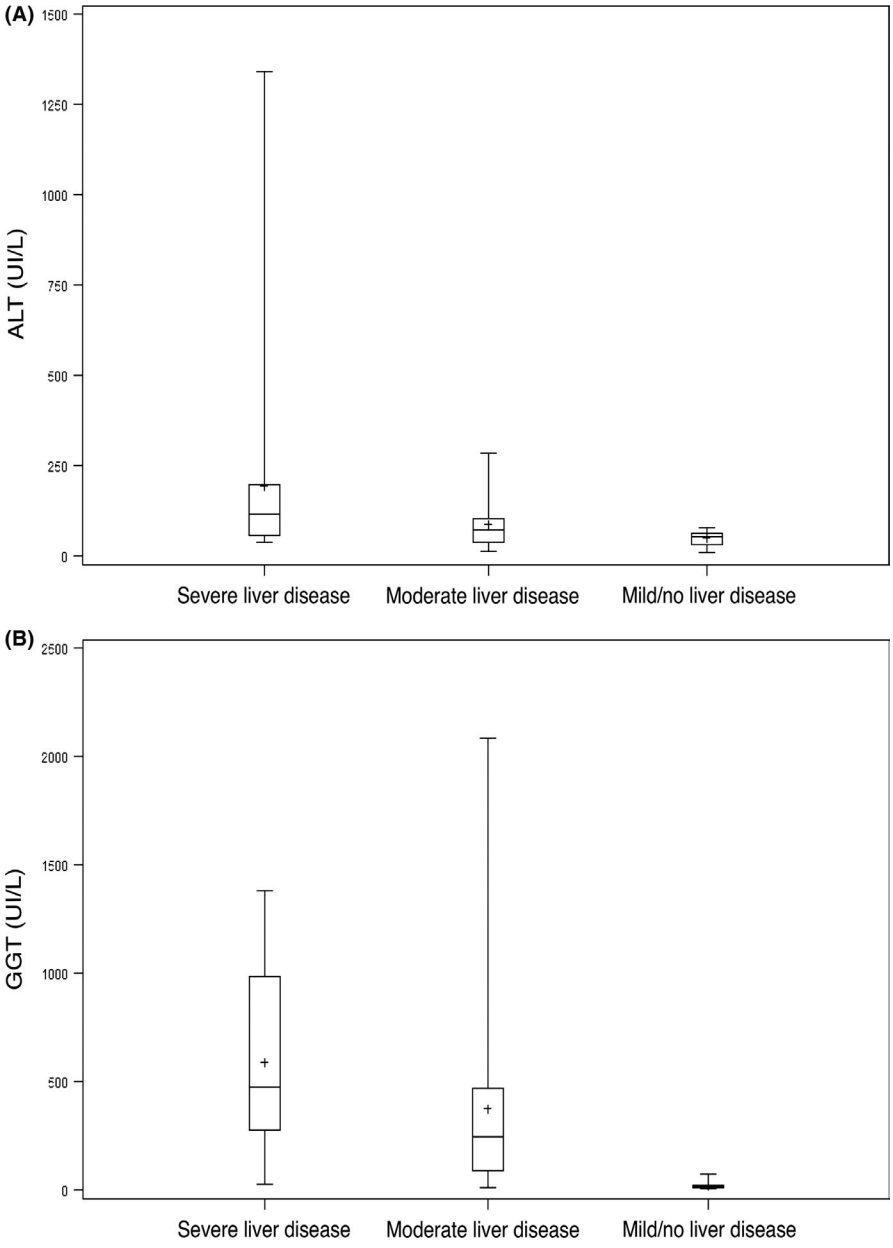

FIGURE 1 Liver parameters according to liver disease. Mean (cross), median (horizontal line), and box 25th-75th quartiles of ALT (A) and GGT (B) levels in UI/L on the vertical axis according to liver disease severity are presented

and ultrasonography at last follow-up. In these 3 patients, the phenotypes were at first defined by isoelectrofocusing as M and MZ: genotyping correctly identified the M_{like} and M_{malton} alleles. Other phenotypes/genotypes were found (MZ, SS, FZ and Z/D256V), but they did not appear to be associated with severe liver disease to date. In 4 patients, the genotype was not determined and they are currently lost to follow-up.

3.4.3 | Risk factors for liver disease

Neonatal cholestasis at diagnosis was significantly associated with severe liver disease in univariate ($P = 0.009$) and multivariate analysis ($P = 0.007$). No other factor was associated with severe liver disease (Table 5 and Figure 2). Liver disease status was missing in 8 patients as of December 2016.

An early diagnosis (before 2 months of age; $P < 0.0001$), PIZZ genotype ($P < 0.0001$), neonatal cholestasis ($P = 0.0008$) and familial screening ($P = 0.04$) were significantly associated with moderate liver disease in univariate analysis. The PIZZ genotype was significantly associated with moderate liver disease, independently from age and sex ($P = 0.003$, Table 6).

4 | DISCUSSION

The liver disease related to AAT deficiency is variable in children, and factors responsible for severe liver damage are unknown. This French paediatric DEFI-ALPHA cohort is one of the largest cohorts of children with AAT deficiency, constituted in order to improve the knowledge of the liver disease of AAT-deficient children.

TABLE 4 Mode of AAT deficiency diagnosis

	Neonatal cholestasis n = 74	Abnormal liver function n = 34	Familial screening n = 24
Gender, n (%)			
Girl	25 (33.8)	12 (35.3)	10 (41.7)
Boy	49 (66.2)	22 (64.7)	14 (58.3)
Mean age at diagnosis, years (SD)	0.3 (0.9)	3.7 (3.7)	2.3 (2.6)
Severity of liver disease, n (%)[a]			
Severe[b]	21 (30.9)	2 (5.9)	1 (4.8)
Liver transplantation, n (%)	12 (57.1)	0	0
Moderate[c]	41 (60.3)	22 (64.7)	10 (47.6)
Mild or none[d]	6 (8.8)	10 (29.4)	10 (47.6)
Missing data	6	0	3
UDCA, n (%)[a]	59 (83.1)	20 (64.5)	13 (59.1)
Missing data	3	3	2
Genotype, n (%)[a]			
PIZZ	68 (93.2)	25 (73.5)	18 (78.3)
PISZ	1 (1.4)	7 (20.6)	2 (8.7)
PIMZ	3 (4.1)	1 (2.9)	0
PISS	0	0	1 (4.3)
$PIMM_{malto}n$	0	1 (2.9)	0
$PIM_{malton}Z$	1 (1.4)	0	0
$PIM_{like}Z$	0	0	0
PIZ/D256V	0	0	1 (4.3)
FZ	0	0	1 (4.3)
Missing data	1	0	1
AAT level, g/l	0.4 (0.1)	0.4 (0.2)	0.4 (0.2)

AAT, alpha1-antitrypsin; UDCA, ursodeoxycholic acid.
Values are means (SD), unless indicated as percentages.
[a]among those with available data
[b]Severe liver disease: portal hypertension, liver failure, liver transplantation or death
[c]Moderate liver disease: significant perturbation of liver biology (ALT and/or GGT > 2N) without criteria of severe liver disease
[d]Mild or no liver disease: no criteria of severe liver disease, ALT and GGT < 2N.

The present study found that children with AAT deficiency could develop liver disease, sometimes severe forms with portal hypertension, and may require LT, as already shown in a previous study.[6] The mode of diagnosis was mostly a neonatal cholestasis, and patients who presented with a neonatal cholestasis developed more often severe liver disease. Furthermore, some genotypes other than ZZ or SZ could also be concerned by liver disease.

Although we confirmed that ZZ and SZ patients may develop severe liver disease, the rate was much lower than the 45% reported by Teckman et al[15] in a US cohort study, using almost the same definition for severe liver disease (PHT or LT). This could be due to differences in healthcare systems and inclusion bias; the American patients were followed in specialised tertiary centres focused on rare paediatric liver diseases. Conversely, Sveger reported a much lower rate of liver disease (defined as prolonged obstructive jaundice with clinical symptoms of liver disease) in Swedish patients (7%) recruited through massive neonatal screening, whereas only patients with known AAT deficiency were included in the present study.[9] Furthermore, there could also be differences in genetic polymorphisms between the North American and European populations, and the gradient between North and South of Europe for the incidence of AAT deficiency is well known.[1,2]

Among the clinical and biological criteria studied, neonatal cholestasis was associated with severe liver disease, while the PIZZ genotype and young age at diagnosis were associated with moderate liver disease, which is in accordance with previous studies.[6,17] Other reported risk factors, such as IUGR, the absence of breastfeeding and male gender, were not found herein,[9,12,13] although severe liver disease was slightly more frequent in boys. This may be in

TABLE 5 Factors associated with severe liver disease

	No severe liver disease n = 117	Severe liver disease n = 28	HR	95% CI	*P*-value
Univariate analysis					
Age at diagnosis					
<2 mo	56	16	1	/	
>2 mo	57	9	0.6	[0.3-1.5]	0.29
Missing	4	3			
Gender					
Girl	48	7	1	/	
Boy	69	21	2.5	[0.9-6.7]	0.07
Familial liver disease	45	11	1.1	[0.5-2.4]	0.85
IUGR	16	6	1.6	[0.6-4.3]	0.32
Breastfeeding	24	4	0.9	[0.3-2.5]	0.82
PIZZ genotype	87	23	2.4	[0.6-10.3]	0.23
Mode of diagnosis					
Neonatal cholestasis	51	22	3.7	[1.4-10.0]	0.009
Diagnosis made because of abnormal liver function	32	2	0.3	[0.1-1.2]	0.08
Familial screening	21	1	0.2	[0.0-1.5]	0.12
	No severe liver disease n = 111	**Severe liver disease n = 28**	**HRa**	**95% CI**	***P*-value**
Multivariate analysis adjusted on age and gender					
Neonatal cholestasis	51	22	4.8	[1.5-15.0]	0.007

HR, hazard ratio; HRa, hazard ratio adjusted; IUGR, intrauterine growth retardation.

relation to males being more prone to cirrhosis,[18] which may explain why AAT-deficient males may be at risk of severe liver disease later in life.

The advantage of a paediatric cohort is to avoid "adult" confounding factors, such as alcohol, hepatitis virus or metabolic disorders. Nevertheless, two of the patients had another liver disease (glycogen storage disease or cystic fibrosis), known to be worsened by AAT deficiency,[19] and therefore, paediatric cohorts do not completely avoid such bias. Another point to consider from this study is that symptomatic severe and moderate liver disease was also found when the diagnosis was made from familial screening. It is therefore important to propose screening and genetic counselling to the family of an index case.

Liver disease in severe AAT deficiency is mainly due to the retention of Z-protein/aggregates in hepatocytes.[7] The selection criteria used herein (low AAT level irrespective of the phenotype) allowed the inclusion of patients with liver disease without Z homozygosis, such as SZ patients, who had severe liver disease about as frequently as ZZ patients. However, SZ patients are approximately 12-fold more frequent than ZZ patients in France,[1] whereas they were 10-fold less frequent in the present cohort. This suggests that only a small fraction of them were included. Similarly, MZ subjects are about 100-fold more frequent than ZZ patients in the general population,[1] but 17-fold less frequent herein. This is evidently related to the threshold of AAT we used (below 0.8 g/L) and points to a continuum of AAT deficiency related not only to the phenotype but also to polymorphisms. It is noteworthy that this threshold was defined in relation with lung disease, and not liver disease,[3,4,14] and that Ferrarotti et al[20] have concluded that 1.1 g/L should be used in order to avoid missing patients with AAT deficiency related to Z or S allele.

Other than ZZ and SZ, this study also found atypical genotypes associated with liver disease; three patients had mutations or variants of the M allele. The M_{malton} allele has previously been associated with severe liver disease,[21,22] and the liver disease in the $M_{like}Z$ patient was more probably due to cystic fibrosis, although it is known that the Z allele is a modifying gene in this condition.[23] The M_{like} allele could thus be considered as a non-clinically significant variant of the wild-type M. Conversely, when patients develop severe liver disease with a supposed MZ phenotype, genotyping should be performed to investigate the presence of a pathological M variant.[24]

The study does have some limitations. The first is the selection bias resulting from the participation of a few centres focusing on paediatric hepatology, irregularly distributed throughout the country. As it is a rare and underrecognised disease in children, this cohort is obviously not exhaustive, and patients have been included from paediatric hepatology centres. Patients with severe liver disease were more likely to be followed and included

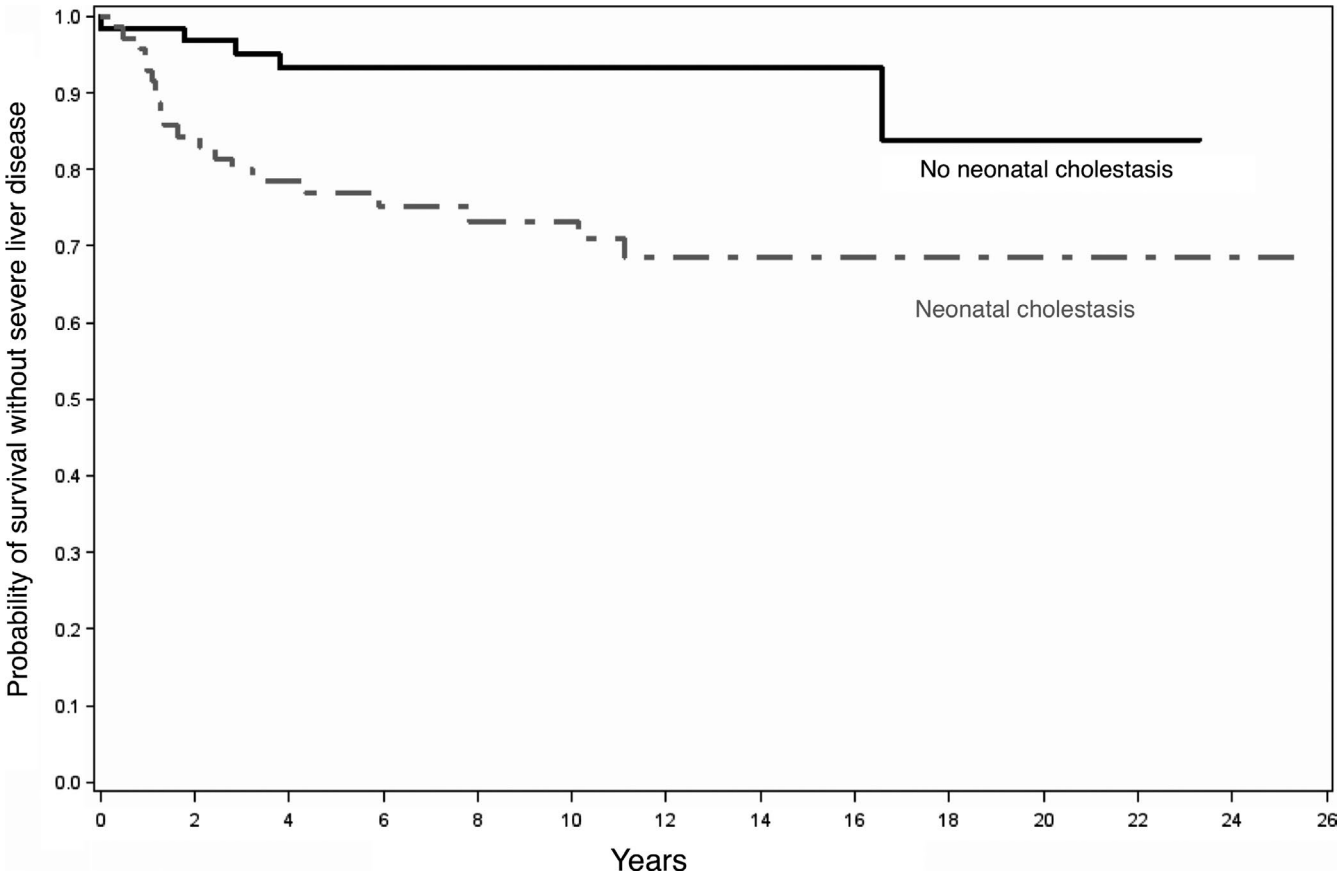

FIGURE 2 Kaplan-Meier analysis comparing survival without severe liver disease in patients, with or without neonatal cholestasis. The survival curves without severe liver disease according to the presence of cholestasis (discontinuous line) or absence of cholestasis (continuous line) are shown ($P < 0.05$)

TABLE 6 Factors associated with moderate liver disease (ALT or GGT > 2N, without PHT)

	ALT and GGT < 2N n = 35	ALT or GGT > 2N n = 76	OR	95% CI	*P*-value
Univariate analysis					
Age at diagnosis					
<2 mo	6	48	1	/	
>2 mo	27	28	0.1	[0.0-0.4]	<0.0001
Missing	2	0			
Gender					
Girl	14	29	1	/	
Boy	21	47	1.1	[0.5-2.6]	0.78
Familial liver disease	16	28	0.7	[0.3-1.6]	0.47
IUGR	1	14	6.3	[0.8-50.7]	0.09
PIZZ genotype	17	70	11.0	[3.5-34.9]	<0.0001
Neonatal cholestasis	7	43	5.6	[2.0-15.1]	0.0008
Diagnosis made because of abnormal liver function	11	22	0.9	[0.4-2.2]	0.80
Familial screening	10	10	0.4	[0.1-1.0]	0.04
	ALT and GGT < 2N n = 35	**ALT or GGT > 2N n = 76**	**ORa**	**95% CI**	***P*-value**
Multivariate analysis					
PIZZ genotype	17	70	6.5	[1.9-22.9]	0.003
Neonatal cholestasis	26	43	2.2	[0.7-7.7]	0.19
Familial screening	10	10	0.4	[0.1-1.1]	0.07

ALT, alanine aminotransferase; GGT, gamma-glutamyltranspeptidase; IUGR, intrauterine growth retardation; OR, odds ratio; ORa, odds ratio adjusted on age binary and gender.

by one of the few large centres (irrespective of their origin), and also, the screening techniques (immunoassay, electrophoresis), as well as detection thresholds, may have differed. Furthermore, the geographical origin of patients, not reported in this study, may be particularly important if the distribution of polymorphisms is uneven across metropolitan France. Second, there is information bias related to the diversity of practices according to the centres. Non-invasive assessment of liver stiffness using transient elastography (FibroScan®, Echosens, Paris, France), or acoustic radiation force impulse (ARFI) imaging for instance, was not commonly available in many centres when the study started. In future, although these techniques are not formally validated in this indication, but increasingly used and validated in many other adults and paediatric liver diseases,[25,26] non-invasive liver stiffness will be assessed. Moreover, it could be interesting to study liver histology, especially in patients presenting with atypical injury. Otherwise, UDCA therapy was started at the initiative of the physicians. The indications and its effectiveness were not been evaluated in the present study, but it would be interesting to assess it.

In conclusion, this study supports that the presence of neonatal cholestasis should lead to investigate AAT deficiency, because it is associated with severe liver disease. It shows that atypical genotypes may be responsible for early liver disease and suggests that these less frequent genotypes should be diagnosed because they may act as cofactor for adulthood liver disease. For this, it might be useful to raise the at-risk threshold for AAT deficiency-related liver disease, for instance at 1.1 g/L. This cohort allows genetic studies with complementary strategies (gene candidates and exome sequencing)[27,28] to assess genetic modifiers and polymorphisms associated with AAT deficiency-related liver disease.

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CONFLICT OF INTEREST

The authors have no relevant conflicts of interest.

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