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Presence of Alpha 1 Antitrypsin risk variants is not associated with histologic severity of pediatric NAFLD

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Abstract

Background: Among adults with nonalcoholic fatty liver disease (NAFLD), alpha-1 antitrypsin (A1AT) heterozygosity has been linked to advanced liver disease; pediatric data remain unclear.

Objective: Determine whether A1AT PiZ or PiS variants are associated with liver disease severity in youth with NAFLD.

Methods: Retrospective study of youth with confirmed NAFLD. Multivariable logistic regression used to determine independent associations between A1AT risk variants and histologic severity (NAFLD activity score (NAS) ≥5 and/or significant fibrosis [stage ≥2]).

Results: The cohort included 269 patients, mean age 12 [± 3] years with NAFLD and A1AT phenotyping (n=260) and/or A1AT levels (n=261). The mean NAS of the cohort was 4.2 [±1.5]; 50% had any, and 18% had significant fibrosis. Most (86%) had the MM A1AT phenotype, while 7% had the MS and 3% the MZ phenotype (the rest had other, non-pathogenic variants). Mean A1AT level was 123 mg/dl [±20]. A1AT levels did not differ by low vs. high NAS (122±2 vs 126 ±19 mg/dl, p=0.12) or by no/mild vs. significant fibrosis (123±20 vs 126±20 mg/dl, p=0.23, respectively). Carriers and non-carriers of the PiS or PiZ variants had similar NAS (mean NAS 3.8 ±1.6 vs 4.2 ±1.4; p=0.25, respectively). Fibrosis severity did not differ by carrier vs non-carrier group: 38% vs 52% had any fibrosis (p=0.17) and 14% vs 18% had significant fibrosis (p=0.80, respectively). Multivariable modeling showed no association between A1AT risk variants and histologic severity.

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Conclusion: While not uncommon, carriage of the A1AT PiZ or PiS risk variants was not associated with histologic severity in children with NAFLD.

Keywords

nonalcoholic steatohepatitis; liver disease; children; obesity

Introduction:

Nonalcoholic fatty liver disease (NAFLD) has become the most common liver disease in children, with an estimated prevalence of nearly 10% in the general population and an incidence that has doubled in the last decade[1–3]. NASH-related cirrhosis or hepatocellular carcinoma have become the fastest-growing indication for liver transplantation among young US adults[4], some of whom presumably develop the disease in childhood. Currently, there are no treatments for pediatric NAFLD, and management is purely dependent on primary/secondary prevention, rendering risk stratification key to preventing end-stage liver disease.

Alpha-1-antitrypsin (A1AT) deficiency is an autosomal codominant condition classically associated with homozygosity for the A1AT Z allele or the concurrent presence of Z and S alleles. It is the most common genetic cause of liver disease in children, with a prevalence in the United States of 1:2,857 to 1:5,097[5]. Beyond the Pi*ZZ or Pi*ZS homozygosity that leads to the development of A1AT deficiency, there is evidence that heterozygosity for the Pi*Z or Pi*S alleles may be a modifier for other types of liver diseases, such as NAFLD.

Recent studies have identified a higher-than-expected prevalence of A1AT heterozygosity in adult patients with NAFLD[6, 7]. Additionally, Pi*Z and Pi*S heterozygosity increase the risk of advanced liver disease and the need for transplantation in these patients[8–10]. In fact, the adjusted odds ratio for developing cirrhosis among heterozygotes for the Pi*Z or Pi*S alleles is 7.3 in adults with NAFLD, as shown in a multicenter study of 1184 individuals[8]. The increased risk of end-stage liver disease in the context of A1AT heterozygosity and NAFLD was also recently shown by Cheeney et al., who revealed the presence of the Z allele in 10% of liver explants for NASH, with 85% of cases genotyping homozygous (n=2) or heterozygous (n=11)[6]. Others have linked A1AT heterozygosity with hyperferritinemia, irrespective of hemochromatosis-related mutations, in the context of NAFLD[11]. The role of polymorphic heterogeneity in the A1AT gene as a modifier of pediatric NAFLD is not clear[12]. Pediatric focused studies are required to support the role of A1AT genotyping, for the purposes of risk stratification, genetic counseling and as a possible future therapeutic target for NAFLD.

The objective of this study was to determine whether Pi*Z and Pi*S heterozygosity is associated with more severe liver disease, while controlling for confounders, in a large cohort of children with confirmed NAFLD.

Methods:

Study design and population:

We conducted a single-center retrospective cohort study using electronic health record data at the Cincinnati Children's Hospital Medical Center (CCHMC) after approval from the Institutional Review Board (IRB#:2022-0100). We identified patients aged 2–21 years with histologically confirmed NAFLD who were followed at our Steatohepatitis Center from January 01, 2010, to June 20, 2021. We excluded patients who had undergone weight loss surgery prior to the liver biopsy, had evidence of other liver diseases (e.g., confirmed A1AT deficiency, autoimmune liver disease, hemosiderosis, autoimmune hepatitis, viral hepatitis [B or C], Wilson's disease), or patients who were on active treatment with vitamin E when the liver biopsy was obtained.

Patients with confirmed NAFLD were identified through a search of an electronic medical database (EPIC). Histological severity was determined according to the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) Scoring System[13]. Serum A1AT levels were determined using nephelometry conducted by the CCHMC laboratory, which had been followed by phenotype testing.

We hypothesized that Pi*Z and Pi*S heterozygosity would be associated with an increased likelihood of advanced histologic disease, as shown by a NAFLD Activity Score (NAS) ≥5 and/or a fibrosis stage ≥2.

Data collection:

Clinical, demographic, laboratory, and histologic data, including NAFLD Activity score (NAS) and fibrosis stage, were collected from the electronic medical record. The initial data to rule out coexisting liver disease (serum A1AT levels, A1AT phenotype, and serum ferritin) were collected within 3 months of the first visit, per the clinical protocol of excluding secondary causes of liver disease. The remaining anthropometric and laboratory data were collected from the time of the liver biopsy (within 3 months of the biopsy, if multiple, whichever was closest).

Statistical analyses:

All statistical analyses were performed using StataMP v13.0 (StataCorp, Texas, USA). The cohort was characterized using descriptive statistics (medians with interquartile ranges [IQR]), Student's t-test, and the chi-square test, when appropriate. Multivariable logistic regression was used to determine whether A1AT variants were associated with histologic severity (NAS ≥5 and/or significant fibrosis [stage ≥2]) while controlling for confounders such as age, sex, and ethnicity. Statistical significance was set at $P < 0.05$.

Results:

Patient characteristics:

The cohort included 269 patients with biopsy-confirmed NAFLD, with a mean age of 12 years [±3], of whom 69% were male and 22% had Hispanic ethnicity. A1AT phenotyping

had been done in n=260 patients, while A1AT levels were available for n=261 of them. The baseline characteristics as well as clinical and laboratory information from the time of liver biopsy are summarized in Table 1.

Clinical outcomes:

The histological data are summarized in Table 2. The mean NAS of the cohort was 4.2 [±1.5] with 50% of patients having any fibrosis (stage 1–4) and 18% having ≥ stage 2 fibrosis. Most patients (86%) had the MM A1AT phenotype, 7% had MS, and 3% had the MZ phenotype. Two patients had the SS phenotype and were included with the MS and MZ heterozygotes for the purposes of the analyses, because studies have shown that it confers low risk of liver disease, compared with the ZZ phenotype[14, 15]. The remaining patients had rare variants that were not associated with A1AT deficiency (Supplemental Table 1). The mean A1AT level was 123 mg/dl [±20] and the mean ferritin level was 78 ng/ml [±88], and they did not correlate with each other (r=−0.01; p=0.86). Ferritin levels did not differ between those with and without A1AT risk variants (119± 211 vs. 74±61 ng/ml, p=0.31).

A1AT levels did not differ between low vs. high (≥5) NAS (122±2 vs. 126 ±19 mg/dl, p=0.12, respectively) or by no/mild vs. significant fibrosis (123±20 vs. 126±20 mg/dl, p=0.23, respectively). Similarly, ferritin levels were not different between the groups (low vs. high NAS: ferritin levels 71±98 vs. 88±71 ng/dL, p= 0.10, respectively; no/mild vs. significant fibrosis: ferritin levels 72±57 vs. 108±176 ng/dL, p=0.20, respectively).

Carriers and non-carriers of the risk variants (PiZ or PiS) had similar age (12±2 vs. 12±3, p=0.54, respectively) and sex distributions (69% male in both groups, p=1.00); however, the proportion of Hispanic children was lower among carriers (7% vs. 23%, p=0.05; respectively). Severity of obesity was similar between the groups (BMI z score 2.5±0.3 vs 2.6 ±0.4, p=0.23, respectively). Carriers and non-carriers of PiS or PiZ variants had similar NAS, and fibrosis severity did not differ by group (Table 2). Multivariable modelling found no association between A1AT risk variants and histologic severity. Specifically, multivariable modelling showed that after controlling for sex, ethnicity, and age, carriers of the risk variants were not more likely to have NAS ≥5 or fibrosis ≥ stage 2 (OR estimate=−0.62, p=0.27 for NAS and OR estimate=0.74, p=0.45 for fibrosis).

Discussion:

In this retrospective analysis of children with histologically confirmed NAFLD at a single US referral center, A1AT heterozygosity was observed in 10% of the children. A1AT heterozygosity was not associated with increased liver disease severity determined histologically using NAS and/or fibrosis scoring while controlling for confounders.

Homozygous A1ATD is one of the most common inherited genetic disorders of liver disease in children. The prevalence of the heterozygous states of A1ATD in the United States ranges from 6 to 12% [16]. The most common heterozygous phenotype is the PiMS, which constitutes approximately 70% of the heterozygous group, whereas the PiMZ phenotype constitutes approximately 28%[7]. There is a wide range in the prevalence of A1AT heterozygosity in adult patients with NAFLD. Valenti et al. showed that A1AT heterozygosity was present

in 10% of 212 patients with NAFLD (vs. 3% of 114 controls)[11], whereas Regev et al. observed PiZ heterozygosity in 5.0% of patients with decompensated liver disease and 1.9% of those with less severe liver disease[7]. In our pediatric cohort, we found a prevalence of heterozygosity for A1AT variants that was similar to that described for the general population (10% of the total cohort, of which, as previously reported, 70% had the PiMS phenotype and 30% had the PiMZ phenotype; Supplemental Table 1).

The association between liver disease and the homozygous PiZZ state has been well established in neonates, children and adults; however, the role of PiZ heterozygosity as a possible cause of liver disease is still being investigated. Recent studies have identified a higher prevalence of A1AT heterozygosity among adult patients with advanced liver disease of varying etiologies; however, the prevalence of PiZ and PiS alleles among cohorts of adults with NAFLD remains lower than expected based on population data, suggesting that heterozygosity is not associated with a higher risk of NAFLD in general[7]. Importantly, these studies have shown an over-representation of the Pi*Z allele among those with more advanced liver disease and need for transplantation (8.8% versus 3%; P = 0.01), suggesting that heterozygosity may play a role in severity of disease[7–10, 17]. In our study, we found no association between A1AT risk variants and histological severity after controlling for confounders. Carriers and non-carriers of PiS or PiZ variants had similar NAS and fibrosis severity. Additionally, A1AT levels did not differ between low and high (≥5) NAS or between no/mild vs. significant fibrosis (> stage 2). This may be reflective of the shorter duration of disease in children than in adults or may again underscore the differences between adult and pediatric NAFLD[18]. Whether A1AT heterozygosity affects the natural history of pediatric NAFLD remains to be determined, as heterozygosity for A1AT deficiency risk alleles might contribute to disease progression once NAFLD is more advanced.

Hyperferritinemia associated with non-parenchymal iron overload in the presence of nearly normal transferrin saturation[19–21] represents a common clinical presentation of NAFLD. Although increased oxidative stress is possibly implicated[20], the reason why only a subset of subjects with metabolic liver disease show alterations in iron parameters is currently unclear. Hyperferritinemia has been reported to be a risk factor for steatohepatitis and fibrosis[22, 23]. A1AT is involved in the regulation of innate immunity, and its deficiency may promote a pro-inflammatory state[24], hence studies have linked A1AT heterozygosity with hyperferritinemia, irrespective of hemochromatosis-related mutations. Valenti et al. showed that A1AT heterozygosity was present in 10% of 212 patients with NAFLD (vs. 3% of 114 controls), and that these patients had higher ferritin levels. In that cohort, A1AT heterozygosity was not associated with liver disease severity but the ferritin was[11]. In our study, the mean ferritin level did not correlate with A1AT levels/heterozygosity or histologic severity of NAFLD. This difference could likely be due to differences in pediatric inflammatory processes or demographic differences, with Valenti's study focusing on an Italian adult population.

It has been well established that Hispanic subjects are at an increased risk of NAFLD, as well as severe NAFLD[25, 26]. In our cohort, although the general proportion of patients of Hispanic ethnicity was higher than that of patients of other ethnicities, these subjects were

less likely to have pathogenic A1AT variants. In agreement with previous reports[27], among the A1AT heterozygous carriers, the proportion of Hispanic children was lower (7% vs. 23%, p=0.05; respectively). Ethnicity was included in the multivariable modelling and did not affect the results of our study.

The strengths of this study include its large sample size and inclusion of children with histologically confirmed NAFLD. Our study has several limitations. First, we retrospectively obtained data, suggesting that some data were missing. Second, this was a single-center study with a small proportion of patients with advanced fibrosis (17% having ≥ stage 2 fibrosis), and type 2 error may have prevented us from determining an association between A1AT levels and/or phenotypes and fibrosis outcomes. Third, in this study, the clinical histology data were not reviewed and scored by a single pathologist, potentially contributing to inter-observer variability in scoring. However, at our institution, we have a large number of patients with NAFLD and multidisciplinary expertise in the field, which minimizes the potential for significant interobserver variability. Finally, the inclusion of patients with confirmed NAFLD may introduce selection bias, as patients undergoing histologic evaluations tend to have more concerning clinical presentations, such as persistently higher elevations in liver enzymes.

Conclusion:

While not uncommon, the presence of A1AT PiZ or PiS risk variants was not associated with greater histologic severity in a single-center cohort of children with biopsy-confirmed NAFLD. Larger studies including children with more advanced fibrosis and assessment of disease progression in this NAFLD inception cohort are needed to investigate this further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Declaration of Funding Source:

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Abbreviations:

NAFLD	nonalcoholic Fatty Liver Disease
CCHMC	Cincinnati Children’s Hospital Medical Center
A1ATD	Alpha 1 Anti trypsin Deficiency

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What is known:

- In adults with liver diseases of varying etiology, heterozygocity for A1AT risk variants (PiZ or PiS) is encountered at an increased frequency among those with advanced liver disease
- Among adults with NAFLD, heterozygotes for A1AT risk variants have more severe liver disease

What is new:

- Up to 10% of youth with histologically confirmed NAFLD carry A1AT risk variants
- Despite the adult literature, A1AT heterozygocity is not associated with more severe liver disease in children and adolescents with confirmed NAFLD

Table 1:

Baseline characteristics of the study patients

Variable	Result (n=269)
Age at first clinic visit, years	12 (±3)
Sex, n male (%)	186 (69%)
Ethnicity, n non-Hispanic (%)	211 (78%)
At liver biopsy:	
Age, years	12 (±3)
BMI, kg/m^2	36 (±7)
BMI z-score	2.5 (±0.4)
ALT, U/L	118 (±89)
AST, U/L	63 (±43)
GGT, U/L	57 (±44)
Alkaline phosphatase, U/L	206 (±113)
A1AT level, mg/dl	123 (±20)
Ferritin, ng/ml	78 (±88)

Data are reported as **means (±SD)** or as proportions

Table 2:

Histology data of the study patients

Scores/staging	Overall n= 269	A1AT Heterozygotes n= 29	Non-heterozygotes n= 231
Steatosis	2.1 (±0.8)	2.0 (±0.9)	2.1 (±0.8)
Lobular inflammation	1.4 (±0.7)	1.4 (±0.7)	1.3 (±0.7))
Ballooning degeneration	0.7 (±0.6)	0.5 (±0.5)	0.7 (±0.6)
NAS	4.2 (±1.5)	3.8 (±1.5)	4.2 (±1.5)
N with NAS≥5 (%)	103/260 (40%)	9/29 (31%)	94/231 (41%)
Fibrosis stage			
N with F1–4 (%)	131/260 (50%)	11/29 (38%)	120/231 (52%)
N with F2–4 (%)	46/260 (18%)	4/29 (14%)	42/231 (18%)

Data are reported as means (±SD) or as proportions