Down-Regulation of Hepatocyte Nuclear Factor-4α and Defective Zonation in Livers Expressing Mutant Z α1-Antitrypsin

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α1-Antitrypsin (AAT) deficiency is one of the most common genetic disorders and the liver disease due to the Z mutant of AAT (ATZ) is a prototype of conformational disorder due to protein misfolding with consequent aberrant intermolecular protein aggregation. In the present study, we found that livers of PiZ transgenic mice expressing human ATZ have altered expression of a network of hepatocyte transcriptional factors, including hepatocyte nuclear factor-4α, that is early down-regulated and induces a transcriptional repression of ATZ expression. Reduced hepatocyte nuclear factor-4α was associated with activation of β-catenin, which regulates liver zonation. Livers of PiZ mice and human patients with AAT deficiency were both found to have a severe perturbation of liver zonation. Functionally, PiZ mice showed a severe defect of ureagenesis, as shown by increased baseline ammonia, and reduced urea production and survival after an ammonia challenge. Down-regulation of hepatocyte nuclear factor-4α expression and defective zonation in livers have not been recognized so far as features of the liver disease caused by ATZ and are likely involved in metabolic disturbances and in the increased risk of hepatocellular carcinoma in patients with AAT deficiency. Conclusion: The findings of this study are consistent with the concept that abnormal AAT protein conformation and intrahepatic accumulation have broad effects on metabolic liver functions. (HEPATOLOGY 2017;66:124-135)
hepatic abnormalities, including elevated liver enzymes, hepatomegaly, and nutritional problems. The risk of life-threatening liver disease in childhood is about 5%, whereas the lifetime risk of cirrhosis may be as high as 50% according to autopsy studies in adults. The risk of HCC is also increased in ZZ patients, although the magnitude of the increased risk is unclear.

The mutant AAT encoded by the Z allele (ATZ) is either degraded by the endoplasmic reticulum–associated degradation or retained within the endoplasmic reticulum of the hepatocytes as polymers in periodic acid Schiff-positive diastase-resistant (PAS-D) inclusions. These inclusions results in a cascade of events leading to activation of multiple pathways including nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and autophagy. However, several questions about the liver disease caused by ATZ remain unanswered, and the natural history and the modifiers involved in the liver disease are still largely unknown.

Starting from investigations of hepatic ATZ accumulation in the PiZ transgenic mouse model, we found that the SERPINA1 and Hnf4a genes are downregulated with aging. In addition, we found activation of β-catenin that regulates liver zonation as hepatocyte nuclear factor-4α (HNF-4α). Finally, we found that livers of PiZ mice and AATD patients exhibited a severe perturbation of liver zonation and that mice showed a defect of ureagenesis.

Materials and Methods

MOUSE STUDIES

Mouse procedures were performed in accordance with the regulations of the Italian Ministry of Health. Male 6-week-old and 36-week-old C57BL/6N (Charles River Laboratories) and PiZ mice were used for the study. For the ammonia challenge, animals were starved for 16 hours prior to the intraperitoneal administration of 5 or 10 mmol/kg of NH₄Cl (Merck) dissolved in water. Serum ammonia and urea were measured using colorimetric assays (BioVision).

HUMAN LIVER SPECIMENS

AATD subjects’ specimens and clinical and pathological data were obtained from the Institute of Pathology, University Hospital of Basel (Basel, Switzerland). The Ethics Committee of the Institute of Pathology, University Hospital of Basel, approved the study.

LIVER STAINING

Mouse liver specimens from phosphate-buffered saline–perfused mice were fixed in 4% paraformaldehyde for 12 hours, stored in 70% ethanol, and embedded into paraffin blocks. PAS-D staining was performed on 10-μm-thick paraffin liver sections. Sections were rehydrated, treated with 0.5% α-amylase type VI-B (Sigma-Aldrich) for 20 minutes, and then stained with PAS reagent according to the manufacturer’s instructions (Bio-Optica). For immunohistochemistry (IHC), 5-μm-thick sections were rehydrated and permeabilized in phosphate-buffered saline/0.2%–0.5% Triton (Sigma) for 20 minutes. Antigen unmasking was performed in 0.01M citrate buffer in a microwave oven. Next, sections underwent blocking of endogenous peroxidase activity in methanol/1.5% H₂O₂ (Sigma) for 30 minutes and were incubated with blocking solution (3% bovine serum albumin [Sigma], 5% donkey serum [Millipore], 1.5% horse serum [Vector Laboratories] 20 mM MgCl₂, 0.3% Triton [Sigma] in phosphate-buffered saline) for 1 hour. Sections were incubated with primary antibody (Supporting Table S1) overnight at 4°C and with universal biotinylated horse antimouse/rabbit immunoglobulin G secondary antibody (Vector Laboratories) for 1 hour. Biotin/avidin-horseradish peroxidase signal amplification was achieved using the ABC Elite Kit (Vector Laboratories) according to the manufacturer’s instructions.

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Diaminobenzidine (Vector Laboratories) was used as peroxidase substrate. Mayer’s hematoxylin (Bio-Optica) was used for counterstaining. Sections were dehydrated and mounted in Vectashield (Vector Laboratories). Image capture was performed using the Leica DM5000 microscope.

IHC on human liver specimens was performed on 7-μm-thick sections of formalin-fixed, paraffin-embedded tissue. Sections were pretreated with Cell Conditioning solution (Ventana Medical Systems) and incubated with primary antibody against glutamine synthetase (GS) (Supporting Table S1). Staining was performed on a Benchmark IHC staining system (Ventana Medical Systems) using iVIEW-DAB as chromogen as described. Positive and negative controls were included in each run. Immunoreactivity was scored by an experienced pathologist (L.T.), as described. Briefly, specimens displaying PAS-D accumulation were classified as no accumulation, 0; minimal accumulation, +; localized consistent accumulation, ++; diffuse and massive accumulation, ++++. Fibrosis was scored according to the METAVIR system: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; F4, cirrhosis.

WESTERN BLOTTING AND GENE EXPRESSION ANALYSES

Proteins from tissues were extracted in RIPA buffer according to standard procedures. Nuclear protein extracts were prepared using the CelLytic NuCLEAR Extraction Kit (Sigma-Aldrich). Primary antibodies were diluted in trishydroxymethylaminomethane-buffered saline (TBS) Tween 20/5% milk (Bio-Rad Laboratories) (Supporting Table S1). Secondary antibodies were enhanced chemiluminescent (ECL) antirabbit horseradish peroxidase and ECL antimouse horseradish peroxidase (GE Healthcare). Peroxidase substrate was provided by the ECL Western Blotting Substrate kit (Pierce). Band intensity was analyzed using Quantity One 1-D Analysis Software, version 4.6.7 (Bio-Rad Laboratories).

For gene expression analyses, total RNA from cells and livers was extracted using the RNeasy mini kit (Qiagen). RNA was retrotranscribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCRs were set up using SYBR Green Master Mix and run in duplicate on a Light Cycler 480 system (Roche). Primers are reported in Supporting Table S2. The running program was as follows: preheating, 5 minutes at 95 °C; 40 cycles of 15 seconds at 95 °C, 15 seconds at 60 °C, and 25 seconds at 72 °C. Mouse β2-microglobulin (B2m) and human β2-microglobulin (B2M) were used as housekeeping genes. Data were analyzed using LightCycler 480 software, version 1.5 (Roche).

GENE SET ENRICHMENT ANALYSIS

Gene Set Enrichment Analysis (GSEA) was performed using the GSEA software (www.broadinstitute.org/gsea). Expression microarrays of livers from PiZ mice (GSE93115), from mice which underwent bile duct ligation (GSE40041), and from mice exposed to CCl4 (GSE86001), diethoxycarbonyldihydrocollidine (GSE77503), diethylnitrosamine (GSE51188), ethanol (GSE86002), or high-fat diet (GSE32095) and relative controls were obtained from previous studies. HNF-4α and β-catenin gene sets were based on differentially expressed genes in microarrays performed on livers from liver-specific Hnf4a-null mice and hepatocytes from liver-specific Apc-null mice, respectively. For the β-catenin gene set only genes with fold change >1.5 over controls were considered for GSEA. No threshold was applied for the HNF-4α gene set because fold changes were not available.

STATISTICAL ANALYSES

Two tailed Student t test and analysis of variance (ANOVA) plus Tukey’s post hoc analysis were used as statistical tests for mean comparisons. The log-rank test was used for survival curve statistical analysis. The χ² test was used to compare IHC score distributions for GS staining. Experimental group sizes are reported in figure legends. Data are reported as average ± standard error.

Results

DOWN-REGULATION OF HEPATIC ATZ AND HNF-4α IN AGING PiZ MICE

Consistent with a previous study, we observed that hepatic ATZ accumulation changes in older PiZ mice compared to younger mice, and the number and the hepatic area occupied by PAS-D gloules progressively decrease with aging (Fig. 1A). This change was associated with a marked decrease in the hepatic expression of SERPINA1 by quantitative PCR, despite
a similar number of SERPINA1 liver genome copies (Fig. 1B; Supporting Fig. S1A). SERPINA1 is a marker of hepatocyte differentiation that is controlled by a complex network of hepatic transcription factors including Cebpα, Foxa2, Hnf1α, Hnf4α, and Nr2f2. SERPINA1 expression itself is under the control of
HNF-4α. Therefore, we investigated the expression levels of these transcription factors in PiZ livers. Compared to age-matched wild-type controls, livers of 36-week-old PiZ mice showed dysregulation of four (Cebpα, Hnf1α, Hnf4α, and Nr2f2) out of the five transcription factors (Fig. 1C). Hnf4α was the only factor significantly down-regulated also in livers of younger, 6-week-old mice. By IHC, hepatocyte nuclei were positive for HNF-4α in wild-type mouse livers, whereas several nuclei were negative in PiZ livers (Fig. 1D). HNF-4α protein levels were also found to be reduced in PiZ livers (Supporting Fig. S1B). Liver staining of HNF-4α in 6-week-old mice showed cell hypertrophy (Fig. 1D). Liver staining of HNF-4α was previously observed also in liver-specific Hnf4α-null mice. (23) Hepatocytes surrounding the central vein have HNF-4α transcriptional activity suppressed by β-catenin and tend to be devoid of PAS-D globules, as shown in PiZ mice with lower levels of ATZ (Supporting Fig. S1C), thus supporting the role of HNF-4α in affecting the levels of ATZ accumulation.

Using microarray data previously generated, we performed GSEA on differentially expressed genes of liver-specific Hnf4α-null mouse livers. Interestingly, a highly overlapping set of genes down-regulated in liver-specific Hnf4α-null mouse livers was also down-regulated in PiZ mouse livers compared to wild-type controls (enrichment score [ES] = −0.66, P = 0.0005) (Fig. 1E; Supporting Fig. S2A). Genes up-regulated in liver-specific Hnf4α-null mouse livers were also found to be enriched (ES = 0.49, P = 0.008) (Supporting Fig. S2A,B). Taken together, these studies show that Hnf4α is down-regulated in PiZ mouse livers, resulting in global transcriptional changes similar to those occurring in liver-specific Hnf4α-null mice.

LIVER ZONATION IS IMPAIRED IN PiZ MICE

Liver zonation is the functional and metabolic organization of hepatocytes along the portocentral axis of the liver lobule and is impaired in livers of liver-specific Hnf4α-null mice. (26) As a consequence of differential oxygen and nutrient availability from perportal to pericentral hepatocytes, several liver functions, such as ammonia detoxification and glucose, lipid, and drug metabolism, are differentially expressed along the portocentral axis. (27) HNF-4α antagonizes the transcriptional activity of β-catenin (CTNNB1), the major determinant of liver zonation. Constitutive activation of β-catenin indeed results in whole-liver expression of pericentral genes and down-regulation of periportal genes. (19) GSEA based on differentially expressed genes in hepatocytes with constitutively active β-catenin showed moderate enrichment for both down-regulated (ES = −0.46, P < 0.0005) and up-regulated (ES = 0.39, P = 0.001) gene sets in PiZ versus wild-type livers (Fig. 2A; Supporting Fig. S3). Consistently, increased levels of activated β-catenin were found in PiZ livers by IHC and western blotting (Fig. 2B,C). Nevertheless, IHC showed no clear β-catenin signal in hepatocyte nuclei (Fig. 2B).

To investigate the specificity of HNF-4α down-regulation and β-catenin activation in PiZ mouse livers, we performed GSEA in a variety of murine models of liver injury. Significant enrichment consistent with HNF-4α down-regulation and β-catenin activation was only found in hepatic injury induced by bile duct ligation but not in other injury models (12–16) (Table 1), suggesting that transcriptional changes occurring in PiZ mouse livers are not a nonspecific response to injury.

We next hypothesized that concomitant down-regulation of HNF-4α and activation of β-catenin perturbs liver zonation in PiZ mice. To test this hypothesis, we evaluated liver zonation by IHC for arginase 1 (ARG1) and cadherin-1 (CDH1) as perportal markers and for GS and ornithine amino transferase (OAT) as pericentral markers (28) in 6-week-old PiZ mice and wild-type controls. In PiZ livers, we observed areas of desaturation for ARG1 and loss of signal zonation for CDH1 (Fig. 3A; Supporting Fig. S4). Consistent with this finding, CDH1 expression was previously found altered in PiZ mice. (29) In contrast to wild-type livers, CDH1 was detected in pericentral areas in addition to the reduced signal intensity in perportal areas (Fig. 3A). Noteworthy, ARG1 is repressed by β-catenin, whereas CDH1, a direct HNF-4α target, is a negative regulator of β-catenin. (31) Although expressed in the pericentral region as in wild-type controls, GS and OAT exhibited in PiZ livers broadened positive signals along the portocentral axis (Fig. 3B), a pattern overlapping with liver Hnf4α-null mice. (26) Perturbation of liver zonation was also detected in older, 36-week-old mice (Supporting Fig. S5).

IMPAIRED UREAGENESIS IN PiZ MICE

Expression of genes involved in ureagenesis is tightly regulated by zonation in mammalian livers and is controlled by HNF-4α and β-catenin. (19,32) Hepatic expression of genes encoding for urea cycle enzymes
carbamoyl phosphate synthetase I (Cps1), ornithine transcarbamylase (Otc), argininosuccinate lyase (Asl), ARG1 (Arg1), N-acetylglutamate synthase (Nags), glutaminase (Gls), and glutamate dehydrogenase (GluD) was down-regulated in PiZ mice compared to controls (Fig. 4A). Consistent with such down-regulation, PiZ mice showed higher baseline blood ammonia levels (Fig. 4B) and impaired ammonia handling, as shown by increased mortality and higher levels of blood ammonia after intraperitoneal ammonia challenge with

![Heatmap](image1.png)

**FIG. 2.** β-Catenin activation in PiZ mouse livers. (A) Heatmap of a GSEA including genes down-regulated in liver-specific Apc-null mouse livers in which β-catenin is constitutively active, showing enrichment in PiZ mice versus wild-type livers. (B) Representative IHC for active β-catenin (CTNNB1) on livers of 6-week-old wild-type and PiZ mice shows increased active β-catenin staining in PiZ mice. □, central vein; ▲, portal vein (n = 3 per group; magnification: 10×; scale bar, 100 μm). (C) Western blot for active and total β-catenin (CTNNB1) on whole-liver extracts of 6-week-old wild-type and PiZ livers showing increased β-catenin activation in PiZ livers. β-Actin was used as a loading control. Abbreviations: ACTB, β-actin; WT, wild type.

| TABLE 1. Enrichment scores from GSEA Performed on Dysregulated Genes From Hnf4a-Null Livers and Apc-Null Hepatocytes in Livers of PiZ and of Hepatic Injury Murine Models |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Up-regulated    | Down-regulated  | Up-regulated    | Down-regulated  | Up-regulated    | Down-regulated  |
|                                | Gene Set        | Gene Set        | Gene Set        | Gene Set        | Gene Set        | Gene Set        |
| PiZ                             | 0.49            | −0.66           | 0.46            | −0.39           |
| BDL (48 hours)                  | 0.73            | −0.77           | 0.66            | −0.79           |
| BDL (28 days)                   | 0.79            | −0.83           | 0.54            | −0.72           |
| OCl₂                            | 0.80            | −0.70           | 0.49            | NS              |
| DDC                             | NS              | −0.48           | 0.44            | −0.52           |
| DEN                             | NS              | NS              | NS              | −0.71           |
| EtOH                            | 0.49            | NS              | NS              | +0.43           |
| HFD                             | NS              | +0.42           | NS              | −0.33           |

Positive enrichment scores (ES) means that most of the genes in the gene set are up-regulated, while negative ES indicates that most of them are down-regulated.

Abbreviations: BDL, bile duct ligation; DDC, diethoxycarbonyl dihydrocolloidine; DEN, diethylaminoethyl; EtOH, ethanol; HFD, high-fat diet; NS, not statistically significant.
NH₄Cl (Fig. 4C,D). In contrast to Fig. 4B, baseline ammonia in PiZ mice which underwent ammonia challenge was not different from controls, likely as a consequence of starvation performed prior to the challenge (Fig. 4D). Liver urea content at 15 minutes postchallenge with NH₄Cl was significantly lower compared to wild-type controls (Fig. 4E), thus suggesting that impairment of ammonia handling is due to defective ureagenesis in PiZ mice.

PERTURBED LIVER ZONATION IN AATD PATIENTS

PiZ mice express multiple copies of the mutant ATZ, whereas most human patients with AATD carry only two copies of the mutant Z allele. To interrogate the clinical relevance of our findings in mice, we evaluated the zonation in liver samples from AATD patients with PAS-D staining of various severity and different stages of fibrosis. Liver samples from subjects with unrelated liver disorders were used as controls (Table 2). In 7 out of 10 control livers, GS showed a purely centrilobular signal (score 0), while only 3 out of 10 samples had an extended GS staining signal but with a negative portal field (score 1) (Fig. 5A,B and Table 2). In contrast, most liver samples from AATD patients showed significantly altered zonation with scores of 1 and 2 (positivity of the entire lobule, albeit with a stronger centrilobular signal) (16/20, P = 0.018), whereas only 4 out of 20 samples had score 0 (Fig. 5A,B and Table 2). Altered GS staining was found in patients without or with minimal fibrosis (Fig. 5C and Table 2), suggesting that the impairment of liver zonation in AATD is independent of fibrosis. Nevertheless, GS diffuse expression is paralleled by a concurrent increase in fibrosis (Table 2).

Discussion

A multitude of intracellular pathways are affected in hepatocytes expressing ATZ. However, Hnf4α gene down-regulation, impaired liver zonation, and ureagenesis have not yet been recognized as features of the liver disease caused by ATZ.

HNF-4α controls the basal expression of several genes involved in the development and maintenance of the hepatocyte metabolic functions. Conditional Hnf4α knockout in adult mouse liver indeed results in a wide range of metabolic defects. In addition, HNF-4α maintains the differentiated state of the hepatocyte and the proper liver architecture in the developing mouse liver. Consistent with this role, down-regulation of HNF-4α in PiZ livers was associated with reduced expression of key hepatocyte transcription factors Cebpα, Hnf1α, and Nr2f2. In aging PiZ mice, down-regulation of HNF-4α was detected.
at an early age compared to other transcription factors that were down-regulated only in older mice. Therefore, hepatocyte dedifferentiation by down-regulation of HNF-4α appears to occur early in response to ATZ-mediated damage in PiZ livers. In agreement with these data, preneoplastic lesions and HCC in PiZ mice have been found to express immature hepatic marker α-fetoprotein, but they were lacking expression of genes expressed in mature hepatocyte genes, such as glucose-6-phosphatase.\(^{36}\)

HNF-4α controls SERPINA1 expression,\(^{22}\) and thus, its reduced expression might serve as a defensive mechanism activated to reduce the burden of toxic ATZ. We indeed observed age-dependent reductions of SERPINA1 expression and PAS-D-positive globules in PiZ mouse livers that were paralleled by a
greater reduction of Hnf4a expression in older compared to younger PiZ mice. In summary, HNF-4α down-regulation might protect hepatocytes from toxic ATZ overload and support liver regeneration by inducing hepatocyte proliferation. However, HNF-4α is a tumor suppressor, and its down-regulation may increase the risks of HCC. Disruption of Hnf4a in mature hepatocytes indeed results in epithelial to mesenchymal transition and HCC. Moreover, HNF-4α expression is reduced in both mouse and human HCC, whereas HNF-4α overexpression prevents hepatocarcinogenesis.

The mechanisms underlying HNF-4α down-regulation remain elusive. ATZ accumulation in the endoplasmic reticulum induces NF-κB, resulting in activation of inflammatory pathways that have been shown to reduce transcriptional activity of HNF-4α. MicroRNA-24 and microRNA-629 have also been involved in cytokine-mediated down-regulation of HNF-4α. Moreover, active NF-κB down-regulates HNF-4α through the expression of microRNA-21, and HNF-4α is the target of other microRNAs involved in other liver diseases, such as nonalcoholic fatty liver disease.
HNF-4α is also a major regulator of liver zonation. The liver is of central importance for whole-body metabolism, executing ammonia detoxification, glucose supply, and xenobiotic metabolism. Periportal hepatocytes receiving nutrient-rich blood from the portal vein are specialized in oxidative energy metabolism, gluconeogenesis, and ammonia detoxification by urea synthesis. Pericentral hepatocytes instead receive blood from the central vein with low nutrient and oxygen content and are specialized in glucose use through glycolysis, glutamine formation from ammonia, and xenobiotic metabolism. Like liver-specific Hnf4α-null mice, PiZ mice exhibited striking alterations of liver zonation with marked expansion of GS and OAT compartment and reduction of ARG1 expressing cells and other urea cycle enzymes. Noteworthy, diffuse and low levels of GS and OAT expression are seen in immature mouse liver as their compartmentalization in pericentral hepatocytes occurs by 2 weeks after birth.

The Wnt/β-catenin pathway is another key player in hepatocyte specification. In perivenous hepatocytes, Wnt/β-catenin signaling drives the expression of perivenous GS. Consistently, we detected increased activation of β-catenin and increased GS expression. In summary, the imbalance between HNF-4α down-regulation and β-catenin activation is responsible for the impaired liver zonation in liver expressing ATZ.

Cirrhosis as a result of several liver diseases is often associated with perturbed metabolic zonation. However, the perturbation of liver zonation in PiZ mice detected in the present study was not a consequence of liver fibrosis and cirrhosis because the onset of fibrosis only occurs in older PiZ mice (10-12 months), whereas the PiZ mice used for this study were 6 weeks old. There is a relatively limited number of conditions resulting in perturbed metabolic zonation in the absence of cirrhosis. Nonalcoholic fatty liver disease and hepatitis C infection in the early stages of the disease are among these conditions affecting liver zonation. However, there is no evidence that these conditions affect HNF-4α expression.

The altered liver zonation and down-regulation of urea cycle enzymes in PiZ mice result in defective

| Age (Years) | PAS-D | Fibrosis | GS Score | Diagnosis |
|------------|-------|----------|----------|-----------|
| 1          | +     | F0       | 0        | AATD, cirrhosis |
| 2          | ++    | F0       | 0        | AATD, steatosis |
| 3          | +     | F3       | 0        | AATD, hemochromatosis |
| 4          | +     | F4       | 0        | AATD |
| 5          | 0     | F0       | 1        | AATD, NASH |
| 6          | ++    | F0       | 1        | AATD |
| 7          | +     | F0       | 1        | AATD |
| 8          | 0     | F0       | 1        | AATD |
| 9          | +     | F3       | 1        | AATD |
| 10         | +++   | F4       | 1        | AATD |
| 11         | +     | F4       | 1        | AATD |
| 12         | +     | F4       | 1        | AATD, NASH |
| 13         | +++   | NA       | 1        | AATD |
| 14         | NA    | NA       | 1        | AATD |
| 15         | +     | F0       | 2        | AATD |
| 16         | +++   | F1       | 2        | AATD |
| 17         | +     | F2       | 2        | AATD |
| 18         | +     | F2       | 2        | AATD |
| 19         | ++    | F3       | 2        | AATD |
| 20         | +     | F4       | 2        | AATD |
| 21         | —     | NA       | 0        | NASH |
| 22         | —     | F1       | 0        | Chronic hepatitis, HBV-positive |
| 23         | —     | F2       | 0        | Chronic hepatitis, HCV-positive |
| 24         | —     | NA       | 0        | Minimal steatohepatitis |
| 25         | —     | NA       | 0        | Steatohepatitis, hemosiderosis |
| 26         | —     | NA       | 0        | Chronic hepatitis, HBV-positive |
| 27         | —     | F0       | 0        | Chronic hepatitis |
| 28         | —     | F1       | 1        | Chronic hepatitis |
| 29         | —     | NA       | 1        | NASH, portal cirrhosis, hemosiderosis |
| 30         | —     | F2       | 1        | Chronic hepatitis, HCV-positive |

Liver biopsies from AATD patients (1-20) and 10 from control subjects (21-30) were analyzed for ATZ accumulation (PAS-D), fibrosis, and GS score by IHC.

Abbreviations: NA, not available; NASH, nonalcoholic steatohepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus.
ureagenesis, as shown by increased blood ammonia and sensitivity to ammonia challenge, and reduced blood urea formation. So far, impaired ureagenesis has not been reported in AATD patients. Therefore, the results of this study raise the question about a defect in ureagenesis in individuals harboring the Z allele that may occur in precirrhotic stages. However, further studies in humans are needed to verify this hypothesis.

HNF-4x is a major regulator of glucose homeostasis, and mutations in the human HNF4A gene result in maturity-onset diabetes of the young type 1, a mono- genic form of type 2 diabetes mellitus. Genetic and biochemical studies of the HNF4A gene and protein in humans have suggested a broader role for HNF-4x in the hepatic and pancreatic regulation of glucose homeostasis and have linked HNF-4x to the development of the common late-onset forms of type 2 diabetes. (49) Interestingly, using a large health care database, patients with AATD were found to have a higher prevalence of diabetes compared to controls. (50)

The hepatotoxicity associated with the Z allele results from a gain-of-toxic function mechanism. Genetic modifiers and/or environmental variables are thought to play a significant role in the development of liver disease. Based on the data presented here, an appreciation of the hepatocyte metabolic changes induced by ATZ might help in understanding how a subset of AATD patients could be predisposed to liver disease. Therefore, this study raises the interesting possibility that liver metabolic alterations may contribute to the phenotype.

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