Diploma thesis

Alpha1-Antitrypsin deficiency and liver injury at the Medical University of Graz

Submitted by

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Statutory declaration

I declare that I have authored this thesis independently, that I have not used other sources than declared and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 11.03.2021 *Matthias Leitner eh*

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List of shortcuts

A1AT	Alpha1-Antitrypsin
AFP	Alpha fetoprotein
ALT	alanine aminotransferase
APRI	Aspartate aminotransferase to platelet ratio index
AST	Aspartate aminotransferase
BMI	Body mass index
CAS	CRISPR associated
COPD	Chronic obstructive pulmonary disease
CRISPR	Clustered regularly interspaced short palindromic repeats
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum associated degradation
GGT	Gamma-glutamyl transferase
HCC	Hepatocellular carcinoma
ICD	International statistical classification of diseases
IMI	Institute of Medical Information technology
INR	International normalized ratio
LFT	Liver function test
LTx	Liver transplantation
mRNA	Messenger ribonucleic acid
NAFLD	Non-alcoholic fatty liver disease
NE	Neutrophil elastase
PAS-D	Periodic acid Schiff - diastase-resistant
PCR	Polymerase chain reaction
PI	Protease inhibitor
PT	Prothrombin time
rAVV	Recombinant adeno associated viral vectors
rER	Rough endoplasmic
RFLP	Restriction fragment length polymorphism
SERPINA1	Serine protease inhibitor clade A
siRNA	Small interfering ribonucleic acid
TNFα	Tumor necrosis factor alpha
UDCA	Ursodeoxycholic acid

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Zusammenfassung

Hintergrund: A1AT-Mangel ist eine Erbkrankheit, die hauptsächlich Lungen- und Lebererkrankungen zur Folge hat. Durch die niedrige Penetranz dieser Lebererkrankungen in genetisch betroffenen Personen, den individuell unterschiedlichen Verläufen und limitierten Behandlungsmöglichkeiten, wird die A1AT assoziierte Lebererkrankung in vielen Personen nicht diagnostiziert. Das Ziel dieser Studie ist, die Anzahl von Personen mit A1AT-Mangel an der Medizinischen Universität Graz zu ermitteln, sowie den natürlichen Verlauf der Lebererkrankung dieser Personen darzustellen.

Methoden: Wir erarbeiteten eine retrospektive Studie, in der wir Daten von Personen mit A1AT-Mangel, die die Medizinische Universität Graz in den letzten 16 Jahren besucht haben, sammelten. Wir ermittelten den Zeitpunkt der Diagnose, den Schweregrad und die Komplikationen der Lebererkrankung (z.B. erhöhte LFTs, Steatose, Fibrose, Zirrhose, HCC, Lebertransplantation (LTx) oder neonatale Lebererkrankungen) sowie den natürlichen Krankheitsverlauf. Diese Ergebnisse setzten wir mit dem molekularen Phänotyp in Verbindung (z.B. ZZ- oder MZ-Phänotyp). Zusätzlich untersuchten wir A1AT-Mangel bei Kindern und stellten die Ergebnisse getrennt dar.

Ergebnisse: Insgesamt wurden 148 Personen mit A1AT-Mangel und bekanntem Phänotyp identifiziert. 93 Personen (62.8%) zeigten den MZ-Phänotyp, 40 Personen (27%) zeigten den ZZ-Phänotyp, 8 (5.4%) zeigten den SZ-Phänotyp, 5 (3.4%) zeigten den MS-Phänotyp und 2 (1.4%) zeigten den MMalton-Phänotyp. 62.5% der ZZ-Phänotypen und 63.4% der MZ-Phänotypen zeigten Zeichen einer Lebererkrankung. 52% der ZZ-Phänotypen mit Lebererkrankung zeigten nur erhöhte LFTs, 28% entwickelten eine Fibrose oder Zirrhose, 4% davon entwickelten ein HCC und 20% benötigten eine LTx, 28% der ZZ Phänotypen zeigten eine neonatale Lebererkrankung. 88.1% der MZ-Phänotypen mit Lebererkrankung zeigten nur erhöhte LFTs, 10.2% entwickelten eine Fibrose oder Zirrhose, 1.7% davon entwickelten ein HCC und 5.1% benötigten eine LTx, 1.7% der MZ-Phänotypen zeigten eine neonatale Lebererkrankung. Es gab keine signifikanten Veränderungen von AST- und ALT-Werten über einen Beobachtungszeitraum von mindestens 10 Jahren. Interessanterweise zeigten ZZ-Phänotypen geringere Raten von Lebersteatose als MZ-Phänotypen (8% vs. 27.1%, p=0.026).

Fazit: Unsere Studienpopulation zeigte höhere Raten an fortgeschrittenen Lebererkrankungen als Populationen in anderen Studien. Wir schlussfolgern daraus, dass die Überweisung an unser Tertiärzentrum eher im fortgeschrittenem Stadium der

Erkrankung stattfindet. Im Gegensatz zu Personen mit fortgeschrittenen Lebererkrankungen, zeigten erwachsene Personen, die nur leichte Erhöhungen von LFTs vorweisen, keine Verschlechterung ihrer Erkrankung über den Beobachtungszeitraum von mindestens 10 Jahren. Wir schlussfolgern daraus, dass Personen mit A1AT-Mangel (insbesondere Kinder) konsequente follow-up-Untersuchungen benötigen, um jedwede Verschlechterung der Lebererkrankung schnellstmöglich zu detektieren. Ein interessantes und unerwartetes Ergebnis unserer Studie war, dass Personen mit ZZ-Phänotyp möglicherweise gegen Lebersteatose geschützt sind. Jedoch sollte dieses Ergebnis im größeren Rahmen untersucht werden.

Abstract

Background: A1AT deficiency is an inherited disease, which primarily leads to lung and liver diseases. Due to the low penetrance of liver disease in genetically affected individuals, the variable natural course and limited specific treatment, many patients with A1AT related liver disease remain undiagnosed. The aim of this study is to determine the number of patients with A1AT deficiency and related liver diseases at the Medical University of Graz and to summarize the natural course of liver disease of these patients.

Methods: In this retrospective study we collected data of patients with A1AT deficiency, who visited the Medical University of Graz over the last 16 years. We assessed the time point of diagnosis, severity and complications of liver diseases (i.e. elevated LFTs, steatosis, fibrosis, cirrhosis, HCC, liver transplantation (LTx) or neonatal liver diseases) as well as the natural course and related our findings to the molecular phenotype (i.e. ZZ or MZ phenotype). In addition, we also investigated A1AT deficiency in children and reported the results separately.

Results: In total, 148 patients with A1AT deficiency and known phenotype were identified. 93 (62.8%) showed the MZ phenotype, 40 (27%) showed the ZZ phenotype, 8 (5.4%) showed the SZ phenotype, 5 (3.4%) showed the MS phenotype and 2 (1.4%) showed the MMalton phenotype. 62.5% of the ZZ phenotypes and 63.4% of the MZ phenotypes showed signs of liver disease. In ZZ phenotypes with liver disease, 52% showed only biochemical signs of liver disease, 28% developed fibrosis or cirrhosis, with 4% developing HCC and 20% requiring LTx. 28% of the ZZ phenotypes presented with neonatal liver diseases in infancy. In MZ phenotypes with liver disease, 88.1% showed only biochemical signs of liver disease, 10.2% developed fibrosis or cirrhosis, with 1.7% developing HCC and 5.1% requiring LTx. Only 1.7% of MZ phenotypes presented with neonatal liver diseases in infancy. There was no significant change in mean AST or ALT values over an observation period of at least 10 years in both ZZ and MZ phenotypes. Interestingly, ZZ phenotypes showed significantly lower rates of liver steatosis than MZ phenotypes (8% vs. 27.1%, p=0.026).

Conclusions: Our study population showed a higher percentage of advanced liver diseases than usually reported in population studies. From this observation we conclude that referral to our tertiary hospital center is more likely taking place only at late disease stages. In contrast to the patients with advanced liver diseases, those adult patients with only mild elevations of LFTs did not progress over an observation period of at least 10 years.

Therefore we conclude, that patients with A1AT deficiency, especially pediatric patients, need to be consequently followed up to detect any deterioration in their liver status early. An interesting and unexpected finding of our study was that patients with ZZ phenotype might potentially be protected from liver steatosis. This, however, needs to be determined in larger setting.

1 Introduction

Alpha1-Antitrypsin deficiency is an inherited disease, which follows an autosomal-codominant inheritance pattern(1). Carl-Bertil Laurell and Sten Eriksson, who were the first to report on this disease in 1963, associated low serum levels of Alpha1-Antitrypsin (A1AT) with pulmonary emphysema(2). Affected patients have low levels of circulating A1AT, which results in various diseases like early-onset lung emphysema and chronic obstructive pulmonary disease (COPD), hepatic disorders and rare disorders like necrotizing panniculitis or vasculitis(1,2). This thesis reports on the hepatic disorders of A1AT deficiency.

1.1 Alpha1-Antitrypsin – Basics and enzymatic function

A1AT is a serine protease inhibitor, which is mainly produced by hepatocytes and released into the bloodstream by the liver(2).

The main function of A1AT is the inhibition of proteases, primarily the neutrophil elastase (NE) secreted by neutrophil granulocytes. NE, a proteolytic enzyme, is released during neutrophil-mediated inflammation processes and serves as a functional defense mechanism against pathogens. Its proteolytic activity also affects connective tissue matrix (mostly alveolar tissue). A1AT deactivates the proteolytic function of NE, therefore, an balance between A1AT and NE is utterly important for the integrity of alveolar tissue. Serum levels of A1AT are reduced in patients with A1AT deficiency, which results in uncontrollable activity of NE, leading to progressive destruction of alveolar tissue(3).

In addition to its classical antiproteolytic activity, A1AT also shows immunmodulatory function, as it is secreted by macrophages and inhibits TNFα upregulation(3).

1.2 Genetics of Alpha1-Antitrypsin deficiency

The gene encoding A1AT is called SERPINA1 (serine protease inhibitor clade A), often described as PI (protease inhibitor), and is located on chromosome 14q32.1(4). Promoters of SERPINA1 are predominantly active in hepatocytes, but may also be active in other cell types such as macrophages(5).

A1AT deficiency is an autosomal-codominant inherited disease. In affected patients, one or both alleles of the SERPINA1 gene differ from the norm variant, leading to different biological and clinical effects(1). The norm variant/wild type and most common allele type

is PiM, the corresponding norm genotype therefore is called PiMM. This genotype leads to physiologic serum levels and normal activity of A1AT(5,6)

Over 150 polymorphisms in the SERPINA1 gene have been reported, leading to different genetic variants of A1AT and associated diseases(5,7). The most common deficient allele types are PiZ, PiS and PiNull, all of them leading to different serum levels and activity of A1AT. The PiZ allele is characterized by a point mutation of normal PiM, leading to a substitution of glutamine to lysine (Glu342Lys), which results in improper folding of A1AT. The improperly folded A1AT polypeptides polymerize and cannot be secreted properly. This leads to a retention of mutant A1AT polymers inside hepatocytes(5).
The PiS allele is characterized by a point mutation, leading to a substitution of glutamine to valine (Glu264Val). Similar to PiZ, this mutation leads to improper folding of A1AT, but polymer formation is slower. In comparison to PiZ, less A1AT is retained inside hepatocytes(5).
The PiNull allele is characterized by a mutation, which forms a premature stop codon. The unfinished mRNA degenerates fast and no A1AT is translated, resulting in the absence of any A1AT(5).

Table 1. Allele types of A1AT and their characteristics

Allele type	Pathophysiology	Amount of secreted protein	Risk of liver disease	Risk of lung disease
PiM	-	100%	no increase	no increase
PiZ	Improper folding and retention inside hepatocytes	~35%	highly increased	highly increased
PiS	Improper folding and retention inside hepatocytes	~60%	increased	increased
PiNull	Premature stop codon and missing protein translation	0%	no increase	severly increased

Table 1: ***Allele types of A1AT and their characteristics*** **(adapted from (5,6)).**

Inheriting two PiZ alleles leads to the ZZ genotype, which has a very high risk of developing lung emphysema/COPD and liver disease over lifetime. ZZ is the most common deficient genotype worldwide and most reports on pathophysiology of A1AT related liver disease are based on this genotype (see „Pathophysiology of liver disease“)(3,6).
Inheriting two PiS alleles leads to the SS genotype, a milder variant with lower risk of developing associated diseases(6).
Inheriting two PiNull alleles leads to the Null/Null genotype, a variant where no A1AT is produced at all, making inheriting individuals highly vulnerable to lung emphysema/COPD, because of the uncontrollable activity of NE(5). Null/Null individuals

have no risk of developing A1AT related liver disease (for further information see „Pathophysiology of liver disease“)(8).

Besides the described homozygous genotypes, heterozygous variants like MZ, MS, MNull, SZ, ZNull or SNull exist, with each of them having different risks of developing lung and liver disease(3,4,6)

The PiZ allele variant is associated with serum A1AT levels less than 35% compared to the PiM norm variant and is the most common deficient variant worldwide(5). The PiS variant has slightly higher serum levels of A1AT, approximately 60% of the norm variant. PiS is the most common deficient variant in European Caucasians(6). PiNull inheritance is associated with undetectable serum levels of A1AT. No A1AT is produced in individuals with two PiNull alleles(5). Table 1 summarizes the most common allele types of A1AT and their risk for developing lung or liver disease(5,6). In addition to these common polymorphisms, less frequent polymorphisms include PiMMalton or PiSi, but are less investigated due to the very low numbers of affected individuals(5).

1.3 Pathophysiology of liver disease

Liver disease in A1AT deficiency primarily occurs in individuals who carry PiZ alleles, particularly in individuals with ZZ genotype(9), due to following mechanisms:

A1AT is primarily produced in hepatocytes(2). The mutant A1AT of ZZ individuals (Z-type) is properly transcribed and translated. After transcription, the translated, but unfolded protein is translocated into the rough endoplasmic reticulum (rER) of hepatocytes, where chaperons assist in the folding process. The normal M-type protein is folded and secreted within minutes, whereas the mutant Z-type protein folds inappropriately(9). The M-type A1AT protein consists of a reactive center loop (to deactivate proteolytic enzymes) and multiple β-sheet structures that form the final confirmation. In the mutant Z-protein, β-sheet A of one molecule is widened and irreversibly linked to the reactive center loop of another molecule, forming polymers of Z-proteins, which aggregate in the rER of hepatocytes (Figure 1). This aggregations can be large enough to be seen in light microscopy as diastase-resistant periodic acid Schiff positive (PAS-D) inclusions (Figure 2)(1,5,9). Some Z-proteins do not polymerize with other proteins, remaining in a monomeric state(9).

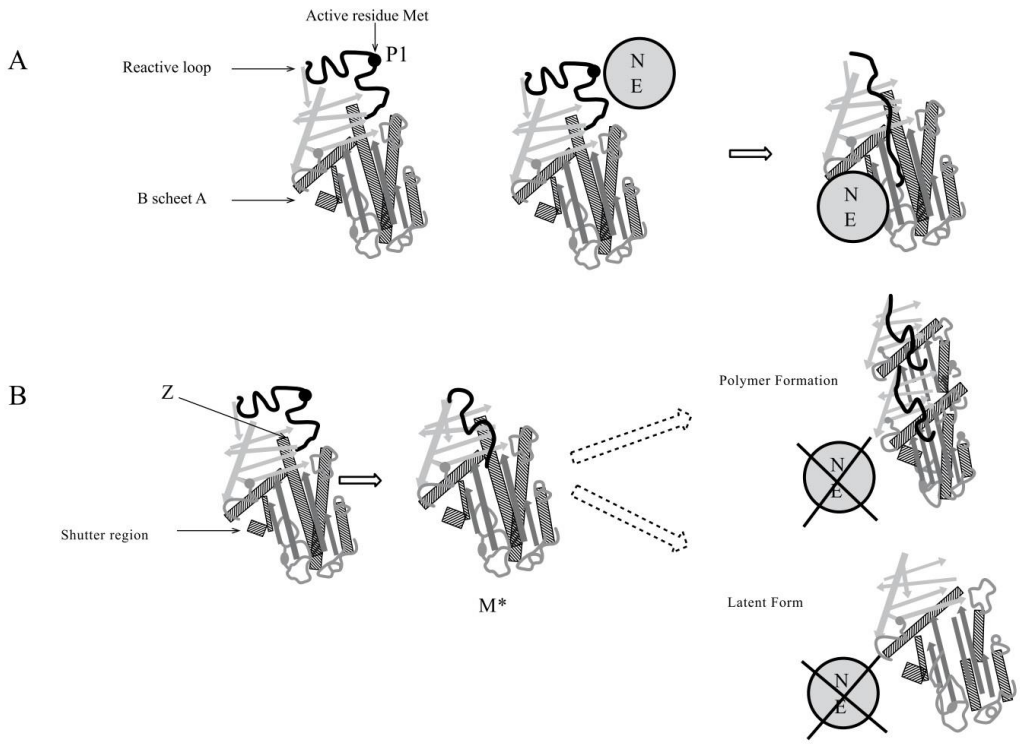

Figure 1: ***Structure of normal and mutated A1AT*****: (A) shows normal A1AT protein structure and inhibition of the neutrophil elastase (NE). After binding NE, the reactive center loop of A1AT moves from the upper to the lower pole of the protein, where it binds to β-sheet A, forming an irreversibly protein formation and deactivating NE irreversibly. (B) shows the pathophysiology of the mutant Z-protein. β-sheet A is widened and the reactive loop center of the same A1AT protein is able to react with β-sheet A (without binding NE previously), forming an intermediate form (M*). This form either reacts with the reactive center loop of another A1AT protein (leading to polymer formation), or binds its own reactive center loop irreversibly (leading to a latent form). Either way, the resulting protein complexes are not able to bind and deactivate NE properly (image taken from (3)).**

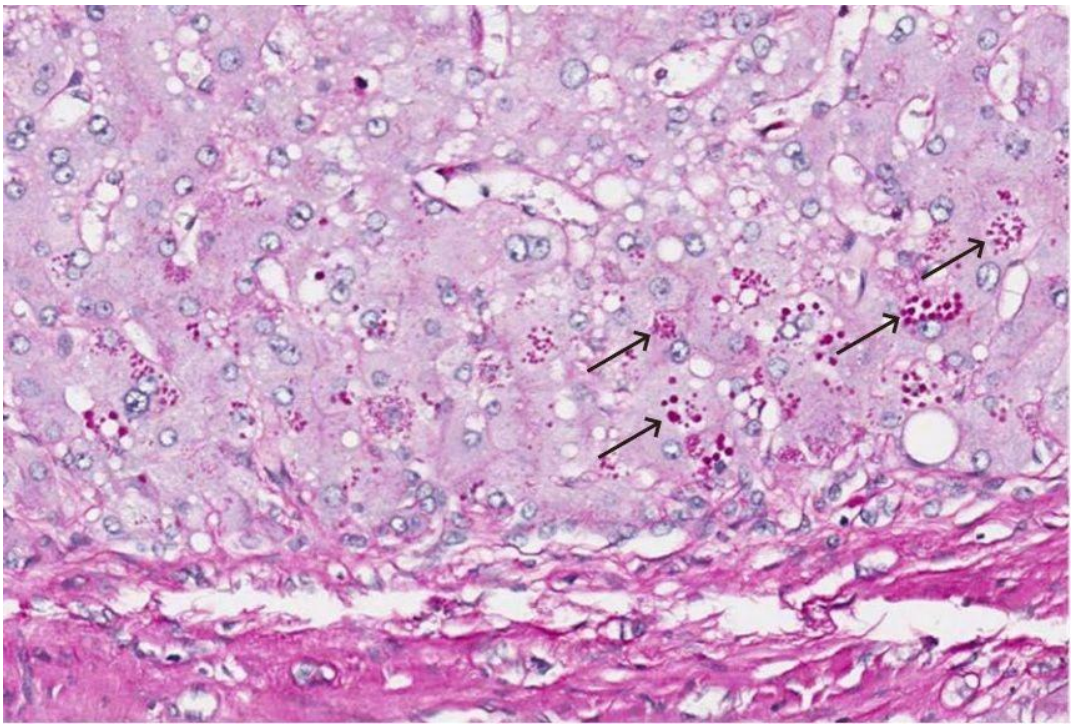

Figure 2: ***Liver histology of A1AT deficiency*****: The PAS with diastase stain shows the diastase-resistant pink intracytoplasmic globules (arrows) that are characteristic of this disease (image taken from**(10)**).**

Due to secretion failure of the Z-protein, 85% of produced A1AT is retained in hepatocytes. 15% of A1AT is secreted and explains, that despite the mutation a small amount of A1AT can still be found in serum of ZZ individuals. Retained A1AT causes ER stress and consequently provokes a stress response to restore cell homeostasis. Monomeric retained proteins are directed to endoplasmic reticulum associated degradation pathways (ERAD), which consist of ubiquitin-dependent and ubiquitin-independent proteasomal pathways. Polymerized proteins, which are too large to be degraded by ERAD pathways, are inducing autophagy, in which whole cell organelles can be degraded. Through autophagy, the masses of polymerized proteins surrounded by rER can be degraded, which reduces intracellular accumulations of retained A1AT. However, these adaptive pathways are not sufficient enough to degrade the continuously accumulating misfolded proteins. In fact, in some hepatocytes the degradation pathways are more efficient than in other hepatocytes, leading to heterogenous accumulations of retained proteins in hepatocytes of the same individual(9).

Hepatocytes with the largest amount of retained proteins activate apoptosis via caspase activity. Hepatocytes with the lowest amount of retained proteins proliferate to obtain functional liver mass. This circle of chronic hepatocellular death and regeneration leads to fibrosis and cirrhosis of hepatic tissue, similar to other chronic hepatic diseases, even leading to malign transformation with a high risk of developing hepatocellular carcinoma (HCC)(9). The pathophysiology of liver injury in patients with a SS genotype is comparable, but with a much slower rate of polymer formation, explaining the milder form of hepatic disease in SS individuals(5).

As only 10-50% of ZZ genotype individuals suffer from Z-protein associated hepatic disorders, this mechanisms do not provide a full explanation of the pathophysiology. Other genetic modifiers and environmental factors, such as gender, BMI, alcoholic or viral hepatitis seem to play a role in outbreak and progression of this hepatic disorder(9,11,12).

The pathomechanism also explains why individuals with Null/Null genotype do not suffer from A1AT related hepatic disorders. As no A1AT is produced in hepatocytes at all, no A1AT is retained in hepatocytes. However, the Null/Null genotype results in non-detectable amounts of A1AT in serum, leading to an extremely high risk of developing lung emphysema/COPD due to the complete loss of protease inhibitory function(5,8).

1.4 Epidemiology

A1AT deficiency is an underdiagnosed disease, therefore general prevalence numbers are marked with uncertainty. The lack of awareness of this disease among physicians, in combination with expensive and not-widely available tests contribute to underdiagnosis. Only 10% of affected individuals are considered to be correctly diagnosed, leaving 90% of patients with A1AT deficiency without diagnosis(3).

A1AT deficiency is a worldwide disease, although the prevalence is highest in western European countries and North America. The reported global number of ZZ, SZ and SS genotypes varies between 1.2 Million and 3.4 Million in different studies(7,12).

The prevalence of A1AT deficiency in newborns has been determined in population studies, with a Swedish cohort study being the largest one. From 1972 to 1974, 200.000 newborns in Sweden were being screened. 127 individuals with ZZ genotype were reported, estimating a prevalence of 1 in 1.600 newborns(2). Studies from other European regions showed, that the prevalence significantly varies among different European countries, with Scandinavian countries having the largest number, and southern countries having the lowest number of ZZ individuals(13). PiZ mutation therefore seems to be arisen in Scandinavia(2,7). The frequency of the PiS allele is highest in Spain and Portugal, with a prevalence of SS individuals of about 1 in 80 newborns(13). In general, the prevalence of ZZ genotype in western European countries is estimated to be 1 in 2.500 newborns(2).

Although the genetic prevalence of A1AT deficiency is high, only a small proportion of affected individuals are diagnosed and develop liver disease over lifetime(11,12)

As there is little data on the number of affected patients with A1AT deficiency in Austria, one of this study's tasks is to determine the number of patients with A1AT deficiency, which have been referred to or diagnosed at the Medical University of Graz.

1.5 Clinical manifestations of liver disease

Liver disease in A1AT deficient individuals typically presents in two peaks. One peak at early childhood and another peak at the age of approximately 50 years(11,14). The reason for this clinical course is not known.

1.5.1 Liver disease in children

Presentation and clinical outcome of liver disease among ZZ children is highly variable. Some children only present with asymptomatic increase in serum liver function tests (LFTs) or bilirubin, whilst other children present either with prolonged jaundice, hepatomegaly/hepatosplenomegaly, failure to thrive or a complex of these symptoms known as neonatal hepatitis syndrome(8). Some children present with a vitamin K deficient coagulopathy with gastrointestinal haemorrhage due to the liver disease(8,11). The occurence of these symptoms ranges between 15-50%, meaning that only a proportion of ZZ individuals develop disease related processes(15).

Disease progression is also highly variable. Most children fully recover from the neonatal diseases, while less than 1% of children with a ZZ genotype develop liver failure in infancy, requiring liver transplantation(11).
About 8% of children develop chronic liver disease until reaching adolescence, including liver fibrosis and liver cirrhosis in compensated and decompensated state. Overall, 16% of affected children with progressive chronic liver disease require liver transplantation (LTx)(11). Transplantation outcomes in children are excellent, with no signs and symptoms of A1AT related lung and liver disease in most children(12,14). A1AT deficiency is, after biliary atresia, the second most common cause of liver transplantation in children (12% of all LTx), whereas only 1% of liver transplantations in adults is due to A1AT deficiency(16,17).
There is a wide range of liver related mortality in children with A1AT deficiency reported among different studies. While one study reported 0% mortality after 20 years of follow-up, another study reported 25.5% mortality among pediatric patients, which have been referred to a tertiary center(14).

There are reported factors associated with a higher risk of developing severe liver disease, including male gender, neonatal hepatitis syndrome, persistent elevated levels of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) or bilirubin and prolonged prothrombin time(PT)(8,11,14). Children with described factors need close monitoring of their disease, to detect advancing liver disease at an early stage.

1.5.2 Liver disease in adults

As already mentioned in „Pathophysiology of liver disease“, liver diseases in ZZ adults are based on a chronic cycle of cell death and regeneration, leading to hepatic fibrosis and cirrhosis in about 10% of affected adults with A1AT related liver disease(9,11).
The natural course is poorly investigated, due to highly variable penetrance and progression of liver disease. Affected individuals can be completely asymptomatic with only elevated LFTs, whilst others develop more complicated forms with compensated hepatic fibrosis or cirrhosis, or even decompensated forms of chronic liver disease, leading to complications such as ascites, esophageal varices and haemorrhage and requiring LTx(18,19). 14.7% of adults with progressive liver disease require LTx(14). Whereas HCC is not reported in children, adults develop HCC with the same incidence as in cirrhosis of other etiologies(11).

The reason why some individuals develop such a severe disease, whilst others remain completely asymptomatic is currently unknown. Genetic and environmental factors seem to play a role and need more investigation(9,11,12). Some factors are established to increase the risk of acquiring severe liver disease, such as higher age (mean age of 34 - 54 years), male gender and increased body mass index (BMI). Other factors, such as alcohol consumption and viral hepatitis also seem to increase the risk of severe liver disease, but study results are conflicting(11,19,20). Vice versa, other studies suggest that presence of A1AT deficiency increases the risk of severe liver disease of other etiologies, such as alcoholic liver disease or non-alcoholic fatty liver disease (NAFLD)(21,22). Liver-related mortality in adults with the ZZ phenotype ranges from 2.3 – 22%(14).
Due to the described variations in liver disease, the disease’s natural course is poorly understood. **Therefore, one task of this study is to report on the natural course of liver disease on the base of changes of LFTs over time, as well as the determination of the number of patients with different severities of A1AT related liver disease.**

1.6 Diagnosis of Alpha1-Antitrypsin deficiency

It is estimated that approximately 90% of patients with A1AT are currently undiagnosed and time between onset of symptoms and correct diagnosis is 6 years on average(5,6,12). There are three strategies commonly used to identify individuals with A1AT deficiency:

measurement of serum A1AT levels, A1AT protein phenotyping in serum and A1AT genotyping(6,12).

Measurement of serum levels of A1AT is available at many laboratories and is typically the first diagnostic approach when A1AT deficiency is suspected (such as in infants with neonatal hepatitis syndrome, prolonged jaundice or failure to thrive or in adults with otherwise unexplained lung or liver diseases). This test quantifies A1AT levels in serum or plasma (normal range between 0.9 and 2.0 g/L). There are several limitations of this test: First, A1AT is an acute phase protein and A1AT levels raise in inflammatory conditions and could therefore obscure an A1AT deficiency, if tested in these conditions. Second, heterozygous individuals often have serum levels of A1AT at or near the normal range, meaning that those individuals cannot be identified via this test. These two reasons lower the sensitivity (between 73.4% and 100% depending on cut-off point) and specifity (between 78% and 94.8% depending on cut-off point) of this test, making further assessments necessary. In individuals with reduced levels of A1AT, protein phenotyping or genotyping should be performed. Individuals, which are highly suspicious to suffer from A1AT deficiency lung or liver disease but have normal levels of A1AT in serum, should also be considered for the more accurate phenotyping or genotyping(3,6,23).

In protein phenotyping, the different isoform patterns of A1AT molecules are evaluated by different migration in electrophoretic fields. With this test, different types of A1AT (like Z-type, S-Type or M-Type) can be identified (Figure 3). One limitation of this test is that it cannot identify individuals with Null/Null genotype, since these genotypes produce no A1AT at all. Another limitation is that ZZ individuals and Z/Null individuals cannot be differentiated, since both produce Z-type A1AT (the same problem applies for differentiation between SS and S/Null and other homozygous variants)(3,5,6).

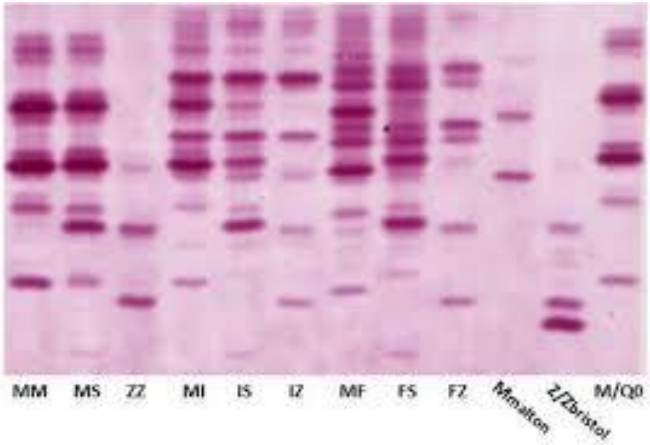

Figure 3: ***Isoelectric focusing and immunofixation of A1AT*****: Different protein phenotypes show different migration in isoelectric focusing and immunofixation(image taken from**(24)**).**

Genotyping allows to identify known mutations by allele-specific amplification. Most genotyping kits are based on polymerase chain reaction (PCR) or restriction fragment length polymorphism (RFLP) and use dried blood spots to identify the most common abnormal allele variants. As PCR needs a known nucleotide sequence and defined primers, the number of different mutations that can be identified is limited. While commonly available genotyping kits identify the most common mutations and alleles, such as PiZ or PiS and some less-common variants, very rare mutations like PiF or PiMheerlen are not recognized(3,5,6,24).

1.7 Assessment of liver disease

Since only 10% of patients with A1AT deficiency develop liver disease, and liver disease can be highly variable among affected individuals, an uniform procedure of liver disease assessment is difficult to establish(11,18,19).

On newly diagnosed patients with A1AT deficiency, taking a medical history and performing a physical examination is required. Further strategies are varying between hepatologists. These strategies include assessment of liver function tests (LFTs), albumin, thrombocytes and INR, performing measurements of non-invasive fibrosis parameters with different scores or elastography, liver sonography, measurement of alpha fetoprotein (AFP) as a hint for HCC or even obtaining a liver biopsy specimen for histopathological analysis. The frequency and role of these different strategies are controversial, since there is no clear evidence(6,19,25). As A1AT deficiency could be increasing the severity of liver diseases of other etiologies, like alcoholic liver diseases or viral hepatitis, assessment of alcohol consumption and serology for viral hepatitis should be performed(21,22).

1.8 Therapy of liver disease

Other than A1AT related lung disease, liver disease currently has no available specific treatment. Lung disease in A1AT deficient individuals can be specifically treated with augmentation therapy of A1AT in intravenous or aerosolized application. The lack of A1AT in serum and therefore the overwhelming activity of NE is compensated by augmentation therapy and slows down the progression of lung emphysema/COPD, although the statistical significance is only marginally(3,5–7,12).

As liver disease does not develop on the base of lacking A1AT in peripheral blood, but on the base of A1AT being accumulated in hepatocytes, A1AT augmentation therapy is ineffective(6,9).

Since there is no currently available specific treatment for liver disease, recommendations for managing A1AT related liver disease is based on prevention of additional risk factors and treatment of potentially existing additional chronic liver diseases. Risk factors such as excessive weight gain and morbid obesity should be avoided, since high BMI is a known risk factor for progression and development of severe liver disease(6,11). Even though alcohol consumption and viral hepatitis are uncertain risk factors, alcohol intake should be minimalized and patients should be vaccinated against hepatitis A and B(6,20).
Chronic liver disease, which progresses to liver fibrosis and cirrhosis are also currently treated like chronic liver disease of other etiologies. This includes screening and treatment of complications of portal hypertension, such as screening and management of esophageal varices, treatment of ascites or hepatic encephalopathy and importantly HCC screening, if the chronic liver disease has progressed to liver cirrhosis. Liver transplantation is the only curative treatment currently available, since the transplanted liver produces A1AT in a physiologic amount(6,7).

1.8.1 Future therapeutic strategies for A1AT related liver disease

There are several experimental approaches, which are currently under investigation and may represent future therapeutic options.
One approach is to reduce the synthesis of Z-type A1AT. With small interfering RNA molecules (siRNA), synthesis of mutant A1AT can be downregulated, which prevents the accumulation of A1AT inside hepatocytes. In a PiZ murine model, a complete reversal of the liver injury was reported(26). Otherwise, since there is less production of A1AT, the risk of developing severe lung disease is much higher. Consequently, patients who would receive such a therapy should likely also receive augmentation therapy(7,15).
Another option targets the intracellular degradation of mutant Z-type protein. Some substances enhance the autophagic pathway in hepatocytes, leading to a lesser burden of accumulated protein and reduced liver injury in murine models. Sirolimus and carpamazebine are known to enhance autophagy, but need extremely high doses to attain this effect(7). Recently, ursodeoxycholic acid (UDCA) has been shown to robustly stimulate hepatic autophagy in patients in a concentration used to treat cholestatic liver

diseases(27). This would make UDCA an interesting compound for the treatment of A1AT related liver disease. However, an earlier clinical trial in children with A1AT related liver disease treated with UDCA showed mixed results(28).

Other options aim to inhibit the polymerization of A1AT molecules or to improve proper folding of A1AT, such as 4-phenyl butyrate, but need more investigation(7,15).

An option, which could treat both liver and lung disease, is gene therapy, in which defective genes are replaced with a functional gene variant. Therefore, the functional gene needs to be delivered to affected cells. Recombinant adeno associated viral vectors (rAVV) are currently under investigation, besides the CRISPR-CAS method(5).

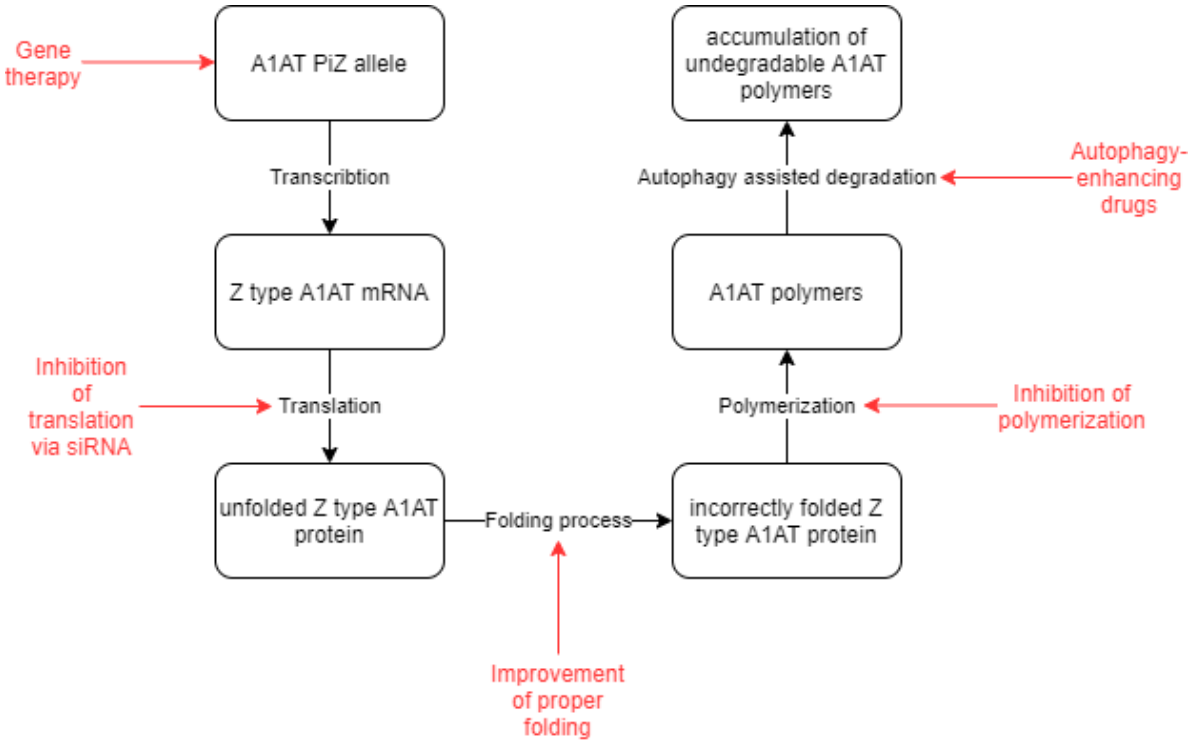

Figure 4: ***Pathophysiology and treatment options of hepatic A1AT deficiency***

Overall, there are many therapeutic approaches currently under investigation, which will hopefully be widely available in the near future.

1.9 Research issue

Due to the low penetrance of liver disease in A1AT deficient individuals, the variable natural course and limited specific treatment of liver disease, many patients with A1AT related liver disease remain undiagnosed. Furthermore, many patients are „lost of follow-up“, when transferred from pediatric to adult care. This leads not only to a loss of important data regarding number of affected patients, disease prevalence and natural

course of liver disease, but also to a loss of medical care and monitoring of this potentially severe disease.

Therefore, one of this study is to determine the number of patients with A1AT deficiency and liver disease which have been diagnosed at or referred to the Medical University of Graz. Another aim of this study is to summarize the natural course of liver disease of these patients. Therefore, we investigate the frequency and severity of A1AT related liver diseases in affected adult and pediatric individuals, as well as changes of LFTs over time, in a retrospective manner.

2 Methods

2.1 Study design & source of data

We performed a retrospective study, where we collected patient data from patients with A1AT deficiency at the Medical University of Graz over a period of 16 years, from January 2004 to February 2020. This data included disease specific characteristics, such as serum levels of A1AT and phenotype as well as general hepatologic parameters, such as AST, ALT, GGT, bilirubin, platelet count, albumin and INR. Personally identifying information and collected data were saved on two password protected computers and were only accessible for associates of this study.

To identify patients with A1AT deficiency, we requested a data search at the Institute for Medical Information Technology (IMI) at the Medical University of Graz, where ICD codes, decreased serum levels of A1AT and key words were used to identify relevant patients.

After identification we used MEDOCS, the patient data documentation system at the Medical University of Graz, to gather patient specific data and laboratory parameters. We extracted data of all relevant patients at all consultations at the Medical University of Graz between January 2004 and February 2020, especially data from the date of diagnosis and the date of latest follow-up.

Ethical approval was provided by the Ethics Committees of the Medical University of Graz (EK 32-246 ex 19/20).

2.2 Patient population

All patients, children and adults, with confirmed diagnosis of A1AT deficiency were included in this study. For statistical analysis of patient characteristics and laboratory parameters, only patients with A1AT related liver disease and known phenotype were further considered.

Liver disease was defined as any abnormal LFTs, i.e. increase of AST >35U/L, increase of ALT >45U/L, presence or history of advanced liver disease (fibrosis/cirrhosis), presence or history of hepatocellular carcinoma, history of liver transplantation or presence or history of neonatal liver disease.

2.3 Study procedures and outcomes

First, the frequency of different phenotypes and liver and lung disease was analyzed. This step provided the information about the number of patients with A1AT deficiency at the Medical University of Graz, as well as the number of patients with associated liver disease. We investigated the age of diagnosis in different phenotypes in order to reproduce the typical two peaks in age, that are characteristic of A1AT related liver disease, and to separate further analysis into adult and pediatric groups(11,14). In further analysis, only data of ZZ and MZ individuals were considered, since the number of patients with other phenotypes were too low to be representative.

Second, we analyzed the frequency and severity of the liver disease in affected individuals, to display the highly variable natural course of liver involvement. Therefore, we investigated the number of patients with advanced liver disease (fibrosis/cirrhosis), HCC, liver transplantation, neonatal liver disease or only increase in AST/ALT (without signs and symptoms of mentioned conditions above). In patients with neonatal liver disease, we investigated the evolution of liver disease over the entire reported observation period. Therefore, the number of patients with complete recovery, advancing liver disease or liver transplantation after neonatal liver disease in infancy are reported. Potential confounders, like alcohol consumption, high BMI and additional liver diseases like liver steatosis and viral hepatitis were considered in analysis and retrieved from MEDOCS.

Third, we report on the natural course of liver disease on the base of changes of LFTs over time. Therefore, AST and ALT values of the date of diagnosis and the date of latest follow-up were compared. To gather representative results regarding changes of laboratory parameters over time, only patients with an observation period ≥10 years were considered in this analysis. In the adult group, we expected an increase of AST and ALT over the observation period (as a sign for advancing liver disease). In the pediatric group, we expected a decrease of AST and ALT over the observation period (as a sign for resolving liver disease in the post infancy period.
Additionally, we compared laboratory parameters between ZZ and MZ individuals with A1AT related liver disease, to gain knowledge about possible differences.

At last, we investigated the number of patients with A1AT related liver disease, which had typical histological features (if histology was available), such as PAS-D positive intracytoplasmic globules or positive immunohistochemical reaction against A1AT(1,9).

2.4 Statistical analysis

Data was collected with Microsoft Excel© 2016. All statistical analysis were performed with IBM SPSS Statistics 26©. In qualitative values (gender, presence of different phenotypes and different liver diseases, presence of confounders) absolute and relative frequencies were measured. Quantitative values (laboratory parameters, age) are presented as means or medians with range.

Differences in laboratory parameters over time were compared with the use of Wilcoxon test. Differences in qualitative values were compared using Chi-squared test. A p-value <0.05 was considered statistically significant.

Statistical graphics were created with IBM SPSS Statistics 26© and Microsoft Excel© 2016.

3 Results

3.1 Number of patients with A1AT deficiency and their phenotype

250 patients were identified by the data search of the IMI. After exclusion of patients, which had either no further data supporting diagnosis of A1AT deficiency (n=32) or inconclusive data (n=11), 207 patients at the Medical University of Graz were defined as having A1AT deficiency. In 59 patients, no phenotype was accessible, either because of missing process of phenotyping or the result of phenotyping could not be retrieved from MEDOCS. Thus, we could extract 148 patients, which have A1AT deficiency with a known phenotype (Figure 5).

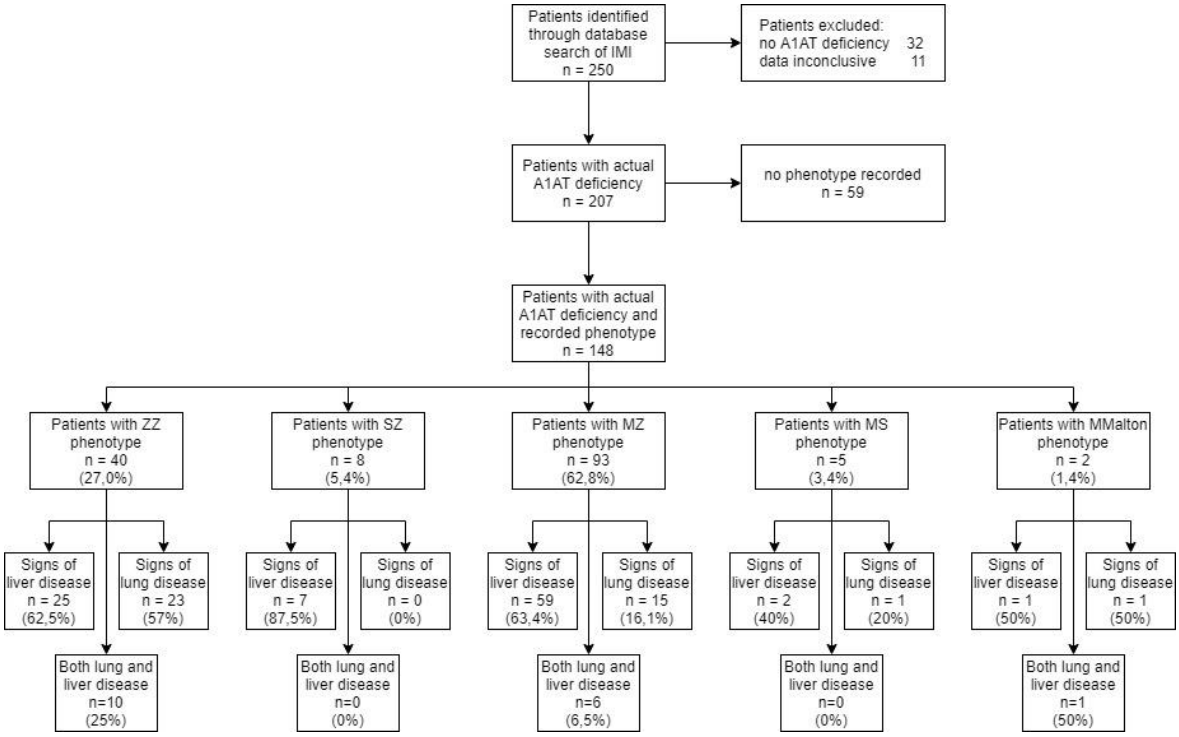

Figure 5: ***Data separation process of patients at the Medical University of Graz*****: This flowchart shows the data separation process of identified patients. Patients were excluded if they had no A1AT deficiency at all or if data were inconclusive (i.e. suspected A1AT deficiency but without confirmation). Further analysis required known phenotype, therefore patients in which phenotype was not traceable were excluded. Liver disease was defined as an increase of AST >35U/L, increase of ALT >45U/L, presence or history of advanced liver disease (fibrosis/cirrhosis), presence or history of hepatocellular carcinoma, history of liver transplantation or presence or history of neonatal liver disease. Lung disease was defined as presence of obstructive ventilation failure, lung emphysema, COPD or history of lung transplantation.**

Most of these patients showed a MZ phenotype (n=93; 62.8%). The ZZ phenotype was reported in 40 patients (27.0%), making it the most frequent found phenotype, in which both alleles carry mutations. The SZ phenotype was found in 8 patients (5.4%), the MS

phenotype was found in 5 patients (3.4%) and the rare MMalton phenotype was found in 2 patients (1.4%) (Figure 6).

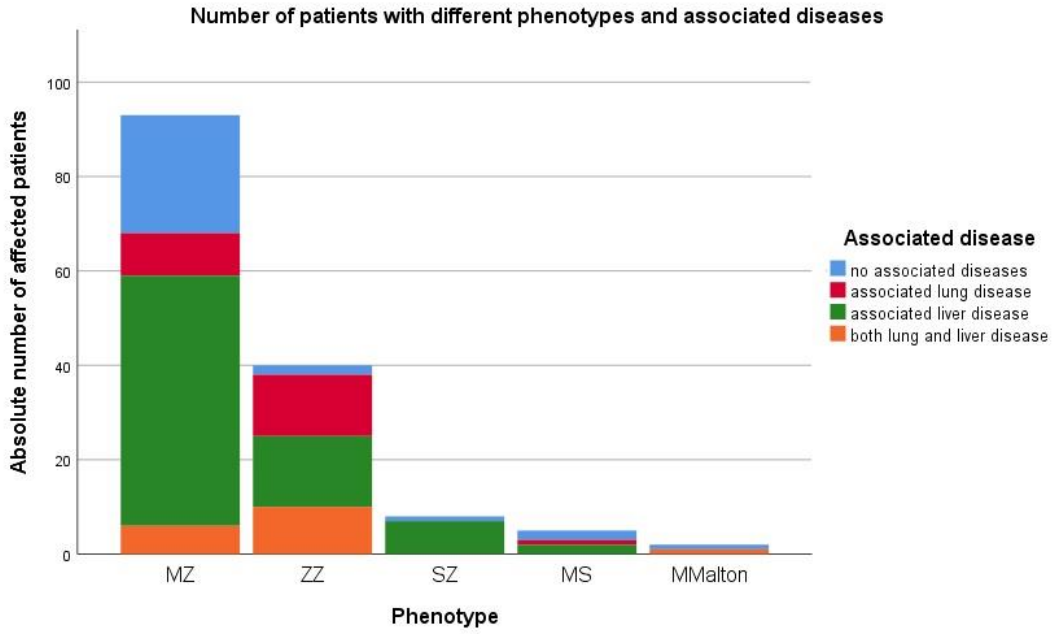

Figure 6: ***Absolute numbers of different phenotypes and associated diseases in patients with A1AT deficiency***

In MZ phenotypes, 25 of 93 patients (26.8%) showed no signs of liver and lung disease, 9 of 93 patients (9.7%) showed only signs of lung disease, 53 of 93 patients (57%) showed only signs of liver disease and 6 of 93 patients (6.5%) showed signs of both liver and lung disease.

In ZZ phenotypes, 2 of 40 patients (5%) showed no signs of liver and lung disease, 13 of 40 patients (32.5%) showed only signs of lung disease, 15 of 40 patients (37.5%) showed only signs of liver disease and 10 of 40 patients (25%) showed signs of both liver and lung disease.

In SZ phenotypes, 1 of 8 patients (12.5%) showed no signs of liver and lung disease and 7 of 8 patients (87.5%) showed only signs of liver disease.

In MS phenotypes, 2 of 5 patients (40%) showed no signs of liver and lung disease, 1 of 5 patients (20%) showed only signs of lung disease and 2 of 5 patients (40%) showed only signs of liver disease.

In MMalton phenotypes 1 of 2 patients (50%) showed no signs of liver and lung disease and 1 of 2 patients (50%) showed signs of both liver and lung disease.

Frequency of liver disease and lung disease in A1AT deficient individuals varied among different phenotypes. Out of all patients with the ZZ phenotype, 25 patients (62.5%)

showed signs of liver disease. In patients with the MZ phenotype, 59 patients (63.4%) showed signs of liver disease and therefore met inclusion criteria for further analysis of liver disease. As there was a relatively low number of patients with the SZ, MS and MMalton phenotype, only patients with the ZZ and MZ phenotype were considered in further analysis of liver disease.

3.2 Age of diagnosis in patients with A1AT deficiency

Overall, the distribution of age, in which A1AT deficiency in different phenotypes was diagnosed, shows the expected two peaks, one between 0 and 5 years of age, one between 50 and 55 years. Importantly, this finding indicates that our study population resembles the typical age distribution pattern, which is also known from larger international studies(5) and thus strongly suggests that our study population is representative of individuals suffering from A1AT deficiency liver disease (Figure 7).

In ZZ phenotypes, one peak of age of diagnosis was at early childhood between the age of 0 and 5 (-10) years. A second peak appeared between 45 and 70 years. No patient with the ZZ phenotype was diagnosed between 10 and 25 years.

Due to the low number of patients with the SZ phenotype, detailed description of the distribution was not useful, but the age of diagnosis was in similar range as in ZZ phenotypes.

In MZ phenotypes, most diagnoses were made in patients between 35 and 60 years and between 0 and 5 years. In contrast to ZZ phenotypes, patients with the MZ phenotype were diagnosed over the entire observed range of age.

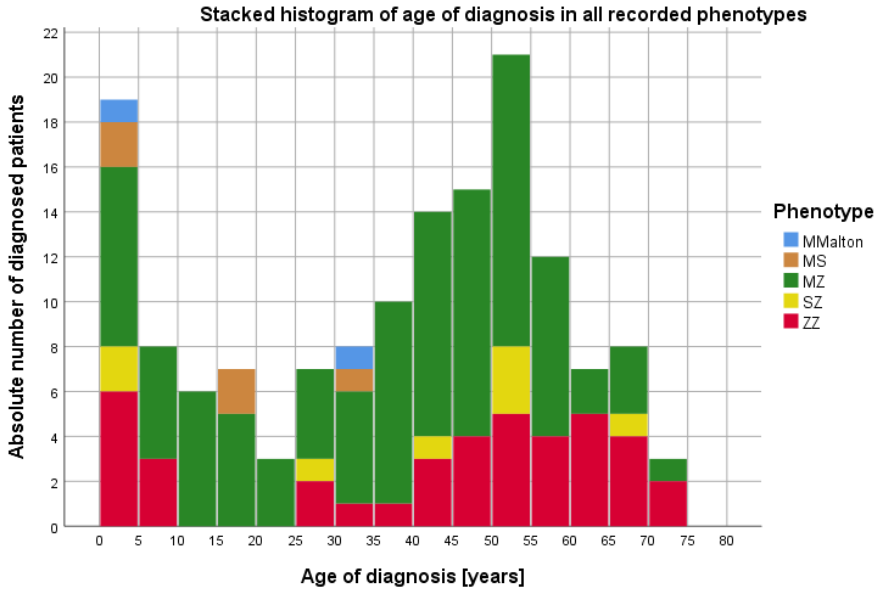

Figure 7: ***Age of diagnosis of A1AT deficiency in different phenotypes***

3.3 Natural course of liver disease

As further analysis required a separation of adult and pediatric patients, all patients, whose age of diagnosis was ≥18 years were put into the adult group. All patients, whose age of diagnosis was <18 years were put into the pediatric group.

Demographic and baseline characteristics are shown in Table 2. Out of all ZZ patients with A1AT related liver disease (n=25), 16 patients (64%) were adults and 9 patients (36%) were children. Out of all MZ patients with A1AT related liver disease (n=59), 48 patients (81.3%) were adults and 11 patients (18.7%) were children. Liver disease-associated cofactors (as shown in Table 2) were only detected in the adult group.

Table 2. Demographic and Baseline Characteristics of patients with A1AT deficiency and liver disease

	ZZ (n=25)	MZ (n=59)
Gender		
Male - no. (%)	15 (60.0)	37 (62.7)
Female - no. (%)	10 (40.0)	22 (37.3)
A1AT-level - mean (range)*	0.40 (0.14 - 1.01)	0.82 (0.56 - 1.25)
Observation characteristics		
Adult patients - no. (%)†	16 (64)	48 (81.3)
Age of diagnosis - median (range)‡	58 (26 - 72)	45 (18 - 71)
Age of latest follow-up - median (range)‡	67 (40 - 86)	55 (18 - 80)
Observation period - mean (range)¶	10 (2 - 15)	8 (0 - 15)
Number of contacts - median (range)ᔓ	17 (5 - 71)	7 (2 - 53)
Pediatric patients - no. (%)ᔕ	9 (36)	11 (18.7)
Age of diagnosis - median (range)‡	0 (0 - 7)	4 (0 - 13)
Age of latest follow-up - median (range)‡	15 (6 - 22)	11 (2 - 27)
Observation period - mean (range)¶	13 (5 - 22)	8 (0 - 21)
Number of contacts - median (range)ᔓ	20 (4 - 27)	5 (1 - 29)
Liver disease-associated cofactors Ψ		
Alcohol consumption (>20g/d) - no. (%)	0 (0.0)	4 (6.8)
BMI > 30 - no. (%)	4 (16.0)	11 (18.6)
Steatosis hepatis - no. (%)	2 (8.0)	16 (27.1)
Viral hepatitis - no. (%)	0 (0.0)	4 (6.8)

*Serum concentration of A1AT measured in g/L (normal serum range: 0.9 - 2.0 g/L)
†Includes patients with A1AT deficiency liver disease, whose age of diagnosis was >18a. Since A1AT deficiency liver disease has two peak ages, one in early childhood and one in 5th decade, we decided to split patients into adult and pediatric patient groups
‡Age of diagnosis and age of latest follow-up both measured in years
¶Observation period describes the period between age of diagnosis and age of latest follow-up, measured in years
ᔓNumber of contacts describes contacts of patients with any medical facility, in which at least AST/ALT measurements were performed
ᔕIncludes patients with A1AT deficiency liver disease, whose age of diagnosis was <18a
ΨLiver disease-associated cofactors only appeared in adult group

Table 2: ***Demographic and Baseline Characteristics of patients with A1AT related liver disease***

Table 3 shows the frequency of different degrees of liver diseases in patients with A1AT deficiency and displays the variable natural course.

In ZZ phenotypes, 13 of 25 patients (52%) showed only biochemical signs of liver disease, displayed as an increase of AST and/or ALT (11 adults, 2 children), with 5 of these patients showing liver disease-associated cofactors (3 with a BMI >30, 1 with steatosis hepatis, 1 showing both BMI >30 and steatosis hepatis). 7 of 25 patients (28%) developed advanced liver disease (5 adults, 2 children), with 1 adult (4%) developing HCC and 5 patients (20%) requiring liver transplantation (4 adults, 1 child). None of these patients showed liver disease-associated cofactors.
7 of 25 (28%) patients had neonatal liver diseases in infancy, 2 of these patients went on to develop advanced liver disease later in life (counted in both table rows).
Overall, in the adult group 11 of 16 patients (68.8%) showed only biochemical signs of liver disease. 5 of 16 patients (31.2%) developed advanced liver disease, 1 of these patients (6.2%) developed HCC and 3 of these patients (18.8%) required liver transplantation.
Overall, in the pediatric group, 2 of 9 patients (22.2%) showed only biochemical signs of liver disease. 7 of 9 patients (77.8%) had neonatal liver disease in infancy and 2 of these patients (22.2%) went on to develop advanced liver disease later in life, with one child (11.1%) requiring liver transplantation.

In MZ phenotypes, 52 of 59 patients (88.1%) showed only biochemical signs of liver disease (42 adults, 10 children), with 19 of these patients showing relevant liver disease-associated cofactors (alcohol consumption, BMI >30, steatosis hepatis, viral hepatitis). 6 of 59 patients (10.2%) developed advanced liver disease (1 of these patients had chronic hepatitis C, 1 showed severe alcohol consumption), with 1 patient (1.7%) developing HCC and 3 patients (5.1%) requiring liver transplantation. All patients who showed advanced liver disease were adults. 1 of 59 patients (1.7%) showed neonatal liver disease in infancy.
Overall, in the adult group 42 of 48 patients (87.5%) showed only biochemical signs of liver disease. 6 of 48 (12.5%) developed advanced liver disease, 1 of these patients (2.1%) developed HCC and 3 of these patients (6.2%) required liver transplantation.
Overall, in the pediatric group, 10 of 11 patients (90.9%) showed only biochemical signs of liver disease. 1 of 11 patients (9.1%) had neonatal liver disease in infancy.

Table 3. Frequency of different liver diseases in patients with A1AT deficiency		
	ZZ	MZ
Patients with A1AT deficiency liver disease - no. (%)*	25 (100)	59 (100)
Adult patients†	16 (64)	48 (81.3)
Increase in AST/ALT only - no. (%)‡	11 (44)	42 (71.1)
Advanced liver disease (fibrosis/cirrhosis) - no. (%)	5 (20)	6 (10.2)
Hepatocellular carcinoma - no. (%)	1 (4)	1 (1.7)
Liver transplantation - no. (%)	3 (12)	3 (5.1)
Pediatric patients¶	9 (36)	11 (18.7)
Increase in AST/ALT only - no. (%)‡	2 (8)	10 (17)
Advanced liver disease (fibrosis/cirrhosis) - no. (%)	2 (8)ᔕ	0 (0)
Hepatocellular carcinoma - no. (%)	0 (0)	0 (0)
Liver transplantation - no. (%)	1 (4)	0 (0)
Neonatal liver diseases - no. (%)ᔑ	7 (28)	1 (1.7)
Neonatal Cholestasis - no. (%)	2 (8)	1 (1.7)
Hepatosplenomegaly - no. (%)	1 (4)	0 (0)
Neonatal Hepatitis Syndrome - no. (%)	4 (16)	0 (0)

*Liver disease is defined as increase of AST > 35 U/L, increase of ALT > 45 U/L, presence or history of advanced liver disease (fibrosis/cirrhosis), presence or history of hepatocellular carcinoma, history of liver transplantation or presence or history of neonatal liver disease

†Includes patients with A1AT deficiency liver disease, whose age of diagnosis was >18a. Since A1AT deficiency liver disease has two peak ages, one in early childhood and one in 5th decade, we decided to split patients into adult and pediatric patient groups

‡Includes patients with biochemical signs of liver disease (increase in AST/ALT), but absence of any signs or symptoms of advanced liver disease, hepatocellular carcinoma or neonatal liver disease

¶Includes patients with A1AT deficiency liver disease, whose age of diagnosis was <18a

ᔕBoth patients had neonatal hepatitis syndrome in early childhood (counted in both table rows)

ᔑIncludes patients with history of prolonged icterus neonatorum, neonatal cholestasis, hepatosplenomegaly or neonatal hepatitis Syndrome

Table 3: ***Frequency of different liver diseases in patients with A1AT deficiency***

3.4 Evolution of liver disease in pediatric patients

Pediatric patients with neonatal liver disease showed different disease outcomes at latest follow-up. In ZZ phenotypes, 4 of 7 children (57.1%) showed no sign of liver disease at latest-follow up. 1 of 7 children (14.3%) showed biochemical signs of liver disease, displayed as an increase of AST and/or ALT. 2 of 7 children (28.6%) went on to develop advanced liver disease later in life, with 1 child (14.3%) requiring liver transplantation. In median, the patients were 15 years of age at latest follow up (Table 4).

In MZ phenotypes, the only child (100%) with neonatal liver disease showed no signs of liver disease at latest follow-up.

Table 4. Follow-up of patients with A1AT deficiency related neonatal liver disease*		
	ZZ	MZ
Patients with neonatal liver disease - no. (%)†	7 (100)	1 (100)
No sign of liver disease - no. (%)‡	4 (57.1)	1 (100)
Increase in AST/ALT - no. (%)¶	1 (14.3)ᘓ	0 (0)
Advanced liver disease (fibrosis/cirrhosis) - no. (%)	2 (28.6)ᘔ	0 (0)
Liver transplantation - no. (%)	1 (14.3)	0 (0)

*This Table describes disease condition at latest follow-up in patients with A1AT deficiency liver disease
†Includes patients with history of prolonged icterus neonatorum, neonatal cholestasis, hepatosplenomegaly or neonatal hepatitis syndrome
‡Describes number of patients, which had neonatal liver disease in infancy, but showed no signs or symptoms of liver disease at latest follow-up
¶Includes patients with biochemical signs of liver disease (increase of AST/ALT), but absence of any signs or symptoms of advanced liver disease or hepatocellular carcinoma
ᘓPatient had neonatal cholestasis in infancy
ᘔBoth patients had neonatal hepatitis syndrome in childhood; 1 developed cirrhosis at age of 7 and required liver transplantation, 1 developed cirrhosis at age of 26

Table 4: ***Follow-up of patients with A1AT deficiency related neonatal liver disease***

3.5 Changes of LFTs over the observation period

3.5.1 Changes of LFTs in adult patients

In adults, we expected an increase of AST and/or ALT over the observation period. Only adult patients with an observation period ≥10 years, with elevated levels of AST and/or ALT and no signs of any other liver disease were included in this analysis. ZZ phenotypes had mean AST and ALT values of 63U/L and 49U/L at diagnosis as well as 47U/L and 44U/L at latest follow-up, respectively (Table 5). There was no statistically significant change in AST and ALT values over the observation period in adult patients with the ZZ phenotype.

In ZZ phenotypes, 4 of 7 patients (57.1%) showed an increase of AST over the observation period, 3 of 7 patients (42.9%) showed a decrease of AST over the observation period.

2 of 7 patients (28.6%) showed an increase of ALT over the observation period, 5 of 7 patients (71.4%) showed a decrease of ALT over the observation period (Figure 8).

MZ phenotypes showed mean AST and ALT values of 40U/L and 61U/L at diagnosis as well as 38U/L and 72U/L at latest follow-up, respectively (Table 5). There was no statistically significant change in AST and ALT values over the observation period in adult patients with the MZ phenotype.

Table 5. Course of AST/ALT in adult patients with A1AT deficiency liver disease*			
	at diagnosis	at latest follow-up	p - Value
ZZ (n=7)†			
AST - mean (range)‡	63 (23 - 164)	47 (32 - 79)	0.866
ALT - mean (range)‡	49 (32 - 73)	44 (24 - 86)	0.398
MZ (n=21)¶			
AST - mean (range)‡	40 (14 - 92)	38 (20 - 136)	0.281
ALT - mean (range)‡	61 (11- 235)	72 (11 - 512)	0.532

*Only patients with an observation period over 10 years and no signs of any other liver disease rather than increase of AST/ALT (asymptomatic patients) are included in calculation of mean and range

†Patients had a mean observation period of 13 years

‡Serum AST and ALT both measured in U/L

¶Patients had a mean observation period of 12 years

Table 5: ***Time course of AST/ALT in adult patients with A1AT deficiency liver disease***

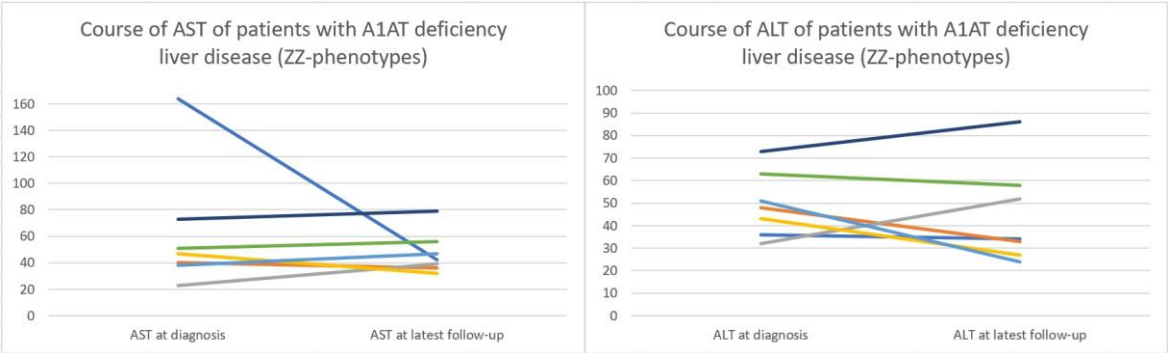

Figure 8: ***Time course of AST and ALT values in adult patients with A1AT related liver disease: Each line represents one of the 7 patients, which were included in this analysis***

3.5.2 Changes of LFTs in pediatric patients

In children, we expected a decrease of AST and/or ALT over the observation period. Only pediatric patients with an observation period ≥10 years and neonatal liver disease were included in this analysis. ZZ phenotypes showed mean AST and ALT values of 56U/L and 59U/L at diagnosis as well as 93U/L and 47U/L at latest follow-up, respectively (Table 6). There was no statistically significant change in AST and ALT values over the observation period in pediatric patients with the ZZ phenotype.

The only MZ phenotype who fulfilled inclusion criteria had an AST and ALT value of 32U/L and 25U/L at diagnosis as well as 28U/L and 17U/L at latest follow-up, respectively (Table 6).

Table 6. Course of AST/ALT in patients with A1AT deficiency neonatal liver disease*			
	at diagnosis	at latest follow-up	p - Value
ZZ (n=4)†			
AST - mean (range)‡	56 (40 - 70)	93 (21 - 276)	0.715
ALT - mean (range)‡	59 (30 - 122)	47 (21 - 114)	0.465
MZ (n=1)¶			
AST‡	32	28	
ALT‡	25	17	

*Only patients with an observation period over 10 years and neonatal liver disease are included in calculation of mean and range

†Patients had a mean observation period of 17 years

‡Serum AST and ALT both measured in U/L

¶Only one patient met the criteria in MZ group. Patient had an observation period of 11 years

Table 6: ***Time course of AST/ALT in pediatric patients with A1AT related liver disease***

In ZZ phenotypes, 3 of 4 patients (75%) showed a decrease of AST over the observation period, 1 of 4 patients (25%) showed an increase of AST over the observation period.
3 of 4 patients (75%) showed a decrease of ALT over the observation period, 1 of 4 patients (25%) showed an increase of ALT over the observation period (Figure 9). The one patient, who showed increasing AST and ALT values, developed liver cirrhosis and required liver transplantation at 7 years of age.

Figure 9: ***Time course of AST and ALT values in pediatric patients with A1AT related liver disease:*** **Each line represents one of the 4 patients, which were included in this analysis.**

3.6 Comparison of liver parameters of ZZ and MZ phenotypes

Mean AST values at time of diagnosis were 63U/L in ZZ phenotypes and 44U/L in MZ phenotypes (p=0.011). Mean ALT values were 53U/L in ZZ phenotypes and 59U/L in MZ phenotypes (p=0.807). Mean GGT values were 193U/L in ZZ phenotypes and 117U/L in MZ phenotypes (p=0.116). Mean platelet count was 180G/L in ZZ phenotypes and 227G/L

in MZ phenotypes (p=0.024). Mean APRI-Score was 0.49 in ZZ phenotypes and 0.25 in MZ phenotypes (p<0.001) (Table 7 and Figure 10).

AST, platelet count and APRI-Score showed statistically significant differences between patients with ZZ phenotype and MZ phenotype, confirming the expected milder liver disease in patients with less severe A1AT deficiency.

Table 7. Comparison of liver parameters in patients with the ZZ and MZ phenotype*

	ZZ (n=25)	MZ (n=59)	p-Value
AST - mean (range)†	63 (23 - 168)	44 (14 - 130)	0.011
ALT - mean (range)†	53 (19 - 123)	59 (11 - 235)	0.807
GGT - mean (range)†	193 (18 - 1164)	117 (9 - 1018)	0.116
Platelet count - mean (range)‡	180 (36 - 404)	227 (75 - 524)	0.024
APRI-Score¶	0.49 (0.1 - 1.56)	0.25 (0.06 - 1.38)	<0.001

*In this table, mean values of liver parameters are compared between patients with the ZZ and MZ phenotype. All patients with A1AT deficiency liver disease are included in calculations of mean and range, compared values are those from date of diagnosis.

†AST/ALT/GGT are measured in U/L

‡Platelet count measured in G/L (10^9/L)

¶APRI means AST to Platelet Ratio Index and serves as a non-invasive fibrosis score. Cut-off for significant fibrosis is >1.5. Cut-off for significant cirrhosis is >2.0.

Table 7: ***Comparison of liver parameters between patients with the ZZ and MZ phenotype and A1AT related liver disease***

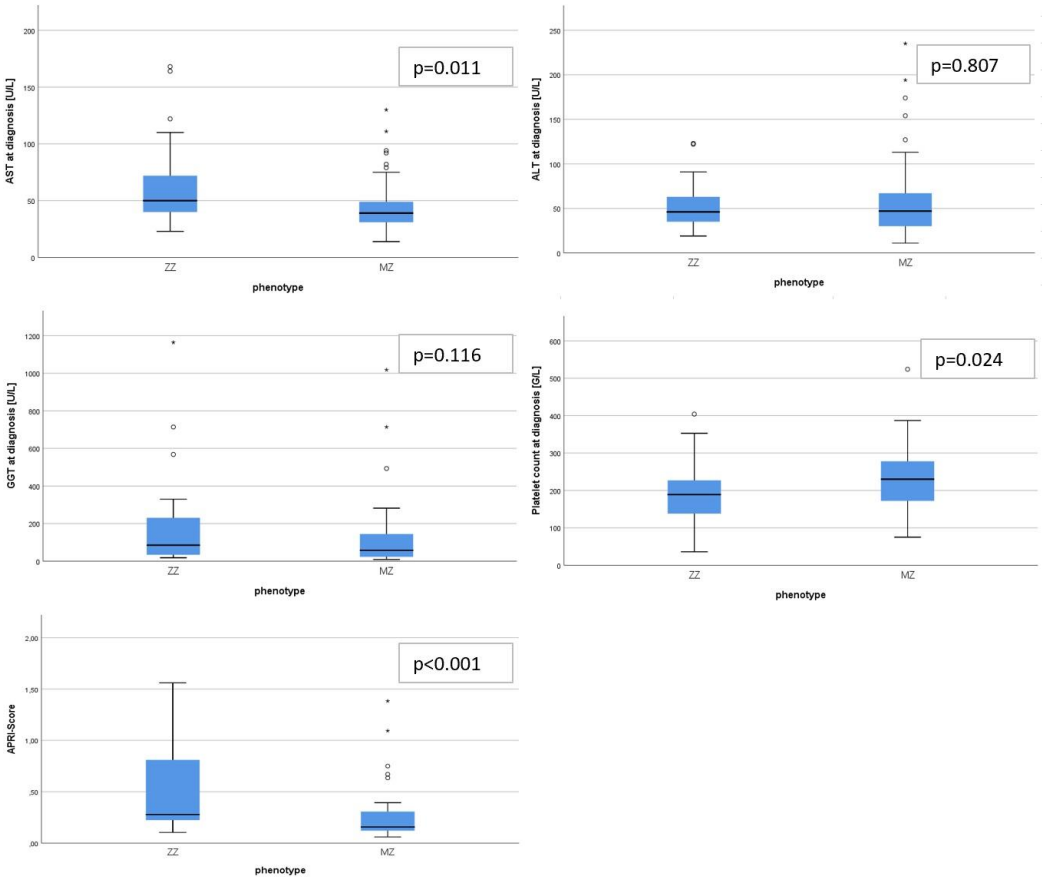

Figure 10: ***AST, ALT, GGT, platelet count and APRI-Score at the time of diagnosis in patients with ZZ and MZ phenotype and A1AT related liver disease***

3.7 Histological features of patients with A1AT related liver disease

In ZZ phenotypes, out of 5 patients, in which liver histology was available, 3 patients (60%) showed typical histological signs of A1AT deficiency. In MZ phenotypes, 1 of 7 patients (14.3%) showed typical histological signs. The patients, which did not show typical signs of A1AT deficiency, showed either signs of chronic viral hepatitis, fibrosis, cirrhosis, HCC or liver steatosis (Table 8).

Table 8. Histological features of patients with A1AT deficiency

	ZZ	MZ
Patients in which liver histology was available - no. (%)*	5 (100)	7 (100)
Patients with histological features of A1AT deficiency - no. (%)†	3 (60)	1 (14.3)
Patients without histological features of A1AT deficiency - no. (%)‡	2 (40)	6 (85.7)

*Describes number of patients, in which at least 1 liver histology was available. This includes liver biopsy as well as histopathological examination of original liver after liver transplantation

†Describes number of patients with histological features of A1AT deficiency, such as PAS-D-positive intrahepatocytic globules or positive immunohistochemical reaction against A1AT

‡These patients had either signs of chronic viral hepatitis, fibrosis, cirrhosis, HCC or liver steatosis but no sign of PAS-D-positive intrahepatocytic globules or positive immunohistochemical reaction against A1AT

Table 8: ***Histological features of patients with A1AT deficiency***

3.8 Liver steatosis in patients with ZZ and MZ phenotype

Our baseline characteristics (Table 2) showed an apparent difference between the frequency of liver steatosis in patients with ZZ and MZ phenotype. 27.1% of MZ phenotypes showed liver steatosis, which corresponds with the frequency of liver steatosis in the standard population(29), whereas only 8% of ZZ phenotypes showed liver steatosis ($p=0.026$ in Chi-squared test). Thus, our study reports lower rates of steatosis in ZZ phenotypes than what would be expected in the standard population.

4 Discussion

A1AT related liver disease is an underdiagnosed disease entity, which is mainly related to the low penetrance of the genetic disease and a poor genotype to clinical phenotype correlation(3). Another factor for the low diagnostic rates is the lack of any therapeutic options, which could mitigate the disease course, except for liver transplantation in end stage liver disease(6,11). We therefore undertook a survey of patients with A1AT related liver disease at our University hospital and retrospectively analyzed available data from all patients with the diagnosis of A1AT deficiency over the last 16 years.

Our analysis provided us with data of the number of patients with A1AT deficiency at the Medical University of Graz, as well as the number of patients with associated liver diseases and their phenotype. The majority of our patients carried at least one Z allele (ZZ, MZ and SZ phenotype), suggesting that the Z allele is the most common mutated allele type in our study population. Other studies report, that the S allele is the most common mutated allele type in European Caucasians(13). One explanation could be that the Z allele is simply more common in the Austrian population than the S allele. Another explanation, which we assume to be more likely, is that this study is not a screening of the standard population, but a study, which investigates patients at a tertiary referral center. Since inheriting the Z allele bears the highest risk of developing associated liver diseases, affected individuals are likely to be diagnosed and followed up at a tertiary hospital rather than patients with the S allele, which causes a less severe clinical course(6). To gather data regarding frequency of different allele types among the standard population, an epidemiologic screening study would be more appropriate. Nevertheless, our data is applicable for patients at a tertiary hospital and shows the most common phenotypes among them.

The analysis of the age of diagnosis in patients with A1AT deficiency showed the expected two peaks of age. In more detail, our results provided data regarding differences in age of diagnosis between ZZ phenotypes and MZ phenotypes. ZZ phenotypes showed the two typical peaks of age, one at early childhood and one between 45 and 70 years of age. MZ phenotypes also showed these two peaks, but contrary to ZZ phenotypes, patients with the MZ phenotype were diagnosed over the entire observed range of age. An explanation for this result is, that the MZ population showed higher relative frequencies of liver disease-associated cofactors and therefore was referred to the hospital more often because of

elevated LFTs. In that case, A1AT deficiency was an incidental finding working up elevated LFTs. Another explanation is, that there were more than twice as much MZ phenotypes as ZZ phenotypes in our study and likewise a higher number of ZZ phenotypes could possibly show a similar distribution of age of diagnosis as MZ phenotypes showed.

The natural course of liver disease in our study population showed higher frequencies of advanced liver disease than usually reported(11). In ZZ phenotypes, 28% of overall patients developed advanced liver disease (31.2% of adult patients; 22.2% of pediatric patients), which is much higher than the reported risk of 10% of developing advanced liver disease(11). As already discussed above, this study is biased by a patient cohort of a tertiary hospital and therefore patients with a more severe variant of liver disease were more likely referred to the Medical University of Graz. An additional explanation may be, that A1AT deficiency is more underdiagnosed than expected. While milder variants with only elevated LFTs could remain undiagnosed, more severe variants like advanced liver disease could be more likely diagnosed and therefore the relative frequency of advanced liver disease could have been raised.
Overall, from our findings that patients showed a higher frequency of advanced liver disease, we conclude that (i) patients are mainly referred to a tertiary hospital center when advanced liver disease is already present, that (ii) diagnosis is still made very late during disease course and that (iii) referral of patients with A1AT related liver disease to a tertiary center is probably late due to non-existing specific therapeutic options.

Neither in adults nor in pediatric patients we observed a significant change in AST and ALT values over an observation period of more than 10 years. In adult patients, we expected an increase in mean AST and ALT values, however the results showed a decrease in mean AST and ALT values in ZZ phenotypes, though the results were not statistically significant.
In pediatric patients, we expected a decrease in mean AST and ALT values, the results showed an increase in mean AST values and a decrease in mean ALT values in ZZ phenotypes, neither result was statistically significant.

While there was no significant change in mean AST and ALT values over the observation period, comparison of liver parameters between ZZ and MZ phenotypes showed different results. As expected, markers of liver injury, such as AST values and markers of liver

fibrosis, such as platelet count and APRI-Score were higher in ZZ phenotypes than in MZ phenotypes. ALT and GGT values however did not significantly differ. Since children were included in our study population, we decided to use APRI-Score rather than FIB-4 as determinants of liver fibrosis, since FIB-4 includes age of patients and the low age of pediatric patients would lead to false-low results. Since ZZ phenotypes are more likely to develop associated liver diseases than MZ phenotypes(6) and show significant differences in AST, platelet count and APRI-Score, these parameters could be more appropriate in measuring severity of liver disease than ALT and GGT. However, this conclusion would require further prospective investigation.

One of the most interesting and unexpected findings of our study was the significant difference of frequency of liver steatosis between ZZ and MZ phenotypes. ZZ phenotypes showed less steatosis than MZ phenotypes, which had steatotic rates comparable to the standard population. Therefore, we hypothesize, that patients with ZZ phenotypes show higher protection against liver steatosis than the standard population. This contrasts with a recent report in patients and mice, which showed higher rates of steatosis in ZZ mice(19). However, our analysis is the first one, which compares ZZ and MZ phenotypes, while the other report compared ZZ phenotypes to healthy individuals(19). Although we cannot support our finding with additional data or additional experiments, we can speculate on a likely molecular explanation for our finding. We speculate that autophagy as an adaptive mechanism is more active in the more severe ZZ phenotype than in the less severe MZ phenotype. Since autophagy is not only a process, in which whole cell organelles are being degraded, but also lipid droplets (a process called lipophagy)(30), higher autophagic activity in ZZ patients should also result in increased lipophagic activity. Therefore, we speculate that ZZ phenotypes might show higher protection against liver steatosis.
However, since this study was not primarily designed to investigate this hypothesis and MZ phenotypes showed higher frequencies of liver disease-associated cofactors, which could also lead to liver steatosis (e.g. alcohol consumption), these results must be taken with caution. A prospective study designed to investigate the frequency of liver steatosis in ZZ phenotypes, MZ phenotypes and the standard population would be appropriate to gain valid results.

One limitation of our study is the retrospective design, which did not allow to do a recent follow-up examination of our patients. The latest follow-up of many patients dates back several years and therefore their current disease status is unknown. As mentioned before, another limitation and bias of this analysis is the fact, that only patients in a tertiary hospital were being investigated and data of asymptomatic patients, which never visited or were referred to a tertiary hospital center are missing.

In conclusion, our study provides an overview of a large number of adults and pediatric patients with A1AT related liver disease at a tertiary hospital center. It is to the best of our knowledge the only study comparing patients with the ZZ and the MZ phenotype. Since the penetrance of the liver disease in genetically affected patients is low and no effective treatment for related liver disease exists, the referral to our tertiary hospital center probably takes place at late disease stages, explaining a high percentage of advanced liver diseases at our center. In contrast to the patients with advanced liver diseases, those patients with only mild elevations of LFTs did not progress over an observational period of at least a decade. From this we conclude, that pediatric patients, which had been diagnosed with A1AT deficiency, need to be consequently followed up to detect any deterioration in their liver status early. This is particularly important since the near future will probably provide us with therapeutic tools to slow down or even heal A1AT deficiency related liver disease. Unfortunately our study did not provide us with information on a long-term follow-up of the pediatric population, either because simply the observation period was too short to detect those with progressive disease, or because patients were simply lost of follow-up. The optimal strategy, of course, would be to screen for A1AT in childhood and follow up those patients with a genetic disposition consequently.

5 Bibliography

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