

# ALPHA-1-ANTITRYPSIN DEFICIENCY IN CHILDREN: LIVER DISEASE IS NOT REFLECTED BY LOW SERUM LEVELS OF ALPHA-1-ANTITRYPSIN — A STUDY ON 48 PEDIATRIC PATIENTS

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## Abstract

**Background:** Alpha-1-antitrypsin ( $\alpha$ 1-AT) is an important protease inhibitor. The phenotypes are characterized by a low total serum  $\alpha$ 1-AT or by an abnormal protein accumulating in the hepatocytes. The aim of our study was to examine a correlation of total serum  $\alpha$ 1-AT, phenotype, and liver involvement in pediatric patients.

**Methods:** 48 patients, deficient for  $\alpha$ 1-AT were included. The phenotypes for  $\alpha$ 1-AT were determined by isoelectric focusing. Liver disease was defined either as elevated transaminases or/and as elevated conjugated bilirubin and  $\gamma$ GT. Patients were reexamined after a mean interval of 2 years.

**Results:** Homozygous  $\alpha$ 1-AD was found in 12 patients, heterozygous in 24 patients. In 12 children rare variants of  $\alpha$ 1-AD were diagnosed. Serum  $\alpha$ 1-AT levels less than 60% of normal were found in all patients with homozygous, in 37% of patients with heterozygous  $\alpha$ 1-antitrypsin deficiency ( $\alpha$ 1-AD), and in patients with the homozygous variant PiM<sub>palermo</sub>. Liver disease was found in 8/12 patients with the phenotype PiZZ and in 15/24 patients with heterozygous  $\alpha$ 1-AD. Three of 4 patients with the phenotype PiMQ0 had severe liver disease despite normal serum levels for  $\alpha$ 1-AT. In 11 patients with heterozygous  $\alpha$ 1-AD liver disease was apparent despite normal serum  $\alpha$ 1-AT levels. In two patients with the variant type Mpalermo serum levels were as low as 11% of normal without any signs of liver disease.

**Conclusions:** Our data clearly show that in the diagnostic workup of neonatal cholestasis measurement of total serum  $\alpha$ 1-AT does not exclude liver disease due to abnormal  $\alpha$ 1-AT variants. We suggest analysis of  $\alpha$ 1-AT-phenotype by isoelectric focussing in patients with unknown liver disease. Heterozygous or rare variant types might remain undiagnosed by measuring total  $\alpha$ 1-AT only.

**Key words:**  $\alpha$ 1-antitrypsin deficiency, children, liver

## INTRODUCTION

Alpha-1-antitrypsin or protease inhibitor (Pi) is a single chain 52 kD glycoprotein consisting of 394 amino acids. The protein is encoded by a gene located on the

distal long arm of chromosome 14 (14q31-32.2) [17]. Structural variants of  $\alpha$ 1-AT in humans are classified according to the protease inhibitor phenotype system as defined by agarose gel electrophoresis or isoelectric focusing of plasma [15]. To date more than 75 different variants are known, some of them leading to severe liver disease in early childhood or early pulmonary emphysema [16, 21]. Several variants of  $\alpha$ 1-AT are associated with a reduction in serum  $\alpha$ 1-AT concentrations, called deficiency variants. The genetic deficiency is inherited in an autosomal co-dominant fashion. Homozygous PiZ  $\alpha$ 1-antitrypsin deficiency has been associated with liver disease in childhood, leading to endstage liver disease in a subset of patients [10]. This variant is characterized by a distinctive histopathological feature of PAS positive inclusion bodies in the endoplasmic reticulum of the hepatocytes [23]. In a prospective screening study of 200.000 newborns in Sweden, 127 children were identified as being deficient for  $\alpha$ 1-AT due to homozygous  $\alpha$ 1-antitrypsin deficiency PiZ [19]. A subset of 14 patients presented with neonatal cholestasis, 3 of them developed severe liver disease. However  $\alpha$ 1-antitrypsin deficiency is still the most frequent metabolic liver disease leading to end stage liver disease. About 10% of patients with the homozygous variant PiZ require liver transplantation during childhood [6]. An increased prevalence of neonatal liver disease is reported in patients with heterozygous PiMZ  $\alpha$ 1-antitrypsin deficiency [1]. However only a very few adult patients with the heterozygous form are reported requiring liver transplantation [9]. The mechanism for liver cell damage in patients with  $\alpha$ 1-antitrypsin deficiency is still unclear. Accumulation of  $\alpha$ 1-antitrypsin in the liver cell is thought to be directly related to liver cell damage [14]. Experiments in transgenic mice carrying the mutant Z allele of the human  $\alpha$ 1-antitrypsin gene showed histological approved liver cell damage in these animals despite normal serum concentrations of  $\alpha$ 1-antitrypsin. It is, therefore, likely that liver injury cannot be caused by low serum concentrations of the protein but by an intracellular accumulation of the abnormal  $\alpha$ 1-antitrypsin gene product [4]. The clinical consequence of these pathophysiological models is that total serum concentrations of  $\alpha$ 1-antitrypsin cannot reveal enough information

about the risk and the outcome of liver disease for the individual patient.

On the basis of these observations we hypothesized:

Determination of total serum  $\alpha$ 1-AT in the diagnostic workup of neonatal cholestasis is an insufficient tool of detecting  $\alpha$ 1-antitrypsin deficiency and rare variants of the protein.

Detection of rare variants of  $\alpha$ 1-AT coinciding with liver disease can only be detected by determination of the  $\alpha$ 1-AT phenotype using isoelectric focusing.

We, therefore, reevaluated 48 children with different types of  $\alpha$ 1-AT deficiency including total serum  $\alpha$ 1-AT,  $\alpha$ 1-AT phenotype, appearance of liver disease by measuring serum levels of AST, ALT,  $\gamma$ GT, GLDH, bilirubin, and coagulation time at the time of diagnosis and after an observation period of 1-18 years (mean 2 years).

## PATIENTS AND METHODS

Our study included 48 pediatric patients, 27 girls and 21 boys, aged between 1 week and 16 years at the time of diagnosis of  $\alpha$ 1-antitrypsin deficiency. Diagnosis of  $\alpha$ 1-antitrypsin deficiency was proven by isoelectric focusing (IEF) of the protein in all patients. Analysis of  $\alpha$ 1-antitrypsin was performed in serum samples by using the IEF technique followed by immunoprinting [15, 22]. Patients were grouped into three subsets: patients homozygous for  $\alpha$ 1-antitrypsin deficiency (PiZ, PiM<sub>Palermo</sub>), patients heterozygous for  $\alpha$ 1-antitrypsin deficiency (PiMZ, PiMS, PiSZ, PiMP, PiVZ, PiMM<sub>Procida</sub>, PiMQ0), and a patient with a normal rare variant of the protein (PiMP<sub>Donauwörth</sub>).

We retrospectively analyzed the prevalence of liver disease in each group at the time of diagnosis. Liver disease was defined as evidence of pathological liver function tests. Pathological elevation of transaminases (ALT > 40 U/l, AST > 45 U/l) and/or elevation of  $\gamma$ GT (newborn:  $\gamma$ GT >120 U/l, infants and children:  $\gamma$ GT > 45 U/l), conjugated bilirubin (> 1,5 mg/dl), and alkaline phosphatase (> 350 U/l) were regarded as hepatitis or cholestasis, respectively. Serum concentrations of  $\alpha$ 1-antitrypsin were determined in each indi-

vidual patient. Liver biopsies were performed in patients with persistent pathological liver function tests only.

All patients underwent a clinical and laboratory reevaluation after a mean period of 2 years after being diagnosed as deficient for  $\alpha$ 1-antitrypsin. Appearance of liver disease was again defined by pathological elevation of liver function tests as described above. Serum concentrations of  $\alpha$ 1-antitrypsin were measured in all patients at the time of reevaluation.

We compared the three subsets of patients regarding appearance of liver disease, total serum concentrations of  $\alpha$ 1-antitrypsin, clinical course of the patients, especially development of cirrhosis.

Differences between groups were analysed using the Wilcoxon-Rank Sum test.

## RESULTS

Abnormal variants of the  $\alpha$ 1-antitrypsin were detected in all patients by isoelectric focusing. Analysis of serum  $\alpha$ 1-antitrypsin and isoelectric focusing of the protein was performed because of appearance of liver disease in 50 %, clinical signs of airway obstruction in 18 %, family history of  $\alpha$ 1-antitrypsin deficiency in 10%. In 12% of the patients  $\alpha$ 1-antitrypsin deficiency was detected during a routine follow up.

Homozygous  $\alpha$ 1-antitrypsin deficiency was diagnosed in 12 patients (PiZZ), heterozygous  $\alpha$ 1-antitrypsin deficiency in 28 patients (PiMZ n = 17, PiMS n = 7, PiSZ n = 2, PiVZ n = 1, PiMP n = 1), rare variants of the protein were found in 8 patients (PiM(Q0) n = 4, PiMP<sub>Donauwörth</sub> n = 1, PiMM<sub>Procida</sub> n = 1, PiM<sub>Palermo</sub> n = 2), respectively. Low serum concentrations of  $\alpha$ 1-antitrypsin were detected in all patients with homozygous deficiency, however 36% of the patients with heterozygous variants showed normal serum levels of  $\alpha$ 1-antitrypsin. Serum levels of  $\alpha$ 1-antitrypsin ranged between 78 and 100% in patients with the rare variant M(Q0), between 9,5 and 12% in patients with the variant PiM<sub>Palermo</sub>, 10% in the phenotype PiMM<sub>Procida</sub>, 100% in the patient with the phenotype PiMP<sub>Donauwörth</sub>. Table 1 summarizes the results of serum  $\alpha$ 1-antitrypsin according to the phenotypes.

Table 1. Pi phenotypes and serum concentrations of  $\alpha$ 1-antitrypsin in 48 pediatric patients with (values of serum  $\alpha$ 1-antitrypsin are given in mg/dl (a) and in percent of normal values for age (b))\*

| Phenotype    | Number of patients    | $\alpha$ 1-AT in mg/dl   | $\alpha$ 1-AT in % $\pm$ SD of age related to normal value |             |
|--------------|-----------------------|--------------------------|--|-------------|
| homozygous   | Z                     | 12                       | 34   | 24 $\pm$ 8  |
|              | M <sub>Palermo</sub>  | 2                        | 19   | 11 $\pm$ 4  |
| heterozygous | MS                    | 7                        | 125  | 81 $\pm$ 11 |
|              | MZ                    | 17                       | 95   | 69 $\pm$ 9  |
|              | SZ                    | 2                        | 56   | 40 $\pm$ 7  |
|              | MP                    | 1                        | 115  | 88 $\pm$ 0  |
|              | VZ                    | 1                        | 60   | 46 $\pm$ 0  |
|              | MM <sub>Procida</sub> | 1                        | 105  | 81 $\pm$ 0  |
|              | M(Q0)                 | 4                        | 190  | 94 $\pm$ 6  |
|              | variant               | MP <sub>Donauwörth</sub> | 1  | 200         |

\* data are expressed as means.

At the time of diagnosis pathological elevation of transaminases was found in 2 patients and significant cholestasis in 6 patients with the phenotype PiZZ, cholestasis was detected in one of the two patients with the phenotype PiSZ. 8 of 17 patients with the phenotype PiMZ had biochemical evidence of cholestasis. Transaminases were elevated in 4 patients with PiMZ and in two patients with the phenotype PiMS. The patient with the rare phenotype PiMP showed no clinical signs of cholestasis. All patients with the rare variant type M(Q0) were severely affected by liver disease (severe neonatal hepatitis in 2 patients, neonatal cholestasis in 2 patients) despite serum levels of  $\alpha$ 1-antitrypsin of 80% of normal range according to patient's age. One of them already had developed cirrhosis at the time of diagnosis. The patient with the rare variant type MP<sub>donauwörth</sub> presenting with a normal serum level for  $\alpha$ 1-antitrypsin had histological evidence of cirrhosis. Two patients with the rare variant M<sub>palermo</sub> in the homozygous state had no chemical signs of liver disease despite very low serum levels of  $\alpha$ 1-antitrypsin (< 20% of normal range according to patient's age). The patient with the variant phenotype MM<sub>procida</sub> had normal serum levels of  $\alpha$ 1-antitrypsin and no clinical evidence of liver disease.

#### CLINICAL COURSE OF THE PATIENTS

The clinical course of the patients was dependent on the phenotype of  $\alpha$ 1-antitrypsin.

Eight of 14 patients with  $\alpha$ 1-antitrypsin deficiency (PiZZ or PiSZ) had clinical evidence of liver disease at the time of diagnosis, in 6 patients neonatal cholestasis, in two patients cholestasis in infancy (3 months and 6 months). Only 3 patients with liver disease due to homozygous phenotypes developed progressive liv-

er disease leading to cirrhosis within 3 years. All of them had the phenotype PiZ. Two of these patients died of chronic liver failure, one patient underwent liver transplantation and died of severe fungal infection 2 weeks after transplantation. Total serum  $\alpha$ 1-antitrypsin was similar in all patients with the homozygous form of the disease (range: 16 - 45% of the normal value corrected for age). There was no difference in the clinical outcome between patients with  $\alpha$ 1-antitrypsin levels less than 20% and more than 20% of normal. The results of the homozygous group of patients are summarized in Table 2, demonstrating the clinical status of the patients at the time of diagnosis and at the time of reevaluation, interestingly 11 patients with phenotype PiZZ exhibited no signs of liver disease.

Eight of 24 patients with heterozygous  $\alpha$ 1-antitrypsin deficiency (PiMZ and PiMS) had neonatal cholestasis. Neonatal hepatitis was observed in 2 patients with the phenotype PiMZ. As in patients with homozygous  $\alpha$ 1-antitrypsin deficiency there was no difference regarding the manifestation of liver disease in patients with nearly normal serum levels of  $\alpha$ 1-antitrypsin (80-95% of normal age related value), compared to those with serum levels of less than 70%. No cirrhosis was observed in the heterozygous group. Liver function tests (AST, ALT,  $\gamma$ GT, bilirubin, alkaline phosphatase) normalized in all patients but two within the first 6 months after birth. Only two patients of this group had slightly elevated liver function tests (ALT of 50 U/l in one patient,  $\gamma$ GT of 65 U/l in another patient) 2 and 3 years after diagnosis, respectively. The results of the heterozygous group of patients are summarized in Table 3, demonstrating the clinical status of the patients at the time of diagnosis and at the time of reevaluation.

Table 2. Clinical status of patients with homozygous  $\alpha$ 1-AD at the time of diagnosis and at the time of reevaluation 1 – 3 years after diagnosis.

|                          | Healthy      |                 | Cholestasis  |                 | Hepatitis    |                 | Zirrhosis    |                 |
|--------------------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|
|                          | At diagnosis | At reevaluation | At diagnosis | At reevaluation | At diagnosis | At reevaluation | At diagnosis | At reevaluation |
| PiZZ                     | 4            | 9               | 7            | 0               | 1            | 0               | 0            | 2               |
| PiSZ                     | 1            | 1               | 0            | 1               | 1            | 1               | 0            | 0               |
| PiM <sub>palermo</sub>   | 2            | 2               | 0            | 0               | 0            | 0               | 0            | 0               |
| MQ0                      | 0            | 1               | 2            | 1               | 2            | 1               | 0            | 1               |
| MP <sub>donauwörth</sub> | 0            | 0               | 0            | 0               | 0            | 0               | 1            | 1               |

Table 3. Clinical status of patients with heterozygous  $\alpha$ 1-AD at the time of diagnosis and at the time of reevaluation 1 – 3 years after diagnosis.

|                         | Healthy      |                 | Cholestasis  |                 | Hepatitis    |                 | Zirrhosis    |                 |
|-------------------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|--------------|-----------------|
|                         | At diagnosis | At reevaluation | At diagnosis | At reevaluation | At diagnosis | At reevaluation | At diagnosis | At reevaluation |
| PiMZ                    | 5            | 14              | 8            | 1               | 4            | 2               | 0            | 0               |
| PiMS                    | 5            | 5               | 0            | 0               | 2            | 2               | 0            | 0               |
| PiMP                    | 0            | 1               | 1            | 0               | 0            | 0               | 0            | 0               |
| PiVZ                    | 1            | 1               | 0            | 0               | 0            | 0               | 0            | 0               |
| PiMM <sub>procida</sub> | 1            | 1               | 0            | 0               | 0            | 0               | 0            | 0               |

The heterogeneous group of patients with rare variants of the  $\alpha 1$ -antitrypsin protein shows a broad spectrum of clinical manifestations. The patients with the phenotypes PiMM<sub>procida</sub>, PiVZ, and PiM<sub>palermo</sub> showed no evidence of liver disease at the time of diagnosis, nor at the time of reevaluation. Their serum levels of  $\alpha 1$ -antitrypsin however were below 30% in 5 patients, in two of them less than 80%. In contrast all patients with the phenotype PiM(Q0) and the patient with the phenotype PiMP<sub>donauwörth</sub> had severe liver disease at the time of diagnosis (neonatal cholestasis in 2 patients, neonatal hepatitis in 3 patients) despite normal levels of  $\alpha 1$ -antitrypsin in serum. Two patients with the phenotype PiM(Q0) and the patient with the phenotype PiMP<sub>donauwörth</sub> developed rapidly progressive cirrhosis. Only one out of these 4 patients had normal liver function tests at the time of reevaluation. The results of the variant groups of patients are summarized in Table 2, demonstrating the clinical status of the patients at the time of diagnosis and at the time of reevaluation.

Unknown environmental factors in addition to the modified genes may play a certain role in the pathogenesis of liver disease.

## DISCUSSION

Homozygous  $\alpha 1$ -antitrypsin deficiency (phenotype PiZZ) is associated with neonatal cholestasis and may lead to cirrhosis in up to 13% of the affected patients [16, 21]. The heterozygous form of the disease as the phenotypes PiMZ and PiMS rarely coincide with liver disease but single cases of cirrhosis are reported in the literature [10, 14]. The homozygous as well as the heterozygous forms of the  $\alpha 1$ -antitrypsin deficiency are associated with low serum levels of  $\alpha 1$ -antitrypsin. In our series of 48 patients with  $\alpha 1$ -antitrypsin deficiency including 10 patients with rare variants of the disease we found an all over prevalence of liver involvement in 67% of the patients at the time of diagnosis. The high prevalence of liver disease in our series might be explained by the design of the study. Patients were retrospectively evaluated and liver disease was the reason of reduced  $\alpha 1$ -antitrypsin in 50 % of the patients.

We detected liver involvement in 58% of our patients with the PiZ homozygous form of the disease, leading to end stage liver disease in 13%. Sveger et al. prospectively screened 200.000 newborns in Sweden for PiZZ  $\alpha 1$ -antitrypsin deficiency [19]. They found clinical signs of liver disease in 18% of their patients with homozygous  $\alpha 1$ -antitrypsin deficiency leading to cirrhosis in 7%. As the patients in our study were referred to our clinic for diagnostical workup of liver disease or respiratory abnormalities this might explain the higher prevalence of liver disease in our patients. However clinical outcome of our patients was similar to Sveger's observations. Only 3 of 14 patients (PiZZ or PiSZ) had clinical evidence of liver disease 1 - 2 years after the diagnosis was established. In Sveger's study 25% of the patients with PiZZ showed abnormal liver function tests 2 years after diagnosis [20]. All of our patients with PiZZ had low serum levels of  $\alpha 1$ -antitrypsin according to the literature. Two of our patients with homozygous  $\alpha 1$ -antitrypsin deficiency were prematurely born infants with neonatal sepsis.

Both children developed cirrhosis. In another child liver function tests deteriorated during a gastrointestinal infection due to Salmonella enteritidis at the age of 2 years. Additional factors as parenteral nutrition and severe systemic infections are known as risk factors for the development of severe liver disease in these children. Environmental factors resulting in an enhanced synthesis of abnormal  $\alpha 1$ -antitrypsin lead to a higher accumulation rate of the protein in liver cells resulting in liver cell damage [12, 13]. These observations support the hypothesis that intracellular accumulation of the mutant protein in liver cells is responsible for liver disease in homozygous  $\alpha 1$ -antitrypsin deficiency [11]. In adults alcohol consumption and viral hepatitis also increase the risk of cirrhosis in patients with homozygous  $\alpha 1$ -antitrypsin deficiency despite normal liver function tests during childhood [3].

15 of 27 patients with heterozygous  $\alpha 1$ -antitrypsin deficiency showed clinical evidence of liver involvement in our series. In 12 of them liver function tests turned to normal within 6 months after the time of diagnosis. Only 2 of them had slightly elevated transaminases during the observation period of 1-3 years. This coincides with other reports in the literature.

Heterozygous  $\alpha 1$ -antitrypsin deficiency is known as being associated with an increased risk of neonatal cholestasis or neonatal hepatitis [1]. However clinical outcome of this group is different from patients with the homozygous form of the disease. Only single reports of cirrhosis due to heterozygous  $\alpha 1$ -antitrypsin deficiency exist [9]. As explained previously additional factors are necessary to increase the intracellular accumulation of mutant protein in liver cells. Factors as viral hepatitis and alcohol abuse are known to be associated with the development of cirrhosis even in patients with heterozygous  $\alpha 1$ -antitrypsin deficiency [5, 9]. In none of our patients cirrhosis was observed. However they still are at a higher risk for liver disease compared to healthy controls. Eight of 17 children with heterozygous  $\alpha 1$ -antitrypsin deficiency and abnormal liver function tests were breast fed. Perlmutter et al. showed that breast feeding is associated with a lower risk of mild neonatal cholestasis in children with heterozygous  $\alpha 1$ -antitrypsin deficiency [13]. The pathomechanism of this observation is still unclear. Liver function tests of the 4 patients returned to normal when breast feeding was discontinued.

Serum levels of  $\alpha 1$ -antitrypsin ranged between normal and 65% of the normal levels adapted to the patients' age. We did not observe any correlation between the serum levels of  $\alpha 1$ -antitrypsin and the appearance of pathological liver function tests in patients with heterozygous  $\alpha 1$ -antitrypsin deficiency. This coincides with other reports in the literature [1, 19].

The heterogeneous group of patients with rare mutants of the  $\alpha 1$ -antitrypsin deficiency showed different clinical outcome. The rare phenotype PiM(Q0) was found in 4 patients as diagnosed by isoelectric focusing in all patients. Two children were prematurely born (gestational age 28 weeks and 36 weeks, respectively). Both of them developed severe liver disease in their first month of life and one child died of progressive liver disease within 6 months. Their postnatal period was affected by several complications due to prematu-

rity, however, the reason for rapidly progressive liver disease remains unclear. Liver biopsies were performed in 3 children, demonstrating cirrhosis in 1 case and severe fatty degeneration of the liver in the other two cases. Only single reports exist about this rare variant. It is characterized by a diminished band pattern by isoelectric focusing of the protein. Mainly premature pulmonary emphysema has been associated with different Pi(Q0) variants (PiQ0 Granite Falls, PiQ0 Bellingham, PiQ0 Mattawa, PiQ0 Riedenburg, PiQ0Ludwigshafen). All of them were homozygous for the null-allele resulting in low serum concentrations of  $\alpha$ 1-antitrypsin [2, 7]. Our patients with PiM(Q0) were heterozygous for the null allele resulting in a slightly reduced concentration for  $\alpha$ 1-antitrypsin. The pathomechanism in this group is unclear but it seems possible that an abnormal structured protein might be responsible for liver cell damage.

The rare variant PiMP<sub>donauwörth</sub> was found in one patient with a normal serum concentration of  $\alpha$ 1-antitrypsin but severe early liver disease. Histology revealed micronodular cirrhosis in this child. This patient developed a rapidly progressive cirrhosis during the first 3 years of life. This variant has also been found in the patient's having normal liver function tests. An abnormal structured protein might have caused liver cell damage by accumulating in the liver cells. However normal serum  $\alpha$ 1-antitrypsin stills remains unclear in this patient [7].

The lowest serum concentrations for  $\alpha$ 1-antitrypsin were found in 2 siblings with the rare variant PiM<sub>palermo</sub>, first described in a Sicilian family [7, 18]. None of them had abnormal liver function tests, nor abnormal lung function tests. This coincides with the report about this family. Possibly a normal protein with a decreased synthesis rate might explain the low serum concentration and the lack of symptoms. This also supports the theory that mainly intracellular accumulation of mutant proteins in the liver cells seems to be responsible for liver cell damage. Low serum concentrations of normally functioning  $\alpha$ 1-antitrypsin do not correlate with liver disease. Low activity of the protease inhibitor could be responsible for pulmonary emphysema in some patients but is not necessarily associated with lung disease.

In one patient we found the rare variant phenotype PiVZ [8]. As in the patients with PiM<sub>palermo</sub> this patient also had very low serum concentrations of  $\alpha$ 1-antitrypsin not associated with liver disease. There is not very much known about this variant type which is regarded as heterozygous for  $\alpha$ 1-antitrypsin deficiency. Patients reported in the literature having the allele Pi\*V do not have an increased risk for liver disease. However our female patient might be of a higher risk for liver disease as she is heterozygous for the Z-allele.

In summary we found an increased prevalence of liver disease in patients with homozygous and heterozygous  $\alpha$ 1-antitrypsin deficiency. However, progression of the disease was only noted in a subset of patients with the variant types PiZZ, PiM(Q0), and PiMP<sub>donauwörth</sub>. The latter two phenotypes were not associated with low serum levels of  $\alpha$ 1-antitrypsin.

In the diagnostical workup of liver disease measuring serum  $\alpha$ 1-antitrypsin might not provide enough

information to rule out  $\alpha$ 1-antitrypsin deficiency. Rare variants as shown in our study can have normal serum levels of  $\alpha$ 1-antitrypsin but a structurally abnormal mutant protein might cause liver cell damage by accumulating in liver cells. Phenotyping and in special cases DNA analysis should be performed in each single patient with unknown liver disease or neonatal cholestasis.

In this study less correlation between  $\alpha$ 1-antitrypsin levels in serum with the clinical phenotype was found. We conclude, therefore, that analysis of the biochemical phenotype with appropriate methods is mandatory in detecting disease causing variants.

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