A novel alpha1-antitrypsin null variant (PiQ0Milano)

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Abstract

Alpha1-antitrypsin deficiency is an autosomal recessive disease characterized by reduced serum levels of alpha1-antitrypsin (AAT) due to mutations in the SERPINA1 gene causing early onset pulmonary emphysema and, occasionally, chronic liver disease. We report an incidental finding of a novel null AAT allele, Q0Milano, consisting of a 17 nucleotides deletion in exon 3 of SERPINA1 gene, in an Italian child with persistently increased liver enzymes and a mild decrease in circulating AAT levels. Q0Milano variant results in an unfunctional protein lacking of AAT active site, as the resultant protein is truncated near PiS locus involved in AAT protein stability.

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Introduction

Alpha1-antitrypsin deficiency (AATD) is an autosomal recessive disease characterized by reduced serum levels of alpha1-antitrypsin (AAT, SERPINA1), a 52 kDa glycoprotein functioning as the main extracellular protease inhibitor. AAT is mainly produced by liver, which releases about 2 g of AAT daily into the circulation under physiological conditions. The normal serum concentration may range between 1.5-3.5 g/L (or 20-48 μmol/L) [1]. AATD is associated with early onset pulmonary emphysema and, occasionally, with chronic liver disease in childhood, hepatocellular carcinoma and/or cirrhosis in adulthood [2]. AAT functions as neutrophil elastase inhibitor, playing a key role in the protection of the lower respiratory tract. AAT serum levels below 11 μmol/L are not sufficient to inhibit elastase in vivo, permitting progressive destruction of alveoli culminating in emphysema [3]. The pathophysiology of liver disease related to AATD is less well understood, but some deficient variants accumulate in endoplasmic reticulum of hepatocytes and are inefficiently secreted, leading to protein aggregation and culminating...
in hepatocytes injury and liver disease[9].

AATD is caused by mutations in SERPINA1, a highly polymorphic genetic locus located on the distal long arm of chromosome 14. More than 100 alleles have been identified. They can be classified according to AAT serum levels and protein functionality; (1) normal variants, all common M types, accounting for 95% of those found in Caucasian individuals, and characterized by normal plasma levels (more than 20 μmol/L); (2) deficient variants associated with reduced AAT serum levels, lower than 20 μmol/L; (3) null variants determining undetectable serum levels; and (4) dysfunctional variants characterized by normal serum levels of dysfunctional AAT protein[10].

The firstly described, and most common cause of AATD, associated with very low serum concentration of the protein, is homozygosity for the PiZ mutation, the most severe AAT deficient variant known with plasma levels among homozygotes of about 5-6 μmol/L, resulting in the development of lung and liver disease[11]. It became later clear that AATD is a heterogeneous disease, caused by several gene defects expressed codominantly, mostly determining reduced serum AAT levels.

The most common deficient alleles are PiS and PiZ, with an allelic frequency of 2%-4% and 1%-2% respectively in Caucasian population, and are both caused by missense mutations responsible for intracellular protein accumulation and degradation. The PiZ mutation leads to a conformational change of AAT reactive site into a β-sheet polymer which forms characteristic periodic acid-Schiff-positive inclusions and can be isolated for liver of AAT PiZZ subjects. Several studies have shown that PiM PiZ in heterozygous state may lead to chronic liver disease, cryptogenic cirrhosis, and chronic active hepatitis[9-11], while the PiS variant is associated to liver disease only if carried in compound heterozygosity with the PiZ allele[12]. Null alleles are very rare (frequency < 0.001, 13% of AATD subjects in Italy[13]) and derived from nucleotide deletion, insertion, or non-sense mutations, causing premature stop codons and producing structurally unstable and truncated protein. Individuals with null-null AAT phenotype are not affected by liver disease, because of the lack of aggregation of mutant proteins in the endoplasmic reticulum, but are associated with an increased risk of emphysema.

In this study we report a novel AAT allele in a child with reduced protein levels.

## CASE REPORT

An 11-years-old male child was referred to our centre for a persistent increase in liver enzymes (aspartate aminotransferase and alanine aminotransferase spanning from 58-239 UI/L, and 98-114 UI/L respectively). All common causes of liver disease were excluded, but mildly decreased AAT serum levels were detected (76 mg/dL). The subject did not show abnormalities in pulmonary function.

Sequencing of the proband revealed a novel null mutation in AAT gene (Table 1), g.9752-9768del (PiQ0[zclio]) (gene ID: 5265, official name SERPINA1, genomic sequence number: NC_000014.8; NCBI Reference Sequence: NC_000014.8). This variant, localized in exon 3 near PiS locus (p.Glu264Val), consists in a 17 bp deletion (AAA CTA CAG CAC CTG GA), resulting in a frameshift causing a new stop codon downstream the deletion site (Figure 1), which leads to a premature termination of protein translation at amino acid 259. The truncated protein lacks of AAT active site centred around Met358-359.

The mutation arose in PiM3 AAT allele, which the proband inherited from his mother, whose genotype was PiM1/A/PiQ0[clio] and had normal liver and pulmonary function, whereas his father, who had normal genotype PiM1/V/PiM3, showed nonalcoholic steatohepatitis associated with hyperferritinemia (Figure 2). Liver biopsy of the proband showed aspecific findings, unrelated to AATD.

## DISCUSSION

The g.9752-9768del mutation (Q0[zclio]) occurs in a key functional region of AAT gene where several other deficiency variants (PiLow/l/PiS [at codon 256], Q0[Carno] at codon 259, T/S at codon 264) have been reported[14-18]. Several mechanisms are responsible for AAT deficiency, including: gene deletion, mRNA degradation, intracellular protein accumulation and degradation, and production of dysfunctional proteins. Only mutations causing intracellular protein accumulation and polymerization of the newly synthesized protein are associated with increased risk of liver disease, as in PiZ and in PiM-Malton Variants[19]. Many previously described null mutations (PiS[carne], PiLongo[Long], PiCarno[Carne]) have been associated with intra-reticular accumulation of unfunctional AAT proteins, which are immediately degraded without any

### Table 1  Sequence of primers used in Alpha1-antitrypsin coding sequence amplification and sequencing

<table>
<thead>
<tr>
<th>Primers forward 5’→3’</th>
<th>Primers reverse 5’→3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT Ex2A</td>
<td>CCCCCCATCTCTGTCTGCG</td>
</tr>
<tr>
<td>AAT Ex2B</td>
<td>ATGAAATCTGGAGCCCTTG</td>
</tr>
<tr>
<td>AAT Ex3</td>
<td>CCCACCTTCCTCCTCTCC</td>
</tr>
<tr>
<td>AAT Ex4</td>
<td>CTGAGATCTTTCGACAGAC</td>
</tr>
<tr>
<td>AAT Ex5</td>
<td>GTCTCAGCTCCTCCCTCC</td>
</tr>
</tbody>
</table>

AAT: Alpha1-antitrypsin; Ex: Exon.
liver damage. Heterozygosity for this novel null mutation is consistent with the lower AAT serum levels (76 mg/dL) measured in the proband, which collocates the patient in intermediate deficiency condition comparable to those of individuals carrying PiMZ genotype.

However, it is unlikely that this genetic variant explained liver disease in the proband, as it was carried in heterozygous state, and it does not affect liver function tests of the mother. Moreover, the absence of periodic acid-Schiff-positive inclusions revealed in liver biopsy excluded hepatic AAT protein accumulation. Thus, the novel null AAT variant was not responsible for liver damage because of the lack of hepatic protein polymerization.

It is likely that other hepatotoxic insults, as non alcoholic steatohepatitis associated with hyperferritinemia, a strongly heritable condition reported in the father of proband, were involved in the development of liver disease.

In conclusion, in this study we report a novel AAT null variant (Q0 Milano) generated by a 17 nucleotides deletion in exon 3 of AAT, which leads to a premature stop codon.

REFERENCES


8 Laurell CB, Eriksson S. The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. COPD 2013; 10 Suppl 1: 3-8 [PMID: 23527532 DOI: 10.1080/00365516309051324]


18 Curiel DT, Chytal A, Courtney M, Crystal RG. Serum alpha 1-antitrypsin deficiency associated with the common S-type (Glu264—Val) mutation results from intracellular degradation of alpha 1-antitrypsin prior to secretion. J Biol Chem 1989; 264: 10477-10486 [PMID: 25672921]


