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CASE REPORT

A novel alpha1-antitrypsin null variant (*PiQ0Milano*)

Raffaela Rametta, Gabriella Nebbia, Paola Dongiovanni, Marcello Farallo, Silvia Fargion, Luca Valenti

Raffaela Rametta, Silvia Fargion, Luca Valenti, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, 20122 Milano, Italy
Gabriella Nebbia, Paola Dongiovanni, Silvia Fargion, Luca Valenti, Internal Medicine, Fondazione IRCCS Ca' Granda Ospedale Policlinico Milano, 20122 Milano, Italy
Marcello Farallo, Pediatric Clinic, Fondazione IRCCS Ca' Granda Ospedale Policlinico, 20122 Milano, Italy
Author contributions: Rametta R performed the genetic analysis and wrote the first manuscript draft; Dongiovanni P supervised genetic analysis; Nebbia G and Farallo M were responsible for the clinical care of the patients; Fargion S and Valenti L supervised the study; Valenti L edited the manuscript; all authors contributed to data interpretation, read and approved the final manuscript draft.
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Correspondence to: Luca Valenti, MD, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Pad. Granelli, via F Sforza 25, 20122 Milano, Italy. luca.valenti@unimi.it
Telephone: +39-2-50320278 Fax: +39-2-50320296
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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, a mild decrease in circulating AAT levels and without any pulmonary disease. 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.

Key words: Alpha1-antitrypsin deficiency; Rare variant; Alpha1-antitrypsin null mutation; Liver disease

Core tip: We report an incidental finding of a novel null alpha1-antitrypsin (*AAT*) allele, Q0Milano, consisting of a 17 nucleotides deletion in exon 3 of *SERPINA1* gene, in an Italian child with persistently increased in 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[3-5]. 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

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

	Primers forward 5′→3′	Primers reverse 5′→3′
AAT Ex2A	CCCCCCATCTCTGTCTTGC	GAGGAGTTCCTGGAAGCCTT
AAT Ex2B	ATGAAATCCTGGAGGGCCTG	CAGGCTGGTTGAGCAACCTT
AAT Ex3	CCCACCTTCCCCTCTCTCC	CACCCTCAGGTTGGGGAATC
AAT Ex4	CTTGAATTTCTTTTCTGCACGAC	AAGGTCGTCAGGGTGATCTC
AAT Ex5	GTCTCTGCTTCTCTCCCCTC	AGGGACCAGCTCAACCCTTC

AAT: Alpha1-antitrypsin; Ex: Exon.

in hepatocytes injury and liver disease[6].

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[7].

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[8]. 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 *PiMZ* 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 ($PiQ0_{Milano}$) (gene ID: 5265, official name SERPINA1, genomic sequence number: NC_000014.8; NCBI Reference Sequence: NG_008290.1). 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 Met^{358}-Ser^{359}.

The mutation arose in *PiM3 AAT* allele, which the proband inherited from his mother, whose genotype was $PiM1A/Q0_{Milano}$ and had normal liver and pulmonary function, whereas his father, who had normal genotype *PiM1V*/*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 ($Q0_{Milano}$) occurs in a key functional region of AAT gene where several other deficiency variants (P_{Lowell}/P_{Duarte} at codon 256, $Q0_{Cairo}$ 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 (Pi_{Duarte}, $Pi_{Hong\ Kong}$, $Pi_{Granite\ Falls}$) have been associated with intra-reticular accumulation of unfunctional AAT proteins, which are immediately degraded without any

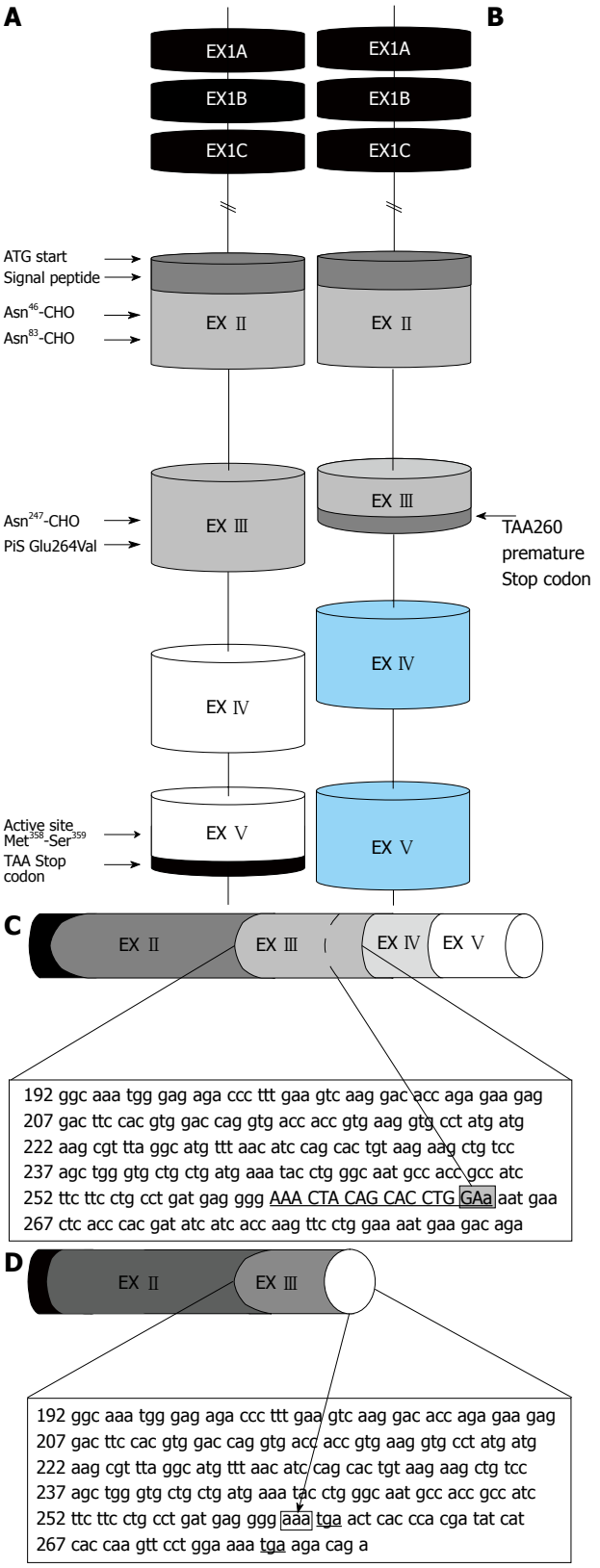

Figure 1 Schematic representation of wild type and mutant Alpha1-antitrypsin gene and protein. A: Alpha1-antitrypsin (*AAT*) wild type gene; B: *AAT* mutated gene: Bule region represents out of frame sequence. Untranslated regions are shown in black; C: AAT wild type protein: Exon 3 wild-type sequence is indicated in square; the deleted nucleotides are underlined; PiS locus is highlighted; D: AAT truncated unfunctional protein; Exon 3 deleted sequence is indicated below; premature stops codons resulting from frameshift were underlined.

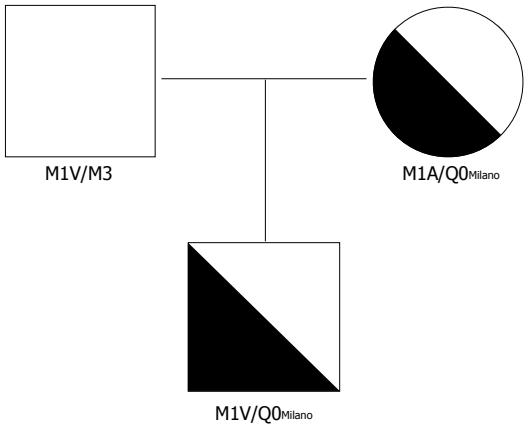

Figure 2 Pedigree of the Q0Milano proband's family. Alpha1-antitrypsin genotypes are listed below.

liver damage[20-22]. 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[23].

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 affected 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[24,25], were involved in the development of liver disease.

In conclusion, in this study we report a novel *AAT* null variant (*Q0Milano*) generated by a 17 nucleotides deletion in exon 3 of *AAT*, which leads to a premature stop codon.

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