Congenital analbuminemia attributable to Compound Heterozygosity for Novel Mutations in the Albumin Gene, Filomena Campagna,1 Francesca Fioretti,2 Marco Burattini,1 Stefano Romeo,2 Federica Sentinelli,2 Maura Bifolco,1 Maria Isabella Sirinian,1 Maria Del Ben,3 Francesco Angelico,3 IV Division of Internal Medicine, Department of Experimental Medicine and Pathology, University of Rome, La Sapienza, Italy; * address correspondence to this author at: Dipartimento di Clinica e Terapia Medica Applicata, Università di Roma “La Sapienza”, Policlinico Umberto I, Viale del Policlinico, 155, 00161 Rome, Italy; fax 039-06-4440290, e-mail marcelloarca@libero.it

Congenital analbuminemia is a rare autosomal recessive disorder characterized by the absence or very low concentrations of serum albumin (HSA) (1). The disorder is conventionally defined as an HSA <1 g/L (as determined by immunoassay) associated with normal liver function and absence of protein loss (2). The incidence of congenital analbuminemia is estimated to be <1 in 1 million births, without sex or race predilection. To date, 39 cases of congenital analbuminemia have been reported, 16 of which were diagnosed in children (3). Despite multiple functions of HSA (4), its absence is a relatively tolerable condition. Except for associated hyperlipidemia, minor edema, and mild fatigability, analbuminemic individuals suffer few adverse symptoms (3, 5, 6). Such relative mildness of symptoms is attributed to a compensatory increase in hepatic biosynthesis of other plasma proteins (7, 8).

Congenital analbuminemia is attributable to defects in the gene coding for HSA. The HSA gene is located on chromosome 4 and is split into 15 exons by 14 intervening introns (9). The identification of multiple mutations that cause analbuminemia clearly indicates that it is a genetically heterogeneous disorder. Six of these mutations introduced stop codons (10–13), and 2 caused splicing defects and determined a premature termination of the translation (14, 15). All were found to be present in the homozygous state. In the present study we characterized another case of congenital analbuminemia caused by 2 newly identified mutations in an Italian family.

The proband was a 29-year-old Italian man with an HSA of 10–12 mg/L as determined by immunoassay. His medical history has been reported elsewhere (16, 17). The proband’s relatives had HSA concentrations in the lower end of the reference interval (father, 35 g/L; mother, 33 g/L; sister, 42 g/L). All gave informed consent before participating in the study.

We used single-strand conformation polymorphism analysis to screen the coding region of the HSA gene in samples from the proband and a control individual. Briefly, the 14 exons and their intron–exon junctions were PCR-amplified from genomic DNAs by use of specific primer pairs as already reported (10). After amplification, 2 μL of PCR products was mixed with 8 μL of single-strand conformation polymorphism analysis buffer containing 950 mL/L formamide, 10 mmol/L NaOH, and 0.5 g/L of both xylene cyanol and bromphenol blue. The mixtures were denatured at 95 °C for 3 min and chilled on ice before electrophoretic separation. Denatured and non-denatured samples were then loaded on a 1X Mutation Detection Enhancement gel (FMC BioProducts) and visualized with silver staining. No abnormal bands were detected in the proband sample, except in exon 13. Direct sequencing of this fragment revealed a silent C-to-T substitution at nucleotide 15229. This variation, which introduced a recognition site for the restriction enzyme SacI (New England Biolabs), was demonstrated by restriction fragment length polymorphism (RFLP) analysis to be a common single-nucleotide polymorphism in samples from 50 control individuals (allele frequency, 0.585).

We next investigated the 14 PCR products by direct sequencing. Both strands were sequenced with the fluorescent dideoxy termination method (BigDye Terminator Cycle Sequencing Kit, Ver. 3.1; Applied Biosystems) on an ABI 310 automated sequencer according to the manufacturer’s protocol (Applied Biosystems). Electropherograms were analyzed with the ABI Prism 310 Data Collector software. The first mutation was identified in exon 10 and consisted of a C→T transition at nucleotide 11999 (Fig. 1A). This mutation changes codon CAG for Gln385 to stop codon TAG (Gln385Stop). The putative protein product should have a length of 384 amino acid residues. The proband was heterozygous for the defect as demonstrated by the sequencing electrophoretogram (Fig. 1A). As this mutation abolished a restriction site for the BseMI enzyme (MBI Fermentas), we used RFLP analysis to evaluate its segregation in the proband’s family. As shown in Fig. 1B, the digestion pattern revealed the presence of an abnormal 340-bp fragment in the proband and his father, whereas the mother and the proband’s sister were normal. This confirmed that the proband was heterozygous for Gln385Stop and demonstrated that he inherited this mutation from his father. The Gln385Stop mutation was named Roma2, for the city of origin of the father. This mutation was not found in 50 DNA samples obtained from healthy individuals.

We were unable to evaluate whether the putative truncated protein produced by the Roma2 allele was present in serum. In all previous cases with a stop codon...
within an exon, no evidence was found for the presence in
the serum of the truncated albumin form.

The second mutation was found in exon 11. The pen-
ultimate nucleotide of exon 11 had undergone an A→G
transition at position 13378 (Fig. 1C). This mutation
changes the codon TAT for Tyr-452 to codon TGT (see
Fig. 1A of the Data Supplement that accompanies the
online version of this Technical Brief at http://www.
clinchem.org/content/vol51/issue7/). This mutation
was also confirmed by restriction analysis using the HpyCH4
III enzyme (New England Biolabs). We found that it was
inherited from the mother (Fig. 1D). We named this
mutation Fondi for the small town in Central Italy where
she was born.

As the GT dinucleotide is known to be a site for intron
splicing, we predicted that the Fondi mutation introduced
a novel, anticipated donor splicing site replacing the first
2 bases of intron 12. To confirm this hypothesis, we
examined HSA cDNA obtained from proband’s leuko-
cytes. Briefly, total RNA was extracted with use of TRizol
(Invitrogen) according to the manufacturer’s protocol.
The RNA was reverse-transcribed by use of MultiScribe
reverse transcriptase (Applied Biosystems) with random
hexamers to obtain total cDNA. The primer pair 5’-
ATTGTGACAAAAAGACAGCTTG-3’ (forward) and
5’-TTTTGTTGCCCTGGGCTTGTGTTT-3’ (reverse) was
designed from mRNA sequences in GenBank by use of
Primer3. We used this pair of primers, spanning exon 10

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Sequence of the HSA gene of the analbuminemic proband and RFLP analysis of samples from the proband’s family.

(A), DNA sequence of exon 10 showing the C→T transition at position 11999 (Roma2 allele). The arrow indicates the nucleotide that is mutated in the patient. The proband is heterozygous for the defect, as seen by the presence of the 2 superimposed peaks representing the normal and abnormal base. (B), RFLP analysis of the Roma2 mutation in the proband’s family and in a control individual (lane C); the BseMII enzyme digested the 406-bp PCR product, giving 3 fragments of 245, 95, and 66 bp. The Roma2 mutation eliminates 1 restriction site for the enzyme, thus producing an abnormal 340-bp fragment. The proband (indicated by the arrow) and his father were heterozygous for this mutation. (C), DNA sequence of exon 11 showing the A→G transition at position 13378 (Fondi allele). The proband is heterozygous for the defect, as seen by the presence of the 2 superimposed peaks. (D), RFLP analysis of the Fondi mutation in the proband’s family and in a healthy control (lane C); the HpyCH4 III enzyme digested the 343-bp PCR product into 2 fragments of 288 and 55 bp. The Fondi mutation introduces 1 restriction site for the enzyme, thus producing 2 abnormal fragments of 178 and 110 bp. The proband (indicated by the arrow) and his mother are heterozygous for the Fondi mutation.
to exon 13, to PCR-amplify the region of HSA cDNA where the mutation was located. All reactions were performed on a T Personal (Biometra) thermocycler in a 20-μL reaction volume. Amplified products were then sequenced using the reported primers as described above. The cDNA electropherogram from the proband showed a double sequence starting from nucleotide 13378, a finding that indicates the presence of both wild-type and mutated alleles (see Fig. 1B of the online Data Supplement). These results confirmed that the mutation disrupts the normal codon TAT for Tyr-452, leaving the residual T nucleotide to join the first 2 CT nucleotides of exon 12 (see Fig. 1A of the online Data Supplement). This effect leads to a reading frameshift that introduces a premature stop codon 12 amino acids downstream in exon 12 (see Fig. 1A of the online Data Supplement). The translation product from the Fondi allele is expected to be a truncated protein consisting of 463 instead of the 585 amino acid residues usually found in mature HSA.

In previously reported cases of analbuminemia attributable to aberrant splicing, the consequences of the defect at the mRNA level were not evaluated (13, 14). We were able to obtain the mutated HSA mRNA from circulating leukocytes and thus directly confirmed that the Fondi allele caused a defect in the ligation of exon 11–exon 12 sequences. How aberrant splicing of the HSA gene affects mRNA processing is poorly known. Studies on Nagase analbuminemic rats, with a splicing error in the HSA gene (18, 19), demonstrated a marked decrease in hepatic mRNA (20). Although we were unable to measure the specific amount of mutated mRNA in our study participants, a substantial amount of cDNA was obtained by reverse transcription-PCR from our proband’s leukocytes, suggesting that the Fondi allele did not cause, at least in leukocytes, a complete degradation of the mutant mRNA.

In summary, we report the first case of congenital analbuminemia attributable to compound heterozygosity for 2 new mutations in the HSA gene. We also demonstrated that circulating leukocytes may be used to investigate the effects of analbuminemia-causing mutations on mRNA processing, making it possible to reevaluate all analbuminemic patients in whom molecular characterization of the HSA gene was carried out only at the genomic level.

References