Analbuminemia is a rare autosomal recessive disorder manifested by the absence or severe reduction of circulating human serum albumin in homozygous or compound heterozygous individuals. It is an allelic heterogeneous defect, caused by a variety of mutations within the albumin gene. The analbuminemic condition was diagnosed in a Turkish female infant on the basis of low albumin concentration (9.0 g/L). The albumin gene was screened by single-strand conformation polymorphism and heteroduplex analysis and submitted to direct sequencing. The proband was found to be homozygous for a T→C transition at nucleotide 13381, the 2nd base of intron 11. The effect of this previously unreported mutation, which inactivates the strongly conserved GT dinucleotide at the splice site consensus sequence of intron 11, was evaluated by examining the cDNA obtained by reverse transcription-PCR from the albumin mRNA extracted from the proband leukocytes. This analysis revealed that the mutation, named Bartin for the geographical origin of the patient’s family, results in the skipping of exon 11. The subsequent frameshift within exon 12 originates a premature stop codon located 5 codons downstream at position 411. The predicted translation product would consist of 410 amino acids. This novel extensive cDNA alteration is responsible for the analbuminemic trait.

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We describe a novel splicing mutation that is the cause of analbuminemia in a 1-year-old female infant, the 1st child of apparently nonconsanguineous parents, born and living in Bartin (Turkey). The patient was born at the 33rd gestational week with a birth weight of 1750 g. The family history is unremarkable for a similar clinical condition. The infant had an uncomplicated neonatal period, and then she presented with breathing difficulty and some respiratory findings at the 3rd month of age. Slight ankle edema had been noticed incidentally at that time. The patient was then hospitalized for respiratory problems and began ongoing follow-up with the diagnosis of reactive airway disease. Complete blood count and liver and renal function test results were within reference intervals, and no proteinuria was observed. Plasma protein electrophoresis showed an extremely low albumin concentration and the increase of phoresis showed an extremely low albumin concentration. Renal function test results were within reference intervals, with the exception of α1, α2, and β2 globulin fractions. The total serum protein was decreased (40 g/L) and only small amounts of albumin (~9.0 g/L) were measured. The mother’s plasma albumin was marginally decreased (38 g/L). The patient showed significant hypercholesterolemia (12.9 mmol/L) with a marked increase of the LDL fraction and only marginal increase of the HDL and the VLDL fractions. Triglyceride concentrations were slightly higher than the upper reference limit.

After we obtained informed consent, we collected blood samples from the proband and her mother. We extracted genomic DNA from whole blood and performed PCR amplification of 14 coding exons of the albumin gene and their intron-exon junctions (12) as previously described (7). The PCR products were subjected to mutation screening by single-strand conformation polymorphism and heteroduplex analysis (7). The only detectable differences in the mother compared with controls were the heteroduplexes and the single-strand conformation polymorphism of the 406-bp PCR product encompassing exon 10 (data not shown). The PCR fragments of exon 10 of the analbuminemic child, her mother, and 2 controls were then submitted to automated direct sequencing.

The results showed that both the patient and her mother were homozygous for the insertion of a T in a stretch of 8 T’s spanning positions 12086–12093 of intron 10 (12) (Fig. 1A, a). This mutation, which was previously described in individuals of Moroccan origin (7), occurs 23 positions downstream from the 5′ splice site in a tract that, compared with the other junctions, does not contain conserved sequence elements. On the basis of available literature data, no direct deleterious effect could be ascribed to the presence of this insertion. This variant probably represents a common polymorphism of the gene. We next examined the possibility of other changes that might have escaped the electrophoretic discrimination. All of the 14 coding exons of the gene were amplified and sequenced. The only mutation found was in exon 11 and the relative intron/exon junctions. DNA sequence analysis of this region showed a homozygous T→C transition at nucleotide 13381 in the analbuminemic patient, the 2nd base of intron 11 (Fig. 1A, b), whereas the mother is clearly heterozygous for the same defect (Fig. 1A, a).

It is well established that nearly all splice sites include invariant dinucleotides at each end of the intron, and that the 14 junctions present in the albumin gene conform with the GT and AG consensus sequences present at the 5′ and 3′ exon/intron splice sites, respectively (12). In humans, mutations that affect pre-mRNA splicing have been shown to account for up to a half of disease-causing gene alterations, potentially representing the most frequent cause of hereditary disorders. The most common consequence of splicing mutations is skipping of one or more
exons, followed by the activation of aberrant 5’ or 3’ splice sites and retention of full introns in mRNA (13).

To establish the consequences of the splicing mutation described, we attempted to amplify albumin cDNA from the proband and a control individual. Leukocytes, separated by ficoll solution, were used for isolation of total RNA. Extracted RNA was used in reverse-transcription reactions by specific albumin reverse primer ALB_1816R (5’-CAG CTG AAC TTG CAG CAA CA-3’) and/or oligo dT primers. The first strand of cDNA was amplified by primers for exon 10 (ALB_1244F:5’-ATT GTG AGC TTT TTG AGC AGC TTG-3’) and exon 13 (ALB_1695R:5’-TTT TGT TGC CTG GGG CTT GTG TT-3’). Amplicons showed different sizes: a PCR band of 452 bp was obtained from the normal sample, and an ~310-bp band was amplified from the proband cDNA (data not shown). Sequencing of the patient PCR product allowed us to establish that the T→C transition at the 2nd position of intron 11 results in the complete skipping of the preceding exon (Fig. 1B, b). The subsequent frameshift within exon 12 originates an anticipated stop codon located at position 411, 5 codons downstream of the 5’ end of the exon. The predicted translation product would consist of 410 amino acids (Fig. 1B, c), with a molecular mass of 47012 and a theoretical pI of 5.20, instead of 5.67 of the normal protein. Two-dimensional electrophoresis (14) confirmed the absence of normal albumin in the proband’s serum, but failed to reveal the presence of a truncated polypeptide chain (data not shown). These results showed that the mutation we report, for which we suggest the name Bartin, affects pre-mRNA maturation by inactivating the 5’ splice site sequence at the 11th exon-intron boundary of the albumin gene, and is a previously unreported mutation causing analbuminemia.

In humans only 2 DNA mutations affecting splicing have been reported to cause analbuminemia, but the consequences of the mutations on the mRNA could not be evaluated (8, 9).

Although in analbuminemic patients the dye-binding methods, serum protein electrophoresis, and immunoassays used for clinical diagnosis invariably indicated nonzero concentrations of albumin (3), in all the cases studied at a molecular level, including the Fondi allele (11) and the Bartin mutation reported here, no evidence was found for the presence in serum of a truncated protein. In those 2 latter cases, however, the mutation did not cause a complete degradation of the variant mRNA, at least in leukocytes. The Bartin protein would almost completely lack the 3rd domain of the molecule. Although it is unclear whether the integrity of the C-terminal end of the protein is crucial for albumin secretion or to prevent its degradation in serum, all the C-terminal truncated or elongated variants of albumin identified to date are present in serum of the heterozygous carrier individuals in amounts ranging from 2% to 30% of the total albumin amount. This finding led to the conclusion that any major structural alteration of the C-terminal region of albumin is probably crucial for the stability of the protein (15).

Two of the 10 different mutations reported to cause analbuminemia in humans, the Fondi allele (13378 A→G) (11) and the Bartin mutation (13381 T→C), lie in close proximity within the exon 11–intron 11 junction. The 2 mutated residues represent the penultimate base of the exon, and the 2nd base of the adjacent intron, respectively. Two other analbuminemia-causing mutations, Vancouver (7706 A→G) (8) and Seattle (7708 G→A) (4), are located very close together at the intron 6–exon 7 junction. In this case the mutated bases represent the penultimate residue of the intron and the 1st base of the adjacent exon, respectively. Taken together, these data suggest that the intron 6–exon 7 and exon 11–intron 11 junctions may represent hot spots in the albumin gene.

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References


