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A novel frameshift deletion in the albumin gene causes analbuminemia in a young Turkish woman

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ABSTRACT

Background: Analbuminemia is a rare autosomal recessive disorder manifested by the absence, or severe reduction, of circulating serum albumin. The analbuminemic trait was diagnosed in a young Turkish woman on the basis of her clinical symptoms (bilateral lower limb edema) and biochemical findings (minimal albumin amount and variable increases in other protein fractions).
Methods: Total DNA from the analbuminemic proband and her parents was PCR-amplified using oligonucleotide primers designed to amplify the 14 exons of the albumin gene (*ALB*) and the flanking intron regions. The products were screened for mutations by single-strand conformation polymorphism (SSCP) and heteroduplex analyses (HA).
Results: HA allowed the identification of the mutation site in exon 12. Direct DNA sequencing of this abnormal fragment revealed that the analbuminemic trait was caused by a homozygous CA deletion at nucleotide positions c. 1614–1615 in the codons for Cys538 and Thr539. The subsequent frameshift should give rise to a putative truncated albumin variant in which the sequence Cys(538)-Thr-Leu-Ser has been changed to Cys(538)-Thr-Phe-Stop. The parents were heterozygous for the same mutation.
Conclusions: Gel-based mutation detection and DNA sequencing substantiate the clinical diagnosis of congenital analbuminemia in our patient and show that the condition is caused by a novel mutation within the *ALB* gene. These results contribute to shed light on the molecular basis of this rare condition.

1. Introduction

Human serum albumin (ALB) is the major protein of blood plasma, where its normal concentration is 35–45 g/l, amounting to about 60% of the total protein. ALB is a multifunctional unglycosylated monomeric protein synthesised and secreted by liver cells. Its polypeptide chain is 585 amino acid long and folds to form a heart-shaped molecule, composed of three homologous domains, with about 67% α-helix but no β-sheet (1). All but one (Cys34) of the 35 cysteine residues are involved in the formation of stabilising disulphide bonds [1]. Among its multiple activities, ALB has two main functions: 1) it acts as a transport and depot protein for a wide variety of endogenous and exogenous ligands, including fatty acids, hormones, steroids, bilirubin, heme, metals, and pharmaceutical drugs; and 2) it plays a crucial role in maintaining the oncotic pressure and volume of blood [1]. In addition, ALB is an important circulating antioxidant and possesses enzymatic properties [2]. Its synthesis is governed by a single copy autosomal gene (GenBank genomic reference sequence NC_000004.10), which contains 15 exons separated by 14 intervening sequences, which are symmetrically placed within the three domains of the ALB molecule that are thought to have arisen by the triplication of a single primordial domain [3]. The gene maps to q11–22 of the human chromosome 4 where the genes of the other members of the ALB superfamily – α-fetoprotein, vitamin D-binding protein, and afamin or α-albumin – are also located [3]. The messenger RNA (GenBank coding reference sequence NM_000477.3) encodes a precursor protein (preproalbumin) of 609 amino acid residues. Cleavage of the 18 residue signal peptide and the 6 residue propeptide yields the mature protein.

Markedly low ALB levels may be associated to acquired disorders, such as impaired synthesis of hepatic proteins (severe cirrhosis or other hepatic diseases), renal or intestinal protein-losing disorders (proteinuria, glomerulopathies, renal diseases, diarrhea, or inflammatory bowel disease), and redistribution into extravascular compartments (septicaemia and other inflammatory states). Also an uncommon neurological

Abbreviations: ALB, serum albumin (protein); *ALB*, albumin gene; FcRn, neonatal Fc receptor.

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disorder of early-onset ataxia with ocular motor apraxia is associated with hypoalbuminemia; the causative mutations have been found in the aprataxin gene [4]. Congenital analbuminemia (MIM 103600) should be suspected in the presence of markedly low ALB levels when the above mentioned clinical states can be ruled out. Usually, the clinical diagnosis is based on routine serum protein electrophoresis, that shows a typical pattern: minimal ALB amount and variable increase in other protein fractions. This needs to be confirmed by molecular diagnosis, aimed at the identification of the causative mutation within the *ALB* gene by DNA sequence analysis. Typically, in the affected individuals, ALB is not completely absent, and analbuminemia is defined as showing an ALB concentration of <1 g/l, or confirmation by genetic studies [5]. In fact, albumin levels of even around 10 g/l have been recently shown to be caused by mutations in the *ALB* gene [5]. The wide range of the values reported for the ALB concentrations in analbuminemic subjects is probably due to the poor accuracy of the commonly used methods for ALB quantification, especially in the presence of low ALB levels [6].

Congenital analbuminemia is a very rare autosomal recessive disorder and consanguinity of the parents is associated with many of the reported cases for which genealogical data are available [1,2,5]. In spite of the fact that the trait is readily detected by routine plasma electrophoretic analysis, only 50 cases have been so far reported worldwide in 42 families and are listed in the continuously updated Register of Analbuminemia Cases [5]. Thus, the incidence is less than one in one million for most populations, apparently without gender or ethnic predilection. Several reports have suggested that the rarity of analbuminemia may be attributed to the perinatal loss of affected foetuses [2,5–7].

In spite of the crucial physiological role of ALB, analbuminemia is usually associated with surprisingly few medical complications. Analbuminemic subjects do not demonstrate serious symptoms, such as marked edema, because of a compensatory increase in other plasma proteins and the tendency of these individuals to have low capillary blood pressure or low-normal blood pressure [1]. The main clinical symptoms are mild edema, hypotension, fatigue, and, especially in women, lipodystrophy, while the most common biochemical signs are gross hyperlipidemia with hypercholesterolemia and elevated LDL cholesterol levels [1]. Although some analbuminemic individuals are treated with lipid-lowering drugs, there is not enough evidence available in the literature to determine if analbuminemics have an increased risk of atherosclerotic disease [5,8].

In this work we report the clinical findings and the molecular characterisation of a new case of analbuminemia diagnosed in a young Turkish woman. Our results show that the condition is caused by a novel frameshift deletion in exon 12 of the *ALB* gene. We suggest for this mutation the name Safranbolu from the city of the origin of the proband.

2. Materials and methods

2.1. Clinical laboratory analyses

The analysis of lipid parameters was performed by Roche P Module using the Abbott Architect 1600 Clinical Chemistry Autoanalyzer. Reagents were from the manufacturers' product line. The serum ALB level was measured in the same machine by the bromocresol green method. Semi automated serum protein electrophoresis was performed on agarose gel at pH 8.6 in a SAS 1/Platinum electrophoresis system (Helena Bioscience Europe).

2.2. Mutational analysis

The mutational analysis of the *ALB* gene was carried out following the principles outlined in the Declaration of Helsinki. After we obtained informed consent, we collected blood samples from the proband and from both parents and extracted genomic DNA from whole blood. Fourteen genomic fragments of the *ALB* gene encompassing the 14 coding exons and their intron–exon junctions [3] were PCR amplified using specific primer pairs as described by Watkins et al. [7]. Genomic DNA from two unrelated healthy volunteers was available as a control. All reactions were performed on a Hybaid thermocycler in a 25 μl reaction volume using PuReTaq Ready-To-Go beads (GE Healthcare) with a final $MgCl_2$ concentration of 1.5 mmol/l. Conditions for amplification with primers A23A and A24A included an initial DNA denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer annealing at 60 °C for 30 s and elongation at 72 °C for 30 s. Finally an extension at 72 °C was performed for 10 min. The other primers were used with the same protocol applying annealing temperatures ranging from 58 °C to 64 °C according to their melting temperature. The PCR products, that ranged from 288 to 464 bp in length, gave sharp bands when checked for homogeneity on a 1.5% agarose gel. The amplicons were mixed with equal amounts of SSCP buffer containing 95% formamide, 10 mmol/l NaOH, 0.25% bromophenol blue and 0.25% xylene cyanol and then submitted to mutation screening by single-strand conformation polymorphism (SSCP) and heteroduplex analysis (HA). An aliquot of each sample was denatured at 94 °C for 3 min and cooled on ice before the electrophoretic separation. Denaturated and non denaturated samples were then loaded on nondenaturing horizontal ultra-fine gels (0.3 mm) composed of 15% acrylamide (acrylamide/piperazine diacrylamide 85:1), 7.5% glycerol in 375 mmol/l Tris–formate buffer (pH 9.0); the electrodes consisted of paper wicks soaked in 1.04 mol/l Tris–borate buffer (pH 9.0) and the gels were run in a Pharmacia Multiphore II apparatus at 8 °C for 90 min at 0.8 W/cm [9]. The bands were visualised by silver staining; briefly, the gel was soaked in 1% nitric acid, rinsed in water and then stained in 0.2% silver nitrate for 20 min. The gel was rinsed several times with 0.28 mol/l sodium carbonate containing 0.0175% formaldehyde, until bands were developed (about 10–15 min). Silver staining was blocked with 5% acetic acid.

In preparation for sequence analysis, 5 μL of PCR products was cleaned up by ExoSAP-IT (USB Corporation; GE Healthcare EUROPE Gmbh). After digestion, 2.5 μl of purified PCR products was subjected to typical sequencing reactions by adding 1 μl of BigDye Terminator (Applied Biosystems) and 5 pmol of primer in a final reaction volume of 10 μl. Cycle conditions consist in a rapid denaturation (96 °C for 10 s) and annealing/extension (60 °C for 3 min) for 25 cycles, followed by a terminal extension at 72 °C for 6 min. Excess of dye terminators were removed by ethanol precipitation twice. Samples were electrophoresed on an automated DNA sequencing instrument (Applied Biosystems 3100), using 50 cm capillary arrays and POP-6 polymer. Data were analysed using the Sequencher software v.4.7 (Genecodes Corp).

2.3. Two-dimensional electrophoresis

Two-dimensional polyacrylamide gel electrophoresis was performed using the immobilized pH gradient system [10]. The first dimension, isoelectric focusing, was carried out on laboratory-made gels, cast on GelBond with a 4–10 non-linear immobilized pH gradient obtained with Acrylamido buffer solutions (Fluka) and the separation was run in the Multiphor II horizontal system (Amersham Biosciences). The gel strips were then equilibrated with SDS, placed on top of vertical 10% gels, and the second dimension was carried out using a Mini PROTEAN II cell (Bio-Rad). The gels were stained with Coomassie blue.

3. Results

3.1. Case report

The patient is a 21 year old female, who was referred to our clinic for evaluation of slight, bilateral lower limb edema developed during