Clinica Chimica Acta 411 (2010) 1711–1715

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

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

Monica Dagnino [a], Gianluca Caridi [a], Zeki Aydin [b], Savas Ozturk [b], Zeynep Karaali [c], Rumeyza Kazancioglu [b], Kivanc Cefle [d], Meltem Gursu [b], Monica Campagnoli [e], Monica Galliano [e], Lorenzo Minchiotti [e,*]

[a] Laboratory on Pathophysiology of Uremia, Istituto Giannina Gaslini IRCCS, Largo G. Gaslini, 5, 16148 Genova, Italy
[b] Haseki Training and Research Hospital, Department of Nephrology, Istanbul, Turkey
[c] Haseki Training and Research Hospital, Department of Internal Medicine, Istanbul, Turkey
[d] Istanbul University, Istanbul Medical Faculty, Division of Medical Genetics, Istanbul, Turkey
[e] Department of Biochemistry "A. Castellani", University of Pavia, viale Taramelli 3B, 27100 Pavia, Italy

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 12 July 2010
Accepted 12 July 2010
Available online 16 July 2010

Keywords:
Human serum albumin
Analbuminemia
Heteroduplex analysis
Hypercholesterolemia
Single-strand conformation polymorphism
DNA sequence

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.

* Corresponding author. Tel.: +39 0382 987724; fax: +39 0382 423108.
E-mail address: loremin@unipv.it (L. Minchiotti).

0009-8981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2010.07.009

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

childhood. The patient had a usual body shape without cellulite deposits or any other finding consistent with lipodystrophy, but she showed an unusually low albumin level (1.3 g/dl, detected by the bromocresol green method). Her parents are consanguineous, since the maternal grandmother of the mother and the paternal grandmother of the father are siblings. The family comes from Safranbolu, a town and district of Karabük Province in the Black Sea region of Turkey. The albumin levels of both parents are close to the lower limit of the normal range (3.6 g/dl the mother and 3.11 g/dl the father). The patient has two brothers and one sister. All are healthy, with the exception of the oldest brother, who has mental retardation with unknown aetiology. The family's history did not reveal foetal or neonatal death of siblings.

The principal clinical laboratory test results obtained on the serum of the patient are reported in Fig. 1 and in Table 1. Densitometric scanning of the serum protein electrophoresis revealed the near complete absence of the albumin peak and the elevation of all the globulin fractions. This compensatory increase leads to only moderate hypoproteinemia (Table 1). The patient demonstrated no clinical symptoms or laboratory findings consistent with a protein-losing disorder. Moreover, there was no evidence of protein malnutrition, malabsorption, or hepatic disease (the patient's hepatic enzymes were normal). Total, VLDL and LDL cholesterol, and triglycerides are all elevated, while the calcium level is below the lower limit of the normal range (Table 1). On the basis of the above reported findings congenital analbuminemia was diagnosed in our patient. In fact, also the abnormal lipid profile and the hypocalcemia are probably related to her low serum albumin concentration. Although further studies are required in order to elucidate the underlying pathophysiologic mechanisms accounting for the association between hypoalbuminemia and dyslipidemia [8], gross hyperlipidemia has been reported in the vast majority of the analbuminemic individuals [1,5,11]. Binding of calcium ions to albumin concerns about 45% of total serum calcium [1]. The binding is weak and not specific, but is highly significant from physiological and clinical standpoints [1]. Consequently, patients with a low serum albumin concentration are expected to have lower serum total calcium concentrations [11].

Table 1
Principal clinical laboratory test results on the serum of the analbuminemic subject.

Test	Proband	Units	Normal reference range
Protein electrophoresis:			
Albumin (absolute)	0.12	g/dl	3.6–5.1
Albumin (relative)	2.4	%	55.8–65.0
α1 Globulins (absolute)	0.48	g/dl	0.1–04
α1 Globulins (relative)	9.7	%	2.2–4.6
α2 Globulins (absolute)	1.38	g/dl	0.4–1.2
α2 Globulins (relative)	28.0	%	8.2–12.5
β Globulins (absolute)	1.17	g/dl	08.–1.3
β Globulins (relative)	23.7	%	7.2–14.2
γ Globulins (absolute)	1.79	g/dl	0.6–1.7
γ Globulins (relative)	36.3	%	11.5–18.6
Protein, total	4.93	g/dl	6.4–8.3
Cholesterol, total	419	mg/dl	130–200
VLDL cholesterol	48	mg/dl	0–40
LDL cholesterol	321	mg/dl	70–130
HDL cholesterol	50	mg/dl	40–60
Triglycerides	240	mg/dl	0–150
Calcium	7.98	mg/dl	8.4–10.2

All the analytes reported in the table were assayed in serum by routine clinical laboratory procedures, as described in the Materials and methods section. % stands for percentage of the total protein content.

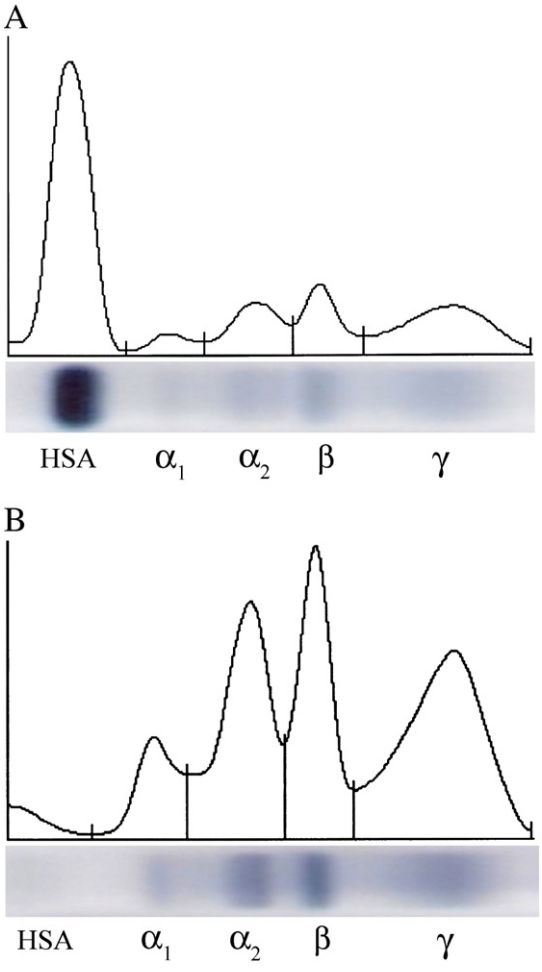

Fig. 1. Agarose gel electrophoresis and densitometric scanning after electrophoresis and protein staining of (A) normal serum and (B) proband's serum. Serum protein electrophoresis was performed as described in the Materials and methods section. Note the almost complete absence of albumin and the compensatory increase of serum globulins in (B).

3.2. Mutational analysis and two-dimensional electrophoresis

In order to confirm the clinical diagnosis of congenital analbuminemia at the molecular level, a mutational analysis of the *ALB* gene from the proband and her parents was carried out as described in Materials and methods. HA analysis clearly indicated that the only detectable change in both homozygous and heterozygous samples occurred in the 386 bp long region amplified by using PCR primers A23A and A24A encompassing exon 12 and the intron 11–exon 12 and exon 12–intron 13 junctions (Fig. 2). Both the heterozygous parents show the presence of four bands corresponding to homoduplex and heteroduplex PCR products (Fig. 2, lanes 2, 2′, 3, and 3′). The homozygous sample (Fig. 2, lanes 1 and 1′) revealed only one band but with a different mobility when compared with controls (Fig. 2, lanes 4, 5, 4′, and 5′). No variation due to conformation polymorphism could be seen under these electrophoretic conditions. The 386 bp long fragment was amplified and submitted to sequence analysis as described in the Materials and methods section. The results showed that the patient is homozygous for a CA deletion near the 3′ end of exon 12, at nucleotide positions c. 1614–1615, according to the Human Genome Variation Society rules, i.e. starting from the initiator codon (Fig. 3B). The electropherograms from the parents confirmed that they are both heterozygous for the same mutation (data not shown). The results of the mutational analysis revealed the inheritance of the trait and showed that analbuminemia in our patient is caused by a novel two base deletion in the *ALB* gene, for which we suggest the name Safranbolu, from the city of the origin of the family. The

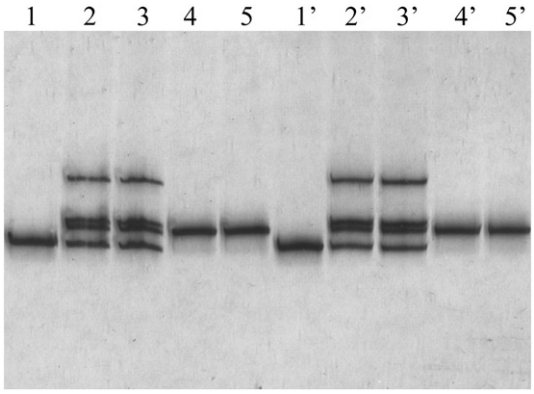

Fig. 2. Heteroduplex analysis of exon 12 from the Turkish family. The DNA encompassing exon 12 and the exon–intron junction from the patient, her mother and father, and two controls were amplified with primers A23A and A24A and the fragments were electrophoresed onto a non-denaturing polyacrylamide gel: *lane 1*, proband; *lane 2*, mother; *lane 3*, father; and *lanes 4–5*, controls. The same samples were denatured and cooled before loading: *lane 1′*, proband; *lane 2′*, mother; *lane 3′*, father; and *lanes 4′–5′*, controls. Heteroduplexes are evident in both parents (*lanes 2, 3, 2′ and 3′*), indicating a heterozygous condition, while an abnormal homoduplex is present in the homozygous proband.

putative effect of the mutation at the protein level is summarised in the following scheme:

				540			
ALB	I	C	T	L	S	E	K
ALB	ATA	TGC	ACA	CTT	TCT	GAG	AAG
Mutant Safranbolu allele	ATA	TG$\overline{C}$	$\overline{A}$CT	TTC	TGA	GAA	GGA
					541		
Putative Safranbolu protein	I	C	T	F	X		

The two nucleotides at positions c. 1614–1615, that are deleted in the Safranbolu allele, are underlined and represent the third base of Cys538 and the first base of Thr539, respectively. The subsequent frameshift changes the codon for Leu540 to Phe and introduces a stop codon TGA at position 541. The predicted translation product (p.Leu540PhefsX2) would consist of 516 amino acid residues instead of the 585 found in mature ALB. This truncation would eliminate nearly half of the third domain of the mature protein, consisting in the C-terminal region of the molecule. Two-dimensional electrophoresis, performed as described in the Material and methods section, confirmed the near complete absence of normal ALB in the proband's serum, but did not reveal the presence of this truncated polypeptide chain.

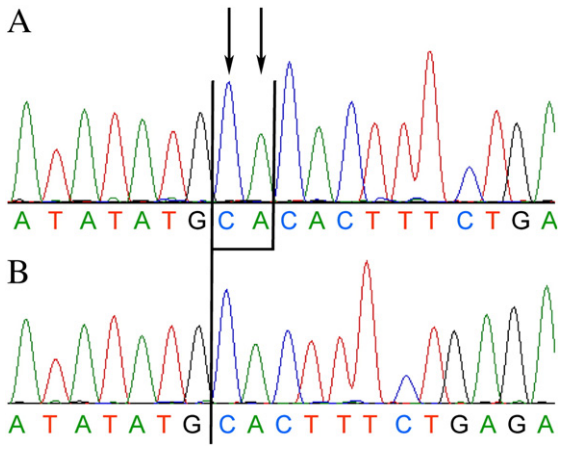

Fig. 3. DNA sequence of the mutated region of exon 12 in the Turkish patient: A, control for the wild type sequence; B, proband. Genomic DNA was prepared as described in the Materials and methods section. The arrows indicate the two bases that are deleted in the patient: CA at nucleotide positions c. 1614–1615. The patient is homozygous for the mutation.

4. Discussion

The two base deletion we have defined in our patient represents the 17th different mutation so far reported in analbuminemic subjects. These seventeen molecular defects, that have been identified in sixteen different individuals [2,5,12,13, and present paper], can be grouped into six nonsense mutations, four mutations affecting splicing, four frameshift/deletion, one frameshift/insertion, and a compound heterozygous nonsense/splice site mutation. The mutations are located in 8 different exons (3, 4, 5, 7, 8, 10, 11, and 12) and in 3 different introns (1, 6, and 11) [5,12,13]. Taken together, these results show that analbuminemia is an allelic heterogeneous disorder caused by homozygous or compound heterozygous inheritance of defects. On the one hand those findings seem to suggest that analbuminemia is the result of widely scattered random mutations [9]; on the other, however, they cannot rule out the presence of regions in the *ALB* gene that are prone to mutations resulting in analbuminemia. A possible hypermutable region was identified in the exon 11–intron 11 junction, as the Fondi allele (c.1427A>G) and the Bartin mutation (c.1428 + 2 T>C) lie in close proximity within this junction [2]. The Safranbolu two base deletion starts 3 nucleotides downstream the single base deletion c.1610delT identified in analbuminemia Locust Valley [14], suggesting that also this region of exon 12 might be prone to deletions resulting in analbuminemia.

The majority of the mutations identified as the genetic cause of analbuminemia are unique, i.e. they have been found in only a single individual or in the members of the same family. To date, only two exceptions to this rule are known: a c.412 C>T transition (analbuminemia Bethesda), and a two base deletion c.228_229delAT (analbuminemia Kayseri) [2,5]. The former mutation was identified in two unrelated individuals, whereas the latter was found in 12 subjects of 8 families, belonging to geographically distant and apparently unrelated ethnic groups [2, 5 and unpublished results)]. Therefore, the Kayseri mutation appears to be the most frequent cause of analbuminemia so far identified.

As a consequence of the previously identified mutations, the protein product was predicted to range in length from 19 to 532 amino acid residues, although no evidence was ever found for the presence in serum of those truncated molecules [2,3]. However, two splicing defects, the Fondi (c.1427 A>G) and Bartin (c.1428 + 2 T>C) mutations, could be verified at the mRNA level, since the variant mRNA is preserved to some degree and could be isolated from white blood cells [2]. The predicted translation product of the Safranbolu mutation would be 516 amino acids in length, the longest of any albumin molecule associated with analbuminemia after albumin Locust Valley, that shows a novel C-terminal sequence containing a proalbumin-like sequence and therefore has the potential to be post-translationally modified from 532 to 517 amino acid residues [14]. Neither of those two putative proteins, however, could be isolated from the sera of the analbuminemic subjects. Although they are significantly longer than the translation products predicted in other cases of analbuminemia, they would still lack a large portion of the third domain. It is well known that in bisalbuminemic heterozygous subjects with truncated or elongated variants the alteration of even a relatively small portion of the C-terminal region of the molecule results in a greatly reduced expression of the mutant allele [2]. Recent studies have shown that the domain III of the ALB molecule is crucial for binding to the neonatal Fc receptor (FcRn), that regulates the serum half-life of ALB through a pH-dependent mechanism that involves salvage from intracellular degradation [15]. Therefore it is likely that the ALB variants associated with analbuminemia, all partly or completely lacking the third domain of the molecule, are incapable of binding to FcRn and consequently go to lysosomal degradation. No

detectable pH-dependent FcRn binding was recently observed for a recombinant truncated ALB variant similar to the clinically observed splice mutant Bartin, that lacks the whole of domain III except the first 25 amino acids [16].

In a very few cases the finding of low albumin levels, with normal liver function and no gastrointestinal or renal protein loss, could not be associated to mutations within the fourteen coding exons and their intron–exon junctions and within the 5′ and 3′ untranslated regions of the *ALB* gene [17 and unpublished results]. In these cases hypoalbuminemia might be due to the presence of mutations within the intronic regions of the *ALB* gene or in more remote regulatory elements, or of mutations in the FcRn gene altering its ALB binding properties. The definition of the causative defects in a larger number of cases is required in order to achieve a better understanding of the molecular basis of analbuminemia.

Acknowledgements

Written informed consent was obtained from the proband for the mutational analysis and for the publication of the results. The authors declare that they have no competing interests. This work was supported by a FIRB 2003 RBNE03B8KK grant from the MIUR, Ministero dell' Istruzione, dell' Università e della Ricerca (Rome, Italy) and by the Cariplo Foundation. MD and GC acknowledge the support of the Renal Child Foundation.

References

[1] Peters Jr T. All about albumin: biochemistry, genetics and medical applications. San Diego: Academic Press; 1996.
[2] Minchiotti L, Galliano M, Kragh-Hansen U, Peters Jr T. Mutations and polymorphisms of the gene of the major human blood protein, serum albumin. Hum Mutat 2008;29:1007–16.
[3] Minghetti PP, Ruffner DE, Kuang WJ, Dennison OE, Hawkins JW, Beattie WG, et al. Molecular structure of the human albumin gene is revealed by nucleotide sequence within q11–22 of chromosome 4. J Biol Chem 1986;261:6747–57.
[4] Ferrarini M, Squintani G, Cavallaro T, Ferrari S, Rizzuto N, Fabrizi GM. A novel mutation of aprataxin associated with ataxia ocular apraxia type 1: phenotypical and genotypical characterization. J Neurol Sci 2007;260:219–24.
[5] The Albumin Website. Available at: http://www.albumin.org.
[6] Lyon A, Meinert P, Bruce GA, Laxdal VA, Salkie ML. Influence of methodology on the detection and diagnosis of congenital analbuminemia. Clin Chem 1998;44: 2365–7.
[7] Watkins S, Madison J, Galliano M, Minchiotti L, Putnam FW. A nucleotide insertion and frameshift cause analbuminemia in an Italian family. Proc Natl Acad Sci USA 1994;91:2275–9.
[8] Rosipal S, Debreová M, Rosipal R. A speculation about hypercholesterolemia in congenital analbuminemia. Am J Med 2006;119:181–2.
[9] Galliano M, Campagnoli M, Rossi A, Wirsing von König CH, Lyon AW, Cefle K, et al. Molecular diagnosis of analbuminemia: a novel mutation identified in two Amerindian and two Turkish families. Clin Chem 2002;48:844–9.
[10] Gianazza E, Astrua-Testori S, Giacon P, Righetti PG. An improved protocol for 2D maps of serum proteins with immobilized pH gradients in the first dimension. Electrophoresis 1985;6:332–9.
[11] Newstead J, Card S, Lyon A. Low serum albumin and abnormal body shape in a young Canadian First Nations woman. Lab Med 2004;35:350–6.
[12] Ruhoff MS, Greene MW, Peters T. Location of the mutation site in the first two reported cases of analbuminemia. Clin Biochem 2010;43:525–7.
[13] Dagnino M, Caridi G, Marsciani M, Bettocchi I, Tassinari D, Bernardi F, et al. A novel frame-shift deletion causing analbuminemia in an Italian paediatric patient. Eur J Clin Invest 2010;40:281–4.
[14] Davis RL, Peters Jr T, Brennan SO. Novel frameshift in the serum albumin gene results in analbuminemia through premature truncation and post translational modification. Clin Biochem 2008;41:1482–5.
[15] Andersen JT, Sandlie I. A receptor-mediated mechanism to support clinical observation of altered albumin variants. Clin Chem 2007;53:1549–52.
[16] Andersen JT, Daba MB, Sandlie I. FcRn binding properties of an abnormal truncated analbuminemic albumin variant. Clin Biochem 2010:367–72.
[17] Paraiso H, Prasad M. Analbuminaemia of unknown origin: a case report. Eur J Intern Med 2008;19:103–4.