Background: Analbuminemia is a rare autosomal recessive disorder in which individuals have little or no circulating albumin, usually the most abundant plasma protein. We describe a new mutation associated with analbuminemia.

Methods: We studied four apparently unrelated patients who had congenital analbuminemia: two of Amerindian and two of Turkish origin. The 14 exons and the flanking intron sequences of the albumin gene were amplified by PCR and screened for mutations by single-strand conformational polymorphism and heteroduplex analysis. The mutated DNA fragments were sequenced directly.

Results: In all four cases, analbuminemia was caused by the same mutation, an AT deletion at nucleotides 2430–2431, the 91st and 92nd bases of exon 3. This novel defect, named Kayseri, produces a frameshift leading to a premature stop two codons downstream. The predicted translation product would consist of 54 amino acid residues.

Conclusions: The AT deletion at nucleotides 2430–2431 is a novel mutation associated with analbuminemia. © 2002 American Association for Clinical Chemistry

Analbuminemia is a rare autosomal recessive disorder in which albumin, which usually accounts for approximately one-half of the total protein in serum and extravascular spaces, is either absent or drastically reduced (1). The condition is acquired by the inheritance of abnormal albumin alleles from both parents, who were found to be consanguineous in all reported cases in which genealogic data were available (1). In spite of missing the many relevant functions of serum albumin, affected adult individuals present with mild symptoms, such as minor edema, fatigue, and gross hyperlipidemia; the relative mildness of the symptoms is attributed to a compensatory increase in hepatic biosynthesis of other plasma proteins (1, 2). In contrast, the disorder appears to be more severe in the fetal state or during early infancy, and the low frequency of analbuminemia reflects the fact that most cases probably do not survive gestation (2).

The existence of analbuminemia is suspected when persistent unexplained hypoproteinemia is observed, and the diagnosis is confirmed by the absence of an albumin band in the serum protein electrophoresis pattern (3). The common dye-binding methods of measuring albumin produce falsely increased results with analbuminemic sera, which can preclude appropriate testing (3). Approximately 35 cases have been described to date in humans, which yields an estimated frequency of 1 in 1 million for most populations. The molecular basis of the disorder was defined for six cases, which were caused by different mutations within the albumin gene (2, 4–6).

In a continuation of our study of this disorder, we report here the molecular defect that is the cause of congenital analbuminemia in four, apparently unrelated, individuals. The first is a young woman of Turkish descent living in Germany, and the second is a Turkish adult male; they will be designated here by their places of origin, Kayseri and Trabzon, respectively. The other two (infants A and B) are infants of a Cree Amerindian band...
living in Saskatchewan, Canada, and one of them, infant A, was the subject of a previous report in which he was indicated as DN (3).

**Materials and Methods**

**Patients**

Four patients were examined in this study. All four presented with low circulating albumin, but the values were >1 g/L, which is conventionally accepted as indicative of analbuminemia.

*Kayseri.* The case designated Kayseri is an analbuminic woman born in 1978, the first child of a mother and a father who were cousins, from the Turkish city of the same name. She presented with generalized edema at the age of 17, and analbuminemia was diagnosed on the basis of the electroreoretic pattern of serum proteins in which albumin represented only 5.2% of the total (~3 g/L), whereas all other fractions were markedly increased.

*Infant A.* Infant A was admitted as a newborn with hypoglycemia and apnea; investigations revealed apparently low serum albumin (15 g/L) by a dye-binding albumin method (3). His serum albumin concentration remained low despite 24-h urine protein excretion rates within reference values (3). He was readmitted on three occasions in his first 6 months of life with respiratory distress and was eventually started on treatment for chronic asthma (3). Serum protein electrophoresis performed at 10 weeks revealed the absence of an albumin band (<3 g/L). The diagnosis of congenital analbuminemia was considered in spite of the misleading false results of conventional albumin methods; this diagnosis was supported when immunoassay indicated that serum albumin was <0.01 g/L (3). He had no symptoms directly attributable to the lack of albumin and presented with recurrent respiratory tract symptoms related to bronchial asthma or pneumonia (3). Reevaluation at age 3 revealed a physically normal infant. Fasting concentrations of cholesterol and triglycerides were increased: cholesterol, 6.9 mmol/L; triglycerides, 3.35 mmol/L.

*Infant B.* This female proband developed diarrhea and subsequent tetany at the age of 5 weeks. The infant had hypomagnesemia and responded to replacement treatment. Hypocalcemia was observed, but it was associated with a low serum albumin result (<10 g/L) by a dye-binding method. Treatment with intravenous albumin was performed to increase the serum albumin to 25 g/L, and the diarrhea resolved. Intravenous albumin treatment was continued for several weeks at an outpatient clinic. The diagnosis of analbuminemia could not be confirmed by serum protein electrophoresis because her serum contained albumin derived from intravenous infusions.

*Trabzon.* This male proband is the son of a marriage between first cousins living in Trabzon, a city at the Black Sea coast of Turkey. He presented with pretibial edema at the age of 30. Routine blood chemistry analysis performed at that time by the dye-binding albumin method showed severe hypoalbuminemia (5 g/L). A diagnosis of congenital analbuminemia was established by elimination of other causes of hypoalbuminemia. The albumin concentrations of the parents were determined, and both of them showed low values: 32 g/L for the mother and 29 g/L for the father.

**Mutational Analysis**

After patients or their parents consented to diagnostic testing, we collected whole blood from the patients and extracted the DNA with a Qiagen QIAamp DNA Blood Mini reagent set (infants A and B) or as described by Watkins et al. (2) (cases Kayseri and Trabzon). The 14 coding exons of the human serum albumin (HSA) gene and their intron-exon junctions (7) were PCR-amplified from genomic DNA using specific primer pairs (2). All reactions were performed on a Hybaid thermocycler in a 25-μL reaction volume using Ready to Go Beads (Amersham Pharmacia Biotech) with a final MgCl₂ concentration of 1.5 mmol/L. Conditions for amplification with primers A05A and A06A included initial DNA denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 64°C for 30 s, and elongation at 72°C for 30 s. A final extension step was performed at 72°C for 5 min. The other primers were used with the same protocol, with annealing temperatures ranging from 58 to 64°C according to the melting temperatures. The PCR products, which were 288–464 bp in length and gave sharp bands when checked for homogeneity on a 3% agarose gel, were submitted directly to mutation screening by single-strand conformation polymorphism (SSCP) and heteroduplex analysis.

For SSCP and heteroduplexes analysis, PCR products were mixed with equal amounts of SSCP buffer containing 950 mL/L formamide, 10 mmol/L NaOH, 2.5 g/L bromphenol blue, and 2.5 g/L xylene cyanol. An aliquot of each sample was denatured at 95°C for 3 min and cooled on ice before electrophoretic separation. Denatured and nondenatured samples were then loaded on nondenaturing horizontal ultrafine gels (gel thickness, 0.3 mm) composed of 15% acrylamide (acrylamide–piperazine diacrylamide, 85:1 by weight) and 75 mL/L glycercol 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 (8). The bands were visualized by silver staining; briefly, the gel was soaked in 10 mL/L nitric acid, rinsed in water, and then stained in 2 g/L silver nitrate for 20 min. The gel was rinsed several times with 0.28 mol/L sodium carbonate containing 0.175 mL/L formaldehyde until bands were developed (~10–15 min). Silver staining was blocked with 50 mL/L acetic acid.
In preparation for sequencing, the PCR products obtained with primers A05A and A06A from genomic DNA of the patient and controls were gel purified (QIAquick Gel Extraction Kit; Qiagen). Both strands were then sequenced with the fluorescent dideoxy termination method (BigDye Terminator Cycle Sequencing Kit; Applied Biosystems Inc.) on an ABI 310 sequencer (Applied Biosystems) using primer A05A and an internal primer, 5’-GTGTGTGTACGAGCATCACAC-3’ (A30M). Electropherograms were analyzed with the ABI Prism 310 Data Collector software.

Results

The four analbuminemic individuals examined in the present study were diagnosed independently, and their blood or DNA samples were sent to Pavia for mutation analyses, which will be described in the same order in which they were performed.

Kayseri

Fourteen genomic fragments of the HSA gene encompassing the 14 albumin-coding exons and their intron-exon junctions from the proband and her heterozygous mother were PCR-amplified and subjected to heteroduplex and SSCP analysis together with two controls. Heteroduplex analysis clearly indicated that the only detectable change in both the homozygous and heterozygous samples occurred in the 356-bp-long region amplified using PCR primers A05A and A06A, which encompassed exon 3 and the intron2-exon3 and exon3-intron3 junctions (Fig. 1). DNA from the heterozygous individual showed the presence of four bands of identical intensity corresponding to homoduplex and heteroduplex PCR products. The homozygous sample revealed only one band, but with a different mobility when compared with controls. No variation attributable to conformation polymorphisms could be seen under these electrophoretic conditions.

The samples were then compared with a previously characterized analbuminemic case named Codogno, which is caused by a 2368C→T substitution in the same exon (5). The electrophoretic pattern obtained for the Codogno PCR products was completely different: no heteroduplex could be seen, whereas the SSCP behavior was clearly different present (Fig. 1). Thus, because albumin Codogno has the only defined exon 3 analbuminemic mutation, the Kayseri allele apparently represents a novel mutation. The 356-bp-long fragment corresponding to this region was amplified and submitted to sequence analysis as described in Materials and Methods. The results obtained with the reverse primer showed that the patient is homozygous for an AT deletion at nucleotides 2430–2431 (7), which represent the 91st and 92nd bases of exon 3 (Fig. 2B). The electropherogram from the mother showed a double sequence starting from nucleotide 2440, which indicates the presence of both the wild-type and the mutated alleles (Fig. 2C). This result confirms the inheritance of the trait. The 2-base deletion leads to a frameshift mutation that introduces a premature stop codon two amino acids downstream (Fig. 3). The predicted translation product from the Kayseri allele therefore consists of only 54 amino acid residues instead of the usual 585 found in mature serum albumin.

Infants A and B

These two cases were examined together, and DNA samples from both parents of infant B and from the mother of infant A were available. PCR amplification of
the 14 exons of the albumin gene and electrophoretic analysis of the products were performed as described above for the Kayseri allele. The patterns obtained from both analbuminemic infants (Fig. 4A) showed the same difference in the region amplified with primers A05A and A06A when compared with controls, and their parents’ samples revealed the presence of four bands corresponding to a homoduplex and heteroduplexes. Sequence analysis showed that the two infants were homozygous for the same AT deletion at nucleotides 2430–2431. This mutation was identical to the one previously identified in the Kayseri proband as suggested by the electrophoretic patterns.

The underlined nucleotides indicate the two bases that are deleted in the Kayseri allele. The numbers above the line refer to the amino acid residues in mature albumin, whereas the numbers below the line refer to the nucleotide numbers from the genomic sequence (7).

Discussion

This study reports a novel mutation in the albumin gene that caused analbuminemia in two Turkish families and in two families of an Amerindian band. The mutation analysis, performed in four homozygous patients and in five heterozygous relatives, revealed the same AT deletion at nucleotides 2430–2431 in exon 3, whereas all other exons examined appeared normal. This mutation, for which we suggest the name Kayseri, causes a frame shift leading to the presence of an anticipated stop

TRABZON

Genomic DNA was extracted from peripheral blood samples obtained from the patient and his father by established procedures (2). PCR reactions, electrophoresis of the products, and DNA sequence analysis were performed as described above. The electrophoretic pattern of the proband, shown in Fig. 4B, was identical to those obtained for the other analbuminemic cases examined here, and the heterozygosity of the father was demonstrated by the presence of the same heteroduplex bands. The result of DNA sequence analysis confirmed that the condition is caused by the same 2-base deletion in the albumin gene.
The DNA encompassing exon 3 and the exon-intron junction from the Amerindian and Trabzon patients, their relatives, and two controls were amplified with primers A05A and A06A, and the fragments were electrophoresed on a nonde-naturing polyacrylamide gel. (A), lane 1, infant B; lane 2, mother of infant B; lane 3, father of infant B; lane 4, infant A; lane 5, mother of infant A; lanes 6 and 7, controls. (B), lanes 1 and 2, controls; lane 3, Trabzon patient; lane 4, father of Trabzon patient.

Fig. 4. Heteroduplex analysis of exon 3 from the two Amerindian infants (A) and from the Trabzon patient (B).

codon, and the predicted translation product consists of only 54 amino acid residues (Fig. 3) with the following sequence: AHKSEVAHRFKDLGEENFKALVLIAFA-QYLQCPFDHVKLNEVTFAKTCC. It is not known whether this putative truncated protein is synthesized, and if so, if it is broken down intracellularly, secreted poorly, or rapidly degraded after release into the bloodstream. Further analysis would require a liver biopsy for RNA analysis or very large amounts of serum, neither of which could be obtained.

Analbuminemia is a genetically heterogeneous disorder (5, 6). In fact, all the human cases described to date at the DNA level resulted from a different type of mutation, and the predicted protein products ranged in length from 19 to 273 amino acid residues. These six mutations are as follows: an exon-splicing defect attributable to a single A→G mutation at nucleotide 7706 in the 3’ splice site of intron 6 in an American Indian girl (4); an adenyl insertion in exon 8 that caused a frameshift and premature stop codon in analbuminemia Roma (2); three single-base mutations that generate stop codons at three different sites, a C→T transition at nucleotide 2368 in exon 3 in analbuminemia Codogno (5), a C→T transition at nucleotide 4446 in exon 4 for an American female (5), and a G→A transition at nucleotide 7708 at the start of exon 7 in a Canadian male (5); and a single G→A transition at nucleotide 118, the first base of intron 1, which inactivates the strongly conserved GT dinucleotide at the 5’ splice site consensus sequence of this intron and causes defective mRNA splicing (6). For all these patients, no data regarding the presence of the abnormal protein products are available (2, 4–6). The present study shows that the same mutation causes analbuminemia in two Turkish and two Amerindian patients, who belong to geographically distant and distinct ethnic groups. Another mutation in the albumin gene, which causes the albumin variant Naskapi (Lys 372→Glu) (9), has been found in high frequency in the Algonquian-speaking people of the eastern United States and Canada (9). Albumin Naskapi is identical to albumin Mersin, which has been detected in polymorphic frequency in Eti Turks of southeastern Turkey (10). Although identical analbuminemia and Naskapi albumin gene variants have been observed in some Eti Turks and Amerindians, there is no known relationship between these populations.

Our results demonstrate how mutation analysis using SSCP with heteroduplex analysis and DNA sequencing can be used to confirm the diagnosis of analbuminemia. The outcome of this study has generated molecular tools that can be used to confirm both analbuminemia in patients and the carrier status of related individuals. The mean serum albumin concentration in three of the parents of the analbuminemic patients was 34 g/L (by dye-binding serum albumin methods), a value near the lower limit of the adult reference interval (35–55 g/L) but otherwise an unremarkable finding. In the context of evaluating analbuminemia status, it is important to remember that serum albumin determinations by dye-binding methods, serum protein electrophoresis, and immunoassay are all likely to generate positive albumin results in analbuminemic patients (3); in addition, these methods can not assess carrier status. Mutation analysis has also allowed confirmation of analbuminemia in a patient who received repetitive intravenous albumin treatments. Our results show that molecular diagnostics can be used in the future to confirm analbuminemia and to assess carrier status in individuals with persistent unexplained hypoalbuminemia.
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