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Detection of a novel splicing mutation causing analbuminemia in a Libyan family ☆

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ABSTRACT

Background and objectives: Analbuminemia is a very rare autosomal recessive disorder. It is an allelic heterogeneous defect caused by a variety of mutations within the albumin gene.
We describe in this report two new cases of analbuminemia in Libyans.

Design and methods: The 14 coding exons of the human serum albumin (HSA) gene and their intron–exon junctions were PCR amplified. The products were screened for mutations by Denaturing High Performance Liquid Chromatography (DHPLC). Samples with altered DHPLC profiles were sequenced.

Results: DNA sequencing revealed the presence of a novol homozygous G➔T transition in the first base of intron 11 (c.1428 + 1G>T), in both children. This mutation destroys the GT consensus donor sequence found at the 5′ end of most intervening sequences and would cause the defective pre-mRNA splicing.

Conclusion: Molecular diagnosis based on DHPLC and DNA sequencing represents a powerful tool to study molecular defects causing analbuminemia.

Introduction

Human serum albumin (HSA) is the most abundant protein synthesized by the liver and comprises about one half of the total proteins in serum, where it has a normal concentration of 35–45 g/L. Among its multiple activities, albumin functions primarily as a carrier for a wide variety of endogenous and exogenous ligands, including steroids, fatty acids, thyroid hormones and pharmaceutical drugs, and plays a crucial role in maintaining the colloid oncotic pressure and the blood volume. HSA consists of a single polypeptide chain of 585-amino acid residues cross-linked by 17 disulfide bonds; the chain is folded into a heart-shaped molecule [1].

Albumin is encoded on chromosome 4 by a single gene composed of 15 exons, the last of which is untranslated, separated by 14 intervening sequences [2].

Analbuminemia, the near absence of albumin in the plasma (<1 g/L), is a very rare autosomal recessive disorder that was first reported by Bennhold et al. [3]. In spite of missing the many relevant functions of albumin, most affected individuals present with surprisingly mild symptoms, such as easy fatigability, slight edema and hyperlipidemia; the relative mildness of the symptoms is attributed to a compensatory increase in hepatic biosynthesis of other plasma proteins [2,4]. In contrast, analbuminemia, thought to be tolerable, appears to be more severe in fetal life or during early infancy, yielding to a significantly increased prevalence of miscarriage and neonatal death.

To date 51 subjects with analbuminemia have been reported worldwide in the continuously updated register [5]. Among these 51 cases, only 18 causative mutations have been elucidated.

In the present study we report two new cases of analbuminemia in a Libyan family and the results of the mutational analysis performed on the albumin gene.

Studied cases

Two brothers were examined in this study. Both of them presented with low circulating albumin, and values obtained with an immunoassay (Albumin/Microalbuminuria, Konelab 20™ Thermo Fisher Scientific Oy, Clinical Diagnostics Finland) were less than 1 g/L, which is conventionally accepted to be indicative of analbuminemia.

Patient 1

The proband analbuminemic patient was a two-month-old male infant, fourth son of first degree consanguineous parents from Tripoli (Lybia). He was born at term (38 week of gestation) but was (small for gestational age) with a birth weight of 2030 g and a length of 44 cm. Pregnancy had been complicated by oligoaminos and fetal bradycardia leading to induced delivery.

☆ Ethics: The work performed in our study is in accordance with the Ethical Guidelines for Journal Publication and has been approved by the Ethical Committee of the Children's Hospital of Tunis.

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He was firstly admitted to the neonatal ward for general care and observation in Lybia because of a slight pretibeal and periorbital pitting edema at the age of 2 weeks. Two weeks later, he was referred to pediatric hospital in Tunisia, with prominent generalized pitting edema as the chief complaint.

On physical examination, body weight was 3750 g, height 48 cm, head circumference 36 cm.

Laboratory investigations showed a decrease in the total protein levels 33 g/L (normal range: 60–80) and an abnormal profile of plasma protein electrophoresis with an extremely low albumin level (0.1 g/L) and increased levels of α1: 4.3g/L; α2: 11.5 g/L; and β globulins: 12.6 g/L (normal ranges: 0.8–2.2; 5.5–11 and 5.2–11.5 g/L respectively). Total immunoglobulins were also decreased: 4.5 g/L (normal range: 6.4–15.4). Urine analysis showed no proteinuria, sweat test was normal and serum liver enzyme levels were normal. A diagnosis of congenital analbuminemia was established after elimination of other causes of hypoalbuminemia.

The patient showed also low calcium: 1.78 mmol/L (normal range: 2.20–2.60), increased TSH: 5.24 mU/L (normal range: 0.27–4.2), normal FT4: 12.11 pmol/L (normal range : 9–23 pmol/l), normal total cholesterol: 4.15 mmol/L (normal range: 1.8–5.2) and slightly elevated triglycerides :1.50 mmol/L (normal range: 0.35–1.45) levels.

After treatment with intravenous albumin the edema disappeared and protein electrophoresis revealed an increase in albumin concentration (28.4 g/L).

The parents were healthy and both had slightly decreased serum albumin concentrations (32.9 and 33.5 g/L) with normal ranges of total cholesterol, triglycerides and TSH levels. Whereas one sister presented low albumin value (32.1 g/L), normal cholesterol and triglycerides ranges but revealed mild hypothyroidism with elevated TSH:6.23 mU/L and normal FT4:19.06 pmol/L levels.

Patient 2

He was a 2-year-old proband's brother who exhibited in the neonatal period a tetany crisis with general edema and marked hypocalcemia leading to his hospitalization but congenital analbuminemia diagnosis was not established.

During the examination, he seemed to be a physically normal infant without edema.

Laboratory results revealed low total proteins level (44 g/L) with the same pattern of typical congenital analbuminemia in plasma protein electrophoresis (albumin 0.2 g/L) and increased levels of α1, α2 and β globulins (3.2; 18.7; and 13.9 g/L respectively). Total immunoglobulins level was within normal range: 8.1 g/L. Calcium was slightly decreased: 2.11 mmol/L whereas total cholesterol and triglycerides levels were slightly increased (6.76 and 1.97 mmol/L respectively). Subclinical hypothyroidism was noted on the basis of high TSH: 8.23 mU/L and normal FT_4: 18.54 pmol/L levels.

Methods

DNA from the probands, their parents and both sisters were isolated from peripheral blood leukocytes after proteinase K digestion and phenol chloroform extraction. Samples were collected after obtaining the informed consent from parents and the study has been approved by the Ethical Committee of the Children's Hospital of Tunis.

Polymerase chain reaction (PCR)

The 14 coding exons of the human serum albumin gene and their intron–exon junctions [6] were PCR amplified using specific primers as previously described [7]. Amplifications were carried out in a 50 μl volume with 300 ng of genomic DNA, 200 μM each of the deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 1 U of GoTaq Flexi DNA Polymerase (Promega®) and 0.2 μM of each primer in 1× Colorless Go Taq Flexi buffer (Promega®).

The PCR reactions were performed under the following conditions: initial denaturation step at 94 °C for 5 min, 40 cycles of 94 °C for 1 min, 55 °C for 2 min and 72 °C for 3 min; and a final extension was performed at 72 °C for 5 min as described [8].

Denaturing high performance liquid chromatography (DHPLC) analysis

Heteroduplexes were obtained by denaturing the PCR products at 94 °C for 10 min and cooling at 56 °C for 60 min.

DHPLC analysis was performed on the automated Wave DNA Fragment Analysis System (Transgenomic®). Eight μl of each PCR product was injected into the mobile phase (buffer A, 0.1 M TEAA, pH 7.0 ; buffer B, 0.1 M TEAA, pH 7.0, containing 25% acetonitrile) using a flow rate of 0.9 ml/min. The heteroduplexes and homoduplexes were eluted from the solid phase (DNASep™ cartridge, Transgenomic Inc) by a linear gradient (5 min gradient, 2% slope per minute) in a 8.8 minute sample run under partially-denaturating conditions. The eluted products were detected by UV analysis at 260 nm. The melting characteristics of the DNA fragments were predicted by use of the Wavemaker™ software. Next, empirical optimization of DNA melting temperatures for amplicons was carried out varying the oven temperature by 0.5 °C above and below the optimal predicted temperatures. DHPLC analysis conditions and amplicons length are listed in Table 1.

A wild-type DNA sample already sequenced for the 14 studied amplicons was also analyzed by this PCR and DHPLC approach to allow comparison of DHPLC profiles. The wild-type DNA sequence showed perfect homology with the normal sequence of the albumin gene (ALB; GenBank accession no. NC_000004.10).

Table 1
Primers and DHPLC conditions used to screen for mutation in the human serum albumin gene.

Exons	Forward primer (5′/3′)	Reverse primer (5′/3′)	Amplicons (Pb)	Predicted melting temperatures (°C)	Buffer B (%)	Flow rate (mL/mn)
1	TGACAAGGTCTTGTGGAGAAA	GATAACCTTGTAAGACTTCAC	464	52.3–54.2	58.1	0.9
2	TGTAGGAATCAGAGCCCAATA	GAATCTGAACCCTGATGACAAG	288	52.8–53.3	54.4	0.9
3	GTTCTCTAGCGTAGCAACCTGT	CAATGGCTTATCAGTCTATGAG	356	52.1–53.2	56.2	0.9
4	CCTGACCAAGCTTAACCAGTATA	TAGAGTGTTGGCCTATGGAGTTA	381	53–54	56.7	0.9
5	TTCTGGGGAGAATGTCGATTAC	TAGAGTGTTGGCCTATGGAGTTA	309	53.1	55	0.9
6	CTGAGCTTATGGAGGGGTGTTTC	GACAGCGTCCTCTAAATTATTCAAC	444	52.4–53.4–54.4	57.8	0.9
7	GCCCTAAGGATAAGTGATTACC	CAACCCACTGTCAGCTATCACCA	394	51.6–52.9–53.1–54.9	56.9	0.9
8	GGTCTGAGGAGAAAGTGTAGCA	GATGAAGAAACATTCTGGGCAG	399	54.5–56	57	0.9
9	ACCCCAAGTCCTTAGCTACTAAG	GATTCCAGAATTGAAACCATCTC	416	52–53.4	57.3	0.9
10	CATGTGGCTTTGAGTAGGAAGAAG	TAGACTACATTCAAATATAGACCTCTCC	458	52–52.5–53	58	0.9
11	ACATCTTAGTTGATTCCGGCCAA	ACATGCACACACACACATTACT	343	52.8–53–53.2	55.7 –55.9	0.9
12	CACCTCTTTTGAATTTCTGCTC	GGCAACACTCCAATACTTTCCTC	386	56–57–58	56.8	0.9
13	CATGCAGATGAGAATATTGAGAC	CCTAAGCCCTAGCCTAACCAAAC	384	54–55.1–56	56.7	0.9
14	CAACTATGTCCGTGAGCTTCCA	GTGGTCGGTGCTGGTCTATATG	339	51–52	55.8	0.9