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CASE REPORT

A novel nonsense variation in the albumin gene (c.1309 A>T) causing analbuminaemia

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Introduction

Congenital analbuminaemia (OMIM # 616,000) is a rare autosomal recessive disorder, characterized by the near-complete absence, or very low levels, of serum albumin. The clinical diagnosis is usually made by serum protein electrophoresis and immunonephelometry [1,2]. However, since albumin levels vary depending on the method for their quantification, and as hypoalbuminaemia may be caused by many different clinical conditions, the mutation analysis of the albumin gene is mandatory in establishing the diagnosis of congenital analbuminaemia [1,2]. The condition is relatively benign in adulthood because the compensatory increase of other plasma proteins does partly take over the functions of albumin. Most adult analbuminaemic individuals are either asymptomatic or oligosymptomatic, with moderate clinical symptoms such as mild oedema, hypotension, and fatigue [1,2]. However, almost all show hypercholesterolaemia and elevated LDL-cholesterol levels, likely increase the risk of premature atherosclerosis and cardiovascular disease, although lack of an adequate follow-up data brings difficulty in confirming this link [2–4]. Furthermore, albumin concentration is considered a remarkably strong prognostic indicator of morbidity and mortality, especially in the elderly and in hospitalized patients [2]. In contrast with the mild symptoms in adulthood, congenital analbuminaemia can have serious consequences during the prenatal period, causing miscarriages and preterm birth, and can lead to death in early childhood, mainly from fluid retention and infections of the lower respiratory tract [2,5,6]. The rarity of the trait has been attributed to the fact that only a few analbuminaemic individuals survive past the pre- and perinatal period [2,5,6]. A confirmation of this hypothesis is provided by a recent survey, showing that congenital analbuminaemia is the second most common direct cause of deaths in children younger than 5 years [7].

The case

Subjects of this study are a 36-year-old male asylum seeker from Syria, brought to our attention by his older brother for a psychiatric consultation. Previous medical history was an acute psychiatric breakdown 3 years previously, treated with sertraline 50 mg, risperidone 0.5 mg and flupentixol/melitracen 0.5 mg/10 mg each once daily, with no other issues except a chronic fatigue. The brother denied that the patient had any history of unintentional weight loss, diarrhoea, or illness in the past. He also reported consanguinity of their parents (first-degree cousins) and the death of a sibling at the age of 4 months. He also reported that the case suffered from recurring infections of the respiratory tract and received regular inpatient treatments.

Repeated examinations during the following months were unremarkable. Due to suspicious mental slowness and before administering any medication, a blood analysis was done. The complete blood count was unremarkable. The chemical analysis was done by the institute of laboratory medicine in Triemli Hospital Zurich with a Roche Cobas 6000 system equipped with the C501 module. The main data of the case and his brother are shown in Table 1. The albumin concentration was measured by both a dye-binding Bromocresol colorimetric assay and by serum protein electrophoresis in a Hydrasys 2 Scan device (Sebia) (Figure 1), which showed the absence of the albumin band and the increase of several other serum protein components. The albumin level was also assessed on Roche Cobas 6000 and an 8000 system with a Tina-quant® turbidimetric immunoassay, with a lower limit of detection for the protein <3 g/l. The data reported in Table 1 confirm that serum protein electrophoresis and immunochemistry methods represent the best techniques for albumin quantification in analbuminaemic individuals, in which the photometric dye-binding assays overestimate this value [2,8].

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The patient's brother showed albumin levels close to the lower limit of the normal range and a compensatory increase, although less significant, of some other serum proteins. Among the other results reported in Table 1, the most significant one is the patient's hypercholesterolaemia with a significant increase in the LDL fraction. The brother also had hypercholesterolaemia, although less serious (Table 1). The cases were started on ezetimibe/atorvastatin 10/40 mg once daily [4]. After 2 months of this therapy, the case's LDL fell to 6.6 mmol/L and the triglyceride to 1.49 mmol/L.

As albumin and other plasma protein have roles in drug binding and transport, levels of the cases' medications were monitoring, finding sertraline, risperidone and their respective metabolites to be sub-therapeutic. Sertraline was continued (reported 98% plasma protein binding [9]), flupentixol/melitracen suspended (reported 99%/89% plasma protein binding), and risperidone (reported 88% plasma protein binding [10]) changed to its newer primary active metabolite paliperidone (9-OH-Risperidone, reported 77% plasma protein binding [10]). On a trial basis, olanzapine (reported 93% plasma protein binding [11]) failed to reach a therapeutic range.

Table 1. Clinical laboratory test results of the case and his brother.

Analyte	Case	Case's brother	Reference range[†]
Albumin* (%)	1.2	56.8	57.4–72.8
Alpha1-globulins* (%)	9.4	3.3	1.2–3.4
Alpha2-globulins* (%)	30.6	12.3	7.2–11.9
Beta-globulins* (%)	32.9	13.6	6.7–11.1
Gamma-globulins* (%)	25.9	14.0	8.2–20.1
Albumin[§] (g/L)	9	37	40.0–49.0
Albumin[¥] (g/L)	< 2	n/a[&]	35.0–52.0
Total proteins (g/L)	51	69	66.0–87.0
Ferritin (µg/L)	70	89	30–400
Creatinine (µmol/L)	65	79	59.0–104.0
Total Bilirubin (µmol/L)	0.4	6	< 21.0
AST (IU/L)	27	23	< 40
ALT (IU/L)	19	29	< 41
Total cholesterol (mmol/L)	8.7	6.2	< 5.0
HDL- cholesterol (mmol/L)	1.35	1.10	< 0.90
LDL- cholesterol (mmol/L)	7.1	4.6	< 4.0
Triglyceride (mmol/L)	2.23	2.32	< 2.26
Vitamin D25 (OH) (nmol/L)	69	n/a[&]	> 75
PTH (pg/mL)	51	n/a[&]	15–65
Total Calcium (mmol/L)	1.94	2.35	2.15–2.50
TSH (mU/L)	2.0	0.9	0.3–4.2
Sodium (mmol/L)	137	138	135–145
Potassium (mmol/L)	3.9	4.2	3.5–4.5
White blood cells (10^9/L)	5.2	n/a[&]	3.9–10.0
Red blood cells (10^{12}/L)	5.4	n/a[&]	4.3–5.7
Haemoglobin (g/L)	151	n/a[&]	135–172
Haematocrit (%)	45	n/a[&]	40–51
MCV (fL)	85	n/a[&]	80–99
MCH (pg)	28	n/a[&]	27–34
MCHC (g/L)	330	n/a[&]	320–360
Platelets (10^9/L)	174	n/a[&]	150–370
INR	1.04	1.08	0.8–1.2

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. TSH: Thyroid-stimulating hormone. PTH: parathyroid hormone. *Values measured by serum protein electrophoresis. [§]measured by a dye-binding Bromocresol method. [¥]measured by Roche Tina-quant® turbidimetric immunoassay. [&]not available

Other investigations were negative: hepatitis A/B/C, HIV, stool pancreatic elastase, a serologic panel screening against multiple intestinal parasites, and haemoglobinopathies (with normal HbF and HbA2). The activities of the erythrocyte enzymes glucose-6-phosphate dehydrogenase and pyruvate kinase were also normal. Gastroenterologists, using ultrasound of the abdomen and extensive specialist investigations, could not ascertain the cause of the low albumin level. A protein-losing enteropathy was excluded since the patient was asymptomatic. It was therefore also considered unnecessary to screen for an inflammatory bowel disease.

Having obtained written informed consent for genetic testing from both brothers, molecular analysis of the albumin gene (gene accession number: NG_009291.1) [12] was performed based on PCR amplification [5], followed by heteroduplex and single-strand conformational polymorphism analyses. Heteroduplex analysis revealed the presence of a molecular defect in the DNA fragment encompassing exon 11 and flanking intronic junctions [11], indicating that the case was homozygous and his brother heterozygous for the same mutation (Figure 2(a)). Automated DNA sequence analysis of this region in an Applied Biosystems 3100xl capillary DNA sequencer allowed us to identify an A > T transversion at nucleotide position c.1309, the first base of codon AAG for p.437Lys, giving rise to a stop codon TAG (Figure 1). This result confirmed that the case is homozygous and that his brother is heterozygous for this nonsense variant (Figure 2(b)). The premature termination codon would encode a truncated protein consisting of only 412 amino acids (p.Lys437Ter according to the Human Genome Variation Society rules) but, as with other variants resulting in congenital analbuminaemia, it is probably not present in the circulation, since an intact C-terminal end of the molecule is required for its long plasma half-life [13,14].

Discussion

We report a new case of congenital analbuminaemia in an oligosymptomatic adult patient, caused by a previously unreported gene variation, for which we propose the name Hama, the town of origin of the case. The family history reveals the death of a sibling in early infancy, consistent with the hypothesis of a crucial role of the protein in the perinatal period [5,6]. The brother, as all the heterozygous carriers of a variation in the gene that in homozygosity result in congenital analbuminaemia, having only one functional allele, shows an albumin level close to the lower limit of the normal range [1,2]. Attention should be paid to individuals in these conditions, and given the potential lethality of congenital analbuminaemia in the earliest periods of life, genetic screening request.

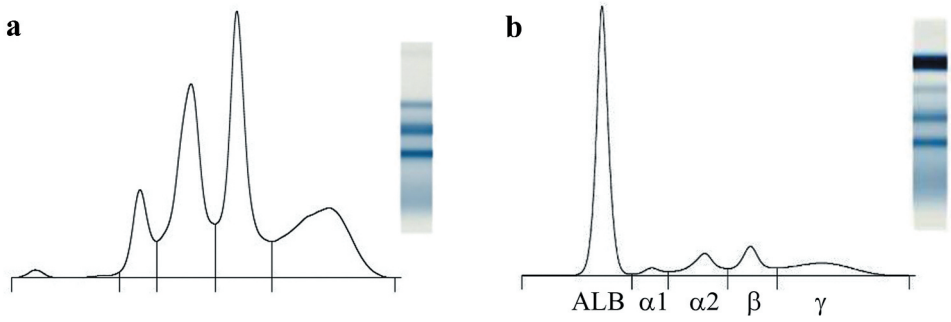

Figure 1. Serum protein electrophoresis of the case (a) and of a normal control from the same run (b). ALB, albumin; α1, α2, β and γ, the globulin fractions. The small peak at the far left of the densitometric scanning in (a) is most likely transthyretin. The lack of albumin causes a compensatory increase of this protein, as well as for the α1, α2, β and γ fractions, and its electrophoretic mobility is very similar to that previously reported for prealbumin in an analbuminaemic individual [16].

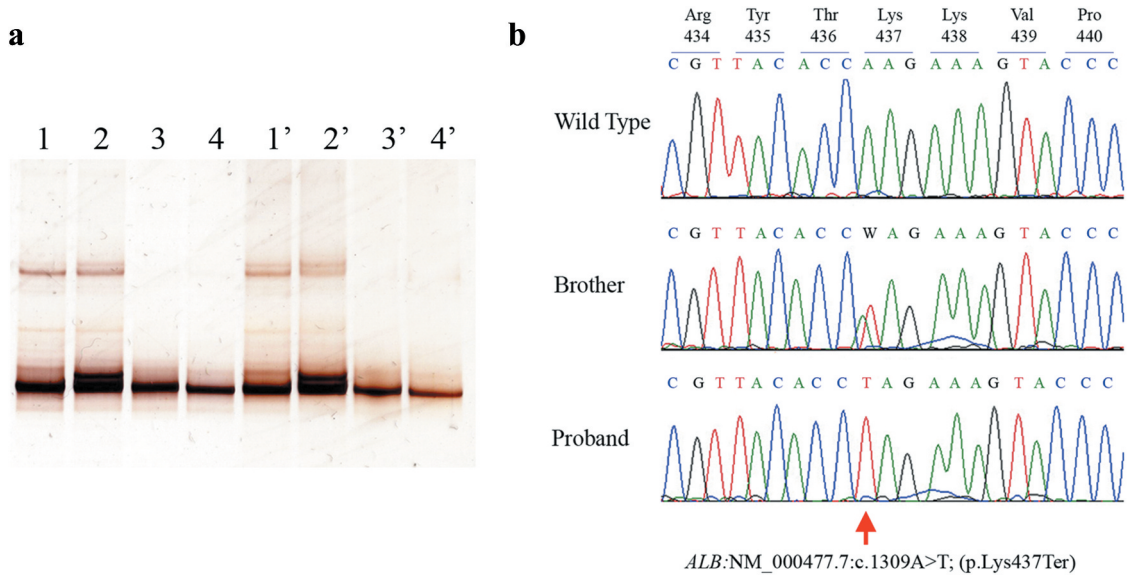

Figure 2. Mutation analysis of exon 11 of the albumin gene (*ALB*) in the two brothers. (a) Heteroduplex analysis. The DNA encompassing this region of the gene in the two brothers and in two controls was amplified with the PCR primers A21A and A22A [5], and the fragments were electrophoresed onto a non-denaturing polyacrylamide gel: lane 1, case; lane 2, brother; lanes 3–4, controls. The same samples were denatured and cooled before loading: lane 1′, case; lane 2′, brother; lanes 3′–4′, controls. (b) Genomic DNA sequence electropherograms of the variant region of the gene in the two brothers, compared with a wild-type sequence. The arrow indicates the A > T transversion at nucleotide position c.1309, the first base of codon AAG for p.437Lys, The case is homozygous for the mutation, and his brother is heterozygous for the wild-type and mutated alleles, as shown by the presence of two superimposed peaks.

The Hama variant is the twenty-eighth different pathogenic variant within the albumin gene so far reported to cause congenital analbuminaemia [2]. Twenty-six are present at the homozygous state, whereas in a single individual was identified with compound heterozygosity for the remaining two [2]. These include a variant in the start codon, frame-shift/insertion, frame-shift/deletions, nonsense variants, and variants affecting splicing [2]. These last (11 cases), together with nonsense variants (8 cases, including albumin Hama), and frame-shift/deletions (6 cases) seem to be the most common causes. These variations are located in 10 different exons and in seven different introns, a scattered distribution that seems to suggest that congenital analbuminaemia is the result of randomly occurring deleterious defects [15]. This hypothesis may be supported by the fact that the vast majority of the molecular defects so far identified, including albumin Hama, are unique, being found only in single individuals or within the same family [2]. In contrast, five variants, Bethesda, El Jadida, Safranbolu, Guimarães and especially Kayseri, are present in unrelated analbuminaemic subjects, indicating the presence of possible hypermutable regions in the gene [2].

These data represent an advance in biomedical science as they increase our knowledge of the phenotype of the analbuminaemic individuals and allow a better understanding of the molecular genetics underlying congenital analbuminaemia.

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