RESEARCH NOTE

Alport syndrome in a Kazakh family: a case study

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Introduction

Alport syndrome is a type-IV collagen inherited disorder caused by mutations in *COL4A3* and *COL4A4* (autosomal recessive) or *COL4A5* (X-linked). In our study, we describe the case of Alport syndrome in a Kazakh family. The 20-year-old male, born in 1993, was diagnosed with chronic nephritic syndrome when he was 16 years old. He had hearing loss and eye lesions. However, his maternal grandparents have not yet developed any kidney disease. They have normal vision and hearing. We have sequenced 51 exons of the *COL4A5* gene in a total of 18 family members. The pedigree consists of three generations including 18 members (eight males and 10 females). We identified missense mutation 1226G>A in exon 20 of *COL4A5* gene, causing amino acid substitutions of Gly to Asp at position 409.

Alport syndrome (AS, MIM: 301050) is an inherited disease that progresses to chronic renal failure (CRF), characterized by haematuria and/or proteinuria often combined with sensorineural hearing loss (SNHL) and eyes lesions (Flinter 1997). The hereditary nephritis with haematuria is caused by pathogenic variants in *COL4A1, COL4A2, COL4A3, COL4A4, COL4A5* and *COL4A6* genes encoding type IV collagen α-chain isoforms (Jais *et al*. 2000; Hudson *et al*. 2003; Kashtan 2009a, b). About 80% of cases are caused by mutations in the *COL4A5* collagen gene and are inherited in an X-linked form of inheritance (Barker *et al*. 1990; Hertz 2009). Autosomal recessive and dominant forms also exist due to mutations in *COL4A3* or *COL4A4*. Autosomal dominant inheritance accounts for about 15% of cases and only 5% less progressively in patients with the autosomal recessive type of disease (Smeets *et al*. 1993; Mochizuki *et al*. 1994; Jefferson *et al*. 1997).

In our study, we describe the case of Alport syndrome in a Kazakh family. The 20-year-old male, born in 1993, was diagnosed with chronic nephritic syndrome, when he was 16 years old. He had hearing loss and eye lesions. However, his maternal grandparents have not yet developed any kidney disease. They have normal vision and hearing.

Materials and methods

Case presentation

In this study 28 members of one Kazakh family were included. The proband is a 20-year-old boy with a familial history of haematuria. He was observed and diagnosed three years ago at the National Research Center for Maternal and Child Health in Astana. His blood analysis showed 70 g/L of total protein level, 35 g/L of albumin and 6.6 mg/L of cholesterol. Analysis of urine showed signs of proteinuria (1.2 g/day) and macrohaematuria. Laboratory data showed decrease of kidney function, characterized by increased level of potassium (5.6 mmol/L), creatinine (120 mmol/L) and urea (9.7 mmol/L) as well as decrease in glomerular filtration rate (56.6 mL/min) in blood. Extrarenal symptoms, such as oedema, or hypertension were not observed (blood pressure 120/80). Renal ultrasonography showed reduced size of both kidneys, decrease of intrarenal haemodynamics with signs of nephrosclerosis. Audiogram detected sensorineural hearing loss of the second degree. In addition, congenital disorders of vision, namely, iris subatrophy of both eyes were also found. During the study of family history we found that the patient's sister also have haematuria without hearing loss and eye disease; moreover, her kidney function was not impaired (figure 1). Mother of the proband (40 years old) had end-stage chronic kidney disease and received renal replacement therapy. Hearing and vision were normal. Proband's three uncles (brothers of proband's mother) died from end-stage chronic kidney disease between the age of 20 and 32 years, all of them had hearing loss. The older brother, who died of chronic renal failure in 32 years, have four daughters. Two daughters

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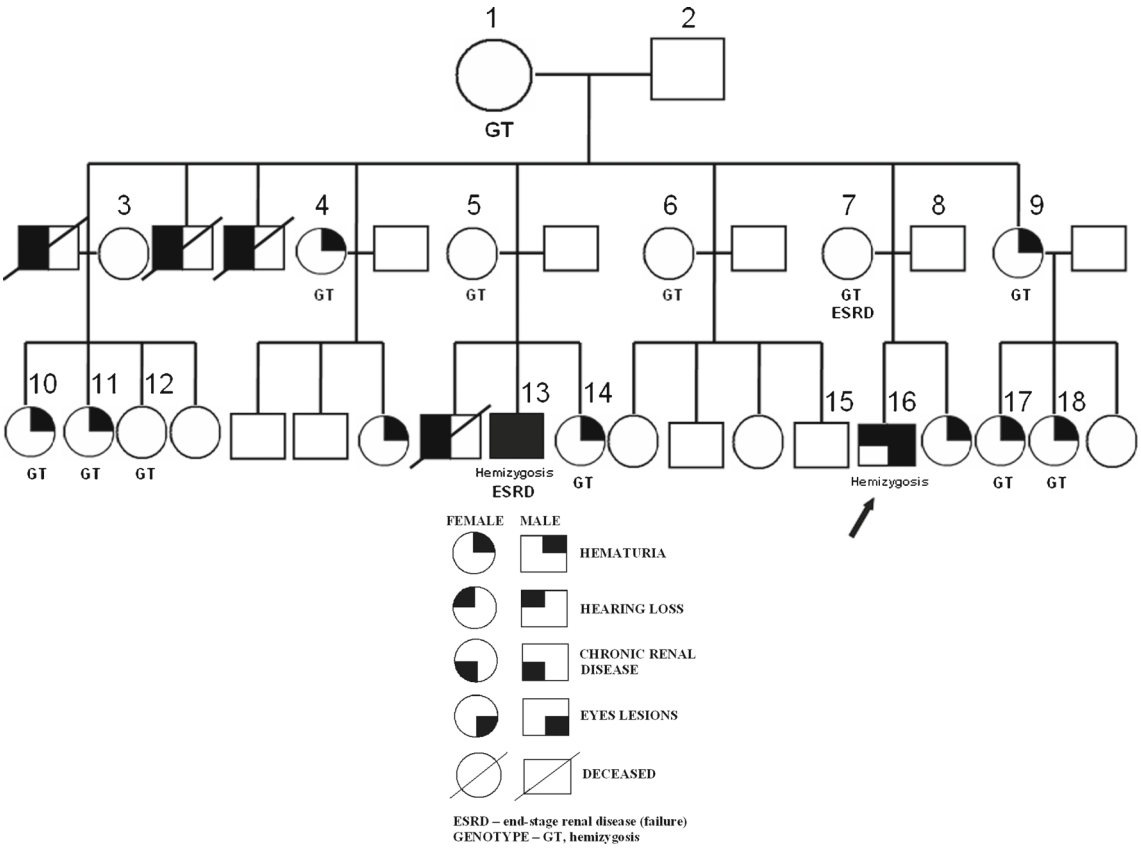

Figure 1. Pedigree of the Alport syndrome in the Kazakh family.

were diagnosed with haematuria without hearing loss and impaired renal function. Two other daughters were healthy. Proband's mother has four sisters (51, 47, 44 and 36 years old). Haematuria without hearing loss and impaired renal function were diagnosed in two sisters, namely, the eldest (51 year old) and the youngest (36 years old). The eldest sister (51 years old) has three children: two sons (26 and 19 years old) are healthy, and one daughter (22 years old) have haematuria without hearing loss and kidney function impairment. The second sister (47 years old), who does not have haematuria, has three children. The eldest son died due to undiagnosed late-stage chronic kidney disease at the age of 18, the second son began receiving renal replacement therapy for end-stage chronic kidney disease at the age of 18 years. Both of them had sensorineural hearing loss. Nine years old daughter was diagnosed with haematuria without hearing loss and impaired renal function. The third sister (44 years old) has four children, namely, two sons (27 and 13 years old) and two daughters (16 and 25 years old). All children are healthy without vision and hearing defects. Younger sister (36 years old) with haematuria has three daughters. Two girls (13 and 18 years old) diagnosed with haematuria without hearing loss and kidney dysfunction. Third daughter (2 years old) was intact, showing normal urine parameters. Proband's maternal grandfather (71 years old) and maternal grandmother (71 years old) have no kidney disease, hearing and vision are normal. Pedigree analysis showed that the proband's mother had end stage renal disease (ESRD). Based on this data, we have identified X-linked form of inheritance of the Alport syndrome. It is known that X-linked Alport syndrome (XLAS) is caused by mutations in *COL4A5*.

Mutation analysis

Genomic DNA was extracted from peripheral whole blood samples using Wizard Genomic DNA Purification kit (Promega, Madison, USA). Analysis was carried out using direct DNA sequencing analysis with protocols that is recommended by the reference laboratory, located at the University of Utah. DNA sequencing was carried out using the BigDye Terminator Cycle Sequencing v3.1 kit (ABI, Foster City, USA). Automated sequencing was performed on 3730XL Genetic Analyzer, Applied Biosystems, Foster City, USA. Analysis was performed using the reference sequence of the gene *COL4A5* (NM_000495.3). Mutations analysis was performed using a database of Alport syndrome http://www.arup.utah.edu and https://grenada.lumc.nl/LOVD2/COL4A/home.php?select_db=COL4A5. All procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki

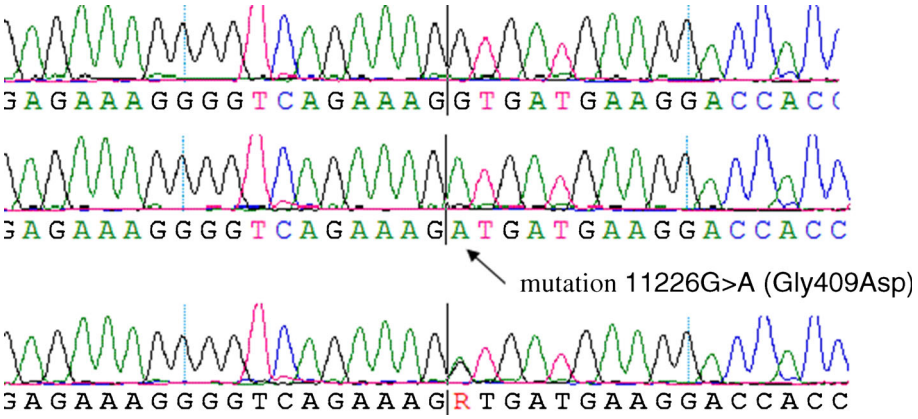

Figure 2. Identification of mutation 1226G>A in the studied family; DNA sequences of exon 20 *COL4A5*.

Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Results and discussion

In this study we have sequenced 51 exons of the *COL4A5* gene in a total of 18 family members. We identified a nucleotide change 1226 G>A, causing amino acid substitutions of Gly to Asp at position 409, in hemizygosis in the exon 20 of *COL4A5* gene (proband 16 and the 13 in pedigree). Sixteen females from pedigree were heterozygous carriers (figure 2; table 1).

More than 700 variants have been described and a further 400 are estimated to be known to individual laboratories but are unpublished (htpp://grenada.lumc.nl/LOVD2/COL4A). Mutation Gly409Asp has been described by Renieri *et al*. (1996). In 1996 according to international database of variations in the gene *COL4A5* Renieri *et al*. (1996) have shown that glycine substitutions are associated with a variable age at onset of ESRD in males. Basically, substitutions of glycines in the collagenous region are supposed to be pathogenic because they create a kink in the folding of the triple helix. There are no reports describing Gly409Asp mutation and clinical manifestations of the Alport syndrome.

Table 1. Clinical manifestations in the family members with/without mutation Gly409Asp in the *COL4A5* gene.

ID	Gender	Age	Haematuria	Eyes lesions	Hearing loss	Mutation Gly409Asp
1	F	71	No	No	No	Yes
2	M	71	No	No	No	no
3	F	50	No	No	No	no
4	F	51	Yes	No	No	Yes
5	F	47	No	No	No	Yes
6	F	44	No	No	No	Yes
7	F	40	ESRD	No	No	Yes
8	M	42	No	No	No	No
9	F	36	Yes	No	No	Yes
10	F	29	No	No	No	Yes
11	F	28	Yes	No	No	Yes
12	F	19	Yes	No	No	Yes
13	M	21	ESRD	Yes	Yes	Yes
14	F	12	Yes	No	No	Yes
15	M	13	No	No	No	No
16	M	20	Yes	Yes	Yes	Yes
17	F	18	Yes	No	No	Yes
18	F	13	Yes	No	No	Yes

Mutation analysis of the proband and his cousin have revealed a mutation (Gly409Asp) in hemizygosis in the *COL4A5* gene. Both had phenotypic effects: the sensorineural hearing loss and eyes disease (angiopathy of retinal vessels).

It should be noted that the grandmother (sample 1) and two aunts (samples 5 and 6) are asymptomatic carriers of Alport syndrome. This fact can be explained by the relationship between disease manifestations in females with *COL4A5* mutations and X-chromosome inactivation (Jais *et al*. 2000; Migeon 2008; Kashtan 2009a, b; Demosthenous *et al*. 2012). In other words, phenotypic expression of X-linked traits in females is highly dependent on the random inactivation of X chromosome. It is well-known that heterozygous females have widely variable disease outcomes, with some affected females exhibiting normal urinalysis and kidney function, while others develop ESRD and deafness (Rheault 2012).

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