GENETIC ANALYSIS OF ALPORT SYNDROME: A CASE STUDY

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ABSTRACT: *Alport syndrome is a genetic condition characterized by glomerulonephritis, end stage kidney disease, hearing loss and can also affect the eyes. This project was undertaken to determine the mode of inheritance and nature of the mutation responsible for Alport syndrome in a family whose two individuals were affected. The mode of inheritance was determined with the help of data which was probably autosomal recessive in this case. The mutation responsible for Alport syndrome lies on chromosome # 2 or X chromosome and was detected by analysis of blood slides under automated karyotype system. However, the mutation was too small to appear in the karyotype analysis.*

Keywords: Alport syndrome, Gentic analysis, Inheritance, Mutation.

INTRODUCTION

Alport syndrome is a progressive hereditary glomerulonephritis that is characterized by hematuria, sensorineural deafness, ocular lesions and progressive renal failure. It results from mutations in type IV collagen genes and is a common hereditary cause for end-stage renal disease [1]. The prevalence of Alport syndrome is 1 in 50,000 births affecting males more often and severely than females [2].

Alport syndrome has three modes of inheritance i.e. X-linked, autosomal dominant and autosomal recessive [3]. It is caused by mutations in the *COL4A3* and/or *COL4A4* genes located on chromosome no 2 or the *COL4A5* gene on the X chromosome [4]. About 80 % of Alport syndrome is inherited in an X-linked manner, 15 % in autosomal recessive and 5 % in autosomal dominant manner [5]. Each mode of inheritance exhibits different symptoms which appear in different ages (Table 1).

Mutations in these genes disrupt the normal function of type IV collagen present in basement membranes of kidneys, ears, eyes and skin, thereby affecting their integrity. Different mutations cause the different phenotypes, but it is difficult to predict the consequences of a certain mutation, because deletion of a whole gene does not necessarily produce a more severe phenotype than does an amino acid substitution [6].

MATERIALS AND METHODS

In this project Alport syndrome was studied in a family whose two members were affected. A questionnaire was prepared to get the history of the syndrome in the affected family and also to obtain information about the present condition of the patient. A pedigree chart was prepared based on this information (Fig. 1).

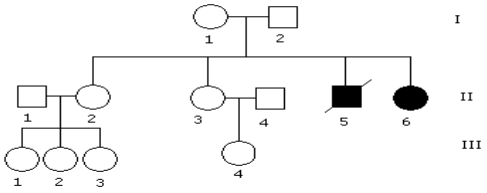

Fig 1: Pedigree chart of the affected family

A complete medical record of both the affected siblings was also obtained to help in the determination of the probable mode of inheritance and also to study the progression of symptoms along with age.

In order to detect the mutation responsible for the syndrome in this family, karyotype analysis was done. For this purpose, 3 ml blood sample was collected from the female patient and transferred to a vial containing heparin to prevent clotting. Slides were prepared with this blood sample using the method of [7].

RESULTS AND DISCUSSION

The family under study consists of four children i.e. two elder daughters, a son and then another daughter. Their only son and the youngest daughter were found to be affected with Alport syndrome. The son died at a young age of 14 due to unsuccessful renal transplant. H. Their youngest daughter has not suffered such severity. The elder daughters were found to be normal and healthy like their parents. They both are married to unrelated persons, eldest one having two daughters and the other one having only one daughter. All of them were found to be healthy. On questioning, it was found that no one else in the family has ever suffered from any sort of renal inefficiency, ocular problems, hearing loss etc. The grand parents were also healthy. No cases of hematuria have ever been reported in the family except these two. Not all the marriages in the family have been consanguineous. However, parents of the affected children are first cousins. Fig 1 is a pedigree of the affected family unto three generations. A complete medical record of the both affected individuals was obtained and progression of various symptoms was studied. Since the age of onset of these symptoms is variable in all the three types of Alport syndrome, it is useful in establishing the probable mode of inheritance [8]. The symptoms and progression of the disease in X linked Alport syndrome and autosomal recessive Alport syndrome resemble each other while autosomal dominant Alport syndrome is typically a slower progressive disorder with the symptoms appearing relatively late in life [9]. The symptoms appear without any gender difference in ARAS, while in XLAS males are more often severely affected (Table. 1). In this case, the symptoms appeared at an early age without any gender difference. Both the siblings suffered from microhematuria and proteinuria from an early age. Hearing loss was also an early development. Red blood cell count or hematocrit (HCT) was found to be low. Moreover creatinine level was also elevated in both siblings. Blood Urea Nitrogen (BUN) level was higher than the normal range in the female patient. The reason for this is the increased permeability of Glomerular Basement Membrane (GBM), thus an indication of some degree of renal failure. Due to some reason the negative charges in the basement membrane of the glomerular capillaries are lost or reduced. This allows proteins,

Table 1: A comparison of onset of various symptoms of Alport syndrome regarding age

SYMPTOMS	AGE AT WHICH THE SYMPTOMS APPEAR IN :			
	ADAS	ARAS	XLAS	
	Males & Females	Males & Females	Males:	Females:
Hematuria	Childhood	Early Childhood	Early Childhood	Early Childhood
Proteinuria	Late Childhood	Childhood	Early Childhood	Later in life
Nerve Deafness	Very late in life (after 50 years of age)	Early Childhood	Late Childhood	Later in life (less requent)
Eye Disorders	Uncommon	Uncommon	Late adolescence	Late adolescence
ESRD	Usually after 50 years of age	Before the age of 30	About 30-40 years of age	About 40-60 years of age
Skin problems	Absent	Absent	Complete absence of staining of epidermal basement membrane	Discontinuous staining of collagen a5 (IV) chain
Hypertension	Does not develop before the onset of chronic renal insufficiency in all cases			

Table 2: Medical record of the male patients

	Normal range	August 1996	Sep 1996
Blood	Negative	+++	+++
Proteins	Negative	+++	++++
Haemoglobin	11.5-17.5 g/dl	9.9 g/dl	8.4 g/dl
MCV	79-96 fl	-	56.3
MCHC	33-35 g/dl	-	29.5
MCH	27-33 pg	-	16.6

Table 3: Medical report of female patient

	Normal Range	1998	2000	2002	2004	2008
Blood	Negative	++	++++	+++	+++	+++
Proteins	Negative	+	+	++	+++	+++
Cholesterol	120-180 mg/dl	212	239	-	169	-
BUN	6-20 mg/dl	34	21	17	16	26
Creatinine	0.50-0.9 mg/dl	0.5	0.5	0.3	0.7	1.23
Haemoglobin	11.5-17.5 g/dl	9.9	9.9	9.60	10.3	8.6
HCT	36-54%	-	30	31.40	33.8	28.6
MCV	76-96 fL	-	65	61.8	62.9	63.7
MCH	27-33 pg	-	32.0	18.9	19.2	19.2
MCHC	33-35 g/dl	-	31.0	30.6	30.5	30.1

particularly albumins, which are also negatively charged to pass through the GBM easily. In Alport syndrome, another condition known as uremia is present. During this condition, kidneys are unable to excrete non-protein nitrogenous compounds-especially urea, creatinine and uric acid. These products are produced due to failure of the body to excrete the metabolic end products of proteins. Creatinine which is a by-product of muscle metabolism is cleared from the body fluids almost entirely by glomerular filtration. Therefore if glomerular filtration rate (GFR) decreases then the creatinine excretion rate also decreases. Thus by estimating creatinine clearance, the efficiency of the kidneys can be determined. The patients who have lost as much as 75% of their nephrons usually are able to excrete normal amounts of fluids. However, urea and creatinine accumulate almost in proportion to the number of destroyed nephrons [10]. Thus from the above given results, it can be concluded that both the siblings had been suffering from some degree of renal insufficiency from an early age. This condition was found to be more severe in the male patient when his kidneys failed to function properly. He died at a young age of 14 due to unsuccessful renal transplant. The cause for his death was probably due to anti-GBM disease, which is a rare complication that may occur in some patients with Alport syndrome after a renal transplant [11]. Hypertension does not usually develop before the onset of chronic renal insufficiency [12] and was observed in the male patient at the time of kidney failure. A high level of cholesterol was also observed in both siblings. On the other hand the female patient has not suffered from such severity. However as seen in table 3, she also suffers from somewhat decreased renal inefficiency. Moreover, according to Atkin and colleagues [13], hearing loss essentially always accompanies renal failure in Alport syndrome as observed in this case. It was concluded so because the symptoms appeared at an early age without any gender difference. No ocular or skin problems were observed in either patient. The pedigree chart (Fig 1) also confirms our conclusion. Thus, the parents of these siblings must be carriers for Alport syndrome on chromosome # 2, the mutation being either on COL4A3 or COL4A4 or on maybe both. An unusual factor was observed in both the siblings i.e. the presence of beta thalassemia minor. As seen in table 2 and 3, the level of MCH, MCV, MCHC and hemoglobin in blood were found to be much lower than the normal range. These decreased values are an indication of the presence of beta-thalassemia trait (minor).

This trait is also present in the female patient while no one else in the family has ever suffered from any form of thalassemia. Although decreased RBC count (i.e. decreased hematocrit) is one of the major symptoms of Alport syndrome, it does not show any correlation between Alport syndrome and beta thalassemia. Moreover, the genes for both diseases lie on separate chromosomes [14].

In order to detect the nature of mutation karyotype analysis was done. Karyotype analysis of mitotic metaphase chromosomes is the single, most important test available in aiding the genetic counselor or physician. In many clinical cases, a karyotype analysis is used to rule out chromosome abnormalities as the possible cause of a disease [15].

Through various staining techniques, the chromosomes are caused to demonstrate reproducible light and dark bands that can be counted and compared to chromosomes of persons having specific disease. Chromosome banding can reveal subtle sub- chromosomal abnormalities, such as small deletions and translocations, the duplication of certain areas within the chromosome and inversions of chromosome material within the same arm of the chromosome [16]. Karyotype analysis of the affected female patient was done by preparing the slides by the method of Moorhead [17]. The chromosomal analysis was performed to detect any chromosomal abnormality in chromosome no 2 or X chromosome as these two carry the genes for collagen type IV. However, the results revealed a normal banding pattern which leads to the conclusion that no significant mutational changes were present. Therefore, in this case, the mutation might be a very small one, such as a base substitution, a missense mutation, a splice site mutation etc. No detectable change was observed in the chromosomal analysis (Fig 2).

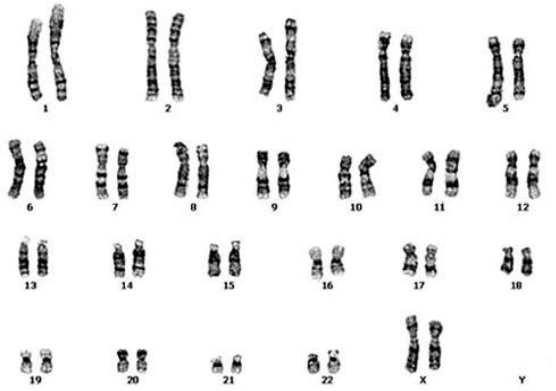

Fig 2: Chromosomal analysis of the female patient.

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