STUDY OF THE COL4A4 GENE AND DESCRIPTION OF NEW MUTATIONS RESPONSIBLE FOR ALPORT SYNDROME

C. Rosado[1], E. Bueno[2], P. Fraile[1], C. Lucas[1], P. García-Cosmes[1], JM Tabernero[1], R. González Sarmiento[2]
1: Service of Nephrology, University Hospital of Salamanca (Spain). 2: Unity of Molecular Medicine. University of Salamanca (Spain).

OBJECTIVES

Autosomal forms represent 20% of all cases of Alport syndrome (15% recessive and 5% dominant). They are caused by mutations in the COL4A3 and COL4A4 genes, which encode alpha-3 and alpha-4 collagen chains.
Our objective is to find, in the patients diagnosed with autosomal Alport syndrome admitted in our hospital, mutations in the COL4A4 gene responsible for this disease.

METHODS

We analyze 6 families with a clinical diagnosis of autosomal Alport syndrome. We carry out a search of mutations in the COL4A4 gene using direct DNA sequencing from the index patient after amplifying it with polymerase chain reaction and mutation analysis with CSGE-heteroduplex. All patients had undergone a previous study of the COL4A3 gene that did not reveal any pathogenic mutation. In order to characterize the missense mutation found we carried out a population genetic study of 100 alleles with CSGE-heteroduplex and *in silico* studies using *Polyphen*, *SNPs3D* and *SIFT*.

RESULTS

From our 6 patients, 4 of them (66.6%) presented a dominant inheritance, one of them (16.7%) had a recessive inheritance, and another one (16.7%) had no previous family history of Alport syndrome.
We have found two pathogenic mutations, responsible of dominant Alport Syndrome, which are not described in the literature. **IVS3+1G>C** is a replacement of Guanine with Cytosine in position +1 of intron 3. It is located in the splicing region, which means that it is considered a pathogenic mutation. Mutation **c.4267C>T; p.P1423S** is considered as pathogenic and responsible for the disease, according to the following criteria:

STUDY	RESULT
Population model	negative
Polyphen	0.989
SNPs 3D	-0.05
SIFT	tolerated

MUTATION	REFERENCE	MEANING
IVS3+1G>C	Our study	Pathogenic (splicing)
c.4267C>T; p.P1423S	Our study	Probably pathogenic (missense)
c.3011C>T; p.P1004L	Boye et al (1998)	Polymorphism
c.3594G>A; p.G1198G	Lemmink et al (1994)	Polymorphism
c.3684G>A; p.K1228K	Badenas et al (2002)	Polymorphism
c.3979G>A; p.V1327M	Longo et al (2002)	Polymorphism
c.4080G>A; p.P1360P	Badenas et al (2002)	Polymorphism
c.4207T>C; p.S1403P	Boye et al (1998)	Polymorphism
c.4932C>T; p.F1644F	Longo et al (2001)	Polymorphism
IVS10-39 T>C	Our study	Polymorphism
IVS 12+30 G>A	Our study	Polymorphism
IVS 12-58 A>G	Our study	Polymorphism
IVS 17+24 A>T	Our study	Polymorphism
IVS 19+21C>A	Our study	Polymorphism
IVS37-61 G>T	Our study	Polymorphism
IVS 38 +40 C>A	Our study	Polymorphism

All other mutations found were polymorphisms. 7 of them have already been described by other researchers and another 7 are described for the first time in this work. They are intronic variants located away from splicing regions.

CONCLUSIONS

We describe here two pathogenic mutations of the COL4A4 gene: **IVS3+1G>C** and **c.4267C>T; p.P1423S**. These mutations are responsible for a dominant autosomal Alport syndrome.

We describe 7 new intronic variants. They are considered as demographic polymorphisms due to their location, away from the splicing regions.