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Brief Communication

Improved genetic counseling in Alport syndrome by new variants of *COL4A5* gene

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ABSTRACT:

There are current requirements of using genetic databases for offering a better genetic assistance to patients of some syndromes, especially those with X-linked heredity patterns (like Alport Syndrome) for the high probability of having descendants affected by the disease. We describe the first reported case of *COL4A5* gene missense c.1499 G > T mutation in a 16-year-old girl confirmed to be affected by Alport Syndrome after genetic counseling. Next Generation Sequencing procedures let discover this mutation and offer an accurate clinical treatment to this patient. Current scientific understanding of genetic syndromes suggests the high importance of updated databases and the inclusion of Variant of Unknown Significance related to clinical cases. All of this updating could enable patients to have a better opportunity of diagnosis and having genetic and clinical counseling. This event is even more important in women planning to start a family to have correct genetic counseling regarding the risk posed to offspring, and allowing the decision to undergo prenatal testing.

Alport Syndrome (MIM #301050, AS) is a hereditary disorder of the basement membrane, resulting in progressive renal failure due to glomerulonephropathy, variable sensorineural hearing loss, and ocular (perimacular retinopathy) anomalies.[1] All of them caused by a defect in type IV collagen so any alteration in the genes *COL4A3, COL4A4* and *COL4A5*; which encode for any of the isoforms of collagen type IV are related to AS. It is a genetically heterogeneous disease, most of the cases have an X-linked inheritance but other patients have autosomal recessive disease.[2] Classical affections are mapped in the 5 α chain of collagen type 4 (*COL4A5* gene) located in X chromosome (Xq22) while less frequent alterations in 3 and 4 α chain of collagen type 4 (*COL4A3* and *COL4A4*) are related to chromosome 2.[3]

Nowadays the important role and benefits of genetic counseling is well known and is commonly applied to familial disorders such as the analysis of *BRCA1/2* in familial breast cancer and the genetic analysis of *MMR* genes (*MLH1, MSH2* and *MSH6*) in Lynch Syndrome. For that reason there are many on-line databases that record disease-causing or association of polymorphisms variations previously published in the literature. The main on-line databases are the Human Gene Mutation Database, HGMD (http://www.hgmd.org/); Online Mendelian Inheritance in Man, OMIM (http://www.omim.org/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), dbSNP (http://www.ncbi.nlm.nih.gov/SNP/; Sherry *et al.* 2001) and an assorted collection of locus-specific mutation databases (LSDBs) (http://www.hgvs.org/dblist/glsdb.html). Moreover there are other databases with information of somatic (COSMIC, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) and mitochondrial mutations (MitoMap, http://www.mitomap.org/MITOMAP).[4] All of these databases are useful tools for clinicians and geneticists in performing genetic diagnosis and reports for family counseling. For that reason our main aim is to update all of these databases with new variants such as the Variant of Unknown Significance described in this case. Moreover with this Variant of Unknown Significance inclusion we will support recent publications that claim for improving databases in renal disease including these kinds of variants.[5]

CASE REPORT

A 16-year-old girl was suspected to be affected by AS. She had positive familial history of AS in the maternal line, as well as clinical evidence of this syndrome. The patient had a clinical diagnosis of haematuria/proteinuria (currently known as thin basement membrane nephropathy) for approximately a year. In databases like *Genereviews* (NCBI: National Center for Biotechnology Information) there are reports that only 30–40% of descendants carrying a mutation will develop the clinical signs of AS (haematuria and renal affection). The biopsy symptoms were the common ones related to AS. Niether audiometry nor ocular examination had any abnormalities. DNA from peripheral blood was extracted by phenol-chloroform extraction procedure after written informed consent. Next Generation Sequencing (NGS) was developed for the analysis of this syndrome in this case. For the capture and amplification of the genes an Illumina Sequencing System was used (HaloPlex Target Enrichment). Ultrasequencing was performed by MiSeq platform (Illumina) and the analysis of the results were developed by DNAnexus software. In the case of describing Variant of Unknown Significance, Sanger sequencing was performed by ABI 3130 genetic analyzer. As reference sequences for this analysis we used *COL4A5* (NM_000495.4), *COL4A3* (NM_000091.4) and *COL4A4* (NM_000092.4), respectively.

After genetic analysis there were no point mutations (or exonic either in adjacent or intronic regions) of *COL4A3* and *COL4A4* genes producing AS. However, there was a new mutation in *COL4A5* gene c.1499 G > T, causing an aminoacidic change p.Gly500Val (p.G500V) (Fig. 1). Although to date there are around 700 mutations in *COL4A5* gene, there were no previous reports of this missense mutation in the main databases such as the Human Gene Mutation Database, ClinVar, Uniprot (http://www.uniprot.org/), Arup (http://arup.utah.edu/database/ALPORT/ALPORT_display.php), or LOVD (http://www.lovd.nl/3.0/home).[3] The main effect of this mutation is caused by a change in a coding codon by another introducing a different aminoacidic residue with a possible pathogenic effect. Certain correlations between genotypes and phenotypes have been established, mainly in men; however, there are not many genotype-phenotype correlations among females, which highlights the relevance of this missense mutation.[3]

Fig. 1 DNAnexus output of Next Generation Sequencing (NGS) results in COL4A5 gene.

Table 1 In silico analysis

In silico software	Results	URL	Prediction
PolyPhen-2	99.6% of being pathogenic.	http://genetics.bwh.harvard.edu/pph2/ (1)	Based on a decision tree that combines a number of protein structural attributes with a prebuilt sequence alignment, generally including only mammalian sequences.
SNPs&GO	High risk 8 score (0–10) and pathologic effect.	http://snps-and-go.biocomp.unibo.it/snps-and-go/ (2)	The prediction of human disease-related single point protein mutations predicting whether a mutation at the protein level is or is not disease-related
AlingGVGD	High risk of being pathogenic. C65 category and score >4.0 (<0.9–>4.0).	http://agvgd.iarc.fr/agvgd_input.php (3)	Combines an alignment with amino acid physicochemical characteristics to calculate the range of variation present at each position in the alignment (GV) and the distance of missense substitutions from that range of variation (GD).
MutPred	86% of risk of being a deleterious variant.	http://mutpred.mutdb.org/ (4)	Classification of an amino acid substitution as disease-associated or neutral in human. In addition, it predicts molecular cause of disease.
SIFT	Pathogenic variant. Value 0.00; pathogenic if values are below 0.05.	http://sift.jcvi.org/ (5)	Whether an amino acid substitution affects protein function, its prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences.

References:
(1) PolyPhen-2. http://genetics.bwh.harvard.edu/pph2/. 2012; 2013.
(2) SNPs&GO. http://snps-and-go.biocomp.unibo.it/snps-and-go/. 2013; 2013.
(3) Align GVGD. http://agvgd.iarc.fr/agvgd_input.php. 2013; 2013.
(4) MutPred. http://mutpred.mutdb.org/. 2013; 2013.
(5) J.Craig Venter Institute. http://sift.jcvi.org/. 2012; 2013.

The aminoacidic change of the described mutation is related to an 85% missense mutation in this gene and affecting the Glycine substitution in the conservative and repetitive region of the molecule (Gly-Xaa-Yaa). In respect of change, Russell, Betts and Barnes 'http://www.russelllab.org/aas/', confirm that the substitution of a Glycine instead of a Valine in extracellular proteins, is unfavourable with a predictive value of −2 (with a maximum possible value of −6).

Although this variant has not been previously described nowadays there is on-line software that can predict its pathogenic effect. *In silico* analysis PolyPhen-2, SNPs&GO, MutPred, SIFT and AlignGVGD scored this mutation with a high risk of being pathogenic and deleterious (details in Table 1).

DISCUSSION

Here we describe a patient with AS in whom we discovered a new mutation in the *COL4A5* gene after genetic analysis. This missense mutation p.Gly500Val (p.G500V) in the position 1499 (c.1499 G > T) of *COL4A5* gene will cause the change in the 500 codon of a Glycine for a Valine. It has not been previously described in any database in relation to AS, so it is currently considered as a Variant of Unknown Significance. Furthermore in previous scientific references, most missense mutations in AS that produce the change between a Gly in a conservative region (as the one we described) are related to pathogenic effect related to AS such as the case of p.Gly500Arg.[6] In the case of this mutation it exists as a change between aminoacids of different size and polarity, which implies a worst clinical prognosis and Russell *et al*.,[7] have postulated that changes in conservative and repetitive regions of a molecule (Gly-Xaa-Yaa) such as the one in this case have a bad prognosis.

Genetic diagnosis and genetic counseling are very important but even more so in diseases like AS. One of the most frequent forms of AS has an X-linked heredity patterns, so in case of the mother being affected, male descendents have a high probability of being affected by the disease. Male AS descendents will be affected by this syndrome but also by deafness and end-stage renal disease (ESRD). Female descendents that inherit the mutation will be considered as carriers and some of them will be affected by severe renal disease and other clinical severe events related to AS. As it is previously mentioned, familial genetic counseling plays an important role among these families to inform them of the clinical risk in the carriers and their future descendents. Once

a genetic diagnosis has been made, a screening of symptomatic or at risk-family members can be performed, as well as offering clinical advantages to these patients (like avoiding unnecessary biopsies). Recent studies have reinforced the important role of genetic diagnosis in AS to have a correct and definitive diagnosis to be made in index patients and at-risk family members.[8]

As it has been proven in this report, all *in silico* analysis, biochemical analysis and clinical data support the pathogenic effect of this Variant of Unknown Significance for AS. However, to totally confirm as pathogenic it should also be evaluated by a functional analysis as it is recommended by the practice guidelines for Variant of Unknown Significance.[9] Although not 100% sensitive, genetic testing can provide a definitive means of making a diagnosis of AS, and is the most reliable way to distinguish females carriers from heterozygous ones. This event is especially important in women planning to start a family to have a correct genetic counseling for the risk to her offspring, and to have the decision to undergo prenatal testing.[8] For these reasons it is imperative that all collagen IV gene mutations related to AS are reported to the appropriate databases. These results will enable clinicians to offer a more accurate genetic test, counseling and prenatal testing in this syndrome, thus improving patients' quality of life.

CONFLICT OF INTERESTS

None.

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