CLINICAL & GENETIC INVESTIGATION OF X-LINKED ALPORT SYNDROME IN THE GREEK POPULATION THROUGH NEXT GENERATION SEQUENCING

The correct diagnosis avoids immunosuppressive treatments

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INTRODUCTION

- Alport syndrome (AS) is the most frequent monogenic inherited glomerulopathy, genetically and clinically heterogeneous.
  - caused by semi-dominant mutations in the X-linked *COL4A5* gene or recessive mutations in the *COL4A3*/*COL4A4* genes.
  - manifests in early childhood with persistent microhematuria and progresses to proteinuria and kidney failure in adolescence or early adulthood, when untreated.
  - on biopsy, pathognomonic features include uniform thinning and progressively alternate thinning, thickening and lamellation of the glomerular basement membrane (GBM), in the presence of podocyte foot process effacement.
- AS patients have been described in all populations but there is no reliable data regarding its exact prevalence. Previous studies mention a prevalence of about 1/50,000.

AIM

- The clinicopathological study and genetic analysis of Greek families with Alport syndrome (AS), applying a next-generation sequencing (NGS) approach.

METHODS

- We investigated 26 unrelated Greek families with 98 patients, that referred for genetic diagnosis due to the presence of familial microscopic hematuria and/or suspicion of AS.

* This research programme was approved by the Cyprus National Bioethics Committee in order for all subjects to provide samples of DNA in the CY Biobank and data were in compliance to the European general data protection regulation (GDPR, (EE) 2016/679).

Table 1. Cumulative clinical and pathological data of the 26 Greek families examined herein

	Mutation carriers (molecularly confirmed)			Biopsy	Pathogenic variants	Hearing loss	Ocular lesions	Tx	MH Only	MH+ Proteinuria, Normal GFR	Impaired Renal Function, CRF or ESRD	ESRD
	Total	♂	♀									
SUM	98 100 %	40♂ 41 %	58♀ 59 %	17 (17,3%) in 15 families (9♂/8♀)	21 different	21 (18♂/3♀)	5 5♂	11 (10♂/1♀) 11,2%	34 (6♂/28♀) 34,7%	31 (18♂/13♀) 31,6%	22 (13♂/9♀) 22,4%	15 (11♂/4♀) 15,3%

- We performed NGS on the **IonTorrent PGM platform**, targeting the genetic diagnosis of patients in a robust manner. Variant calling and annotation were eimpemented by Ion Torrent Suite V4.0.2 Variant Caller & Ion Reporter Software V4.0 (Life Technologies, CA, USA).

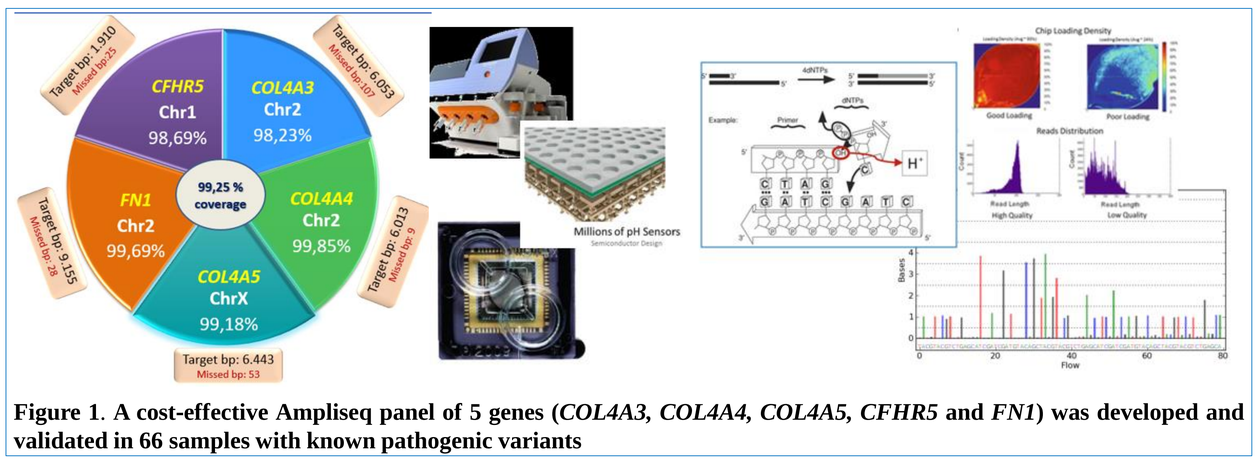

Figure 1. A cost-effective Ampliseq panel of 5 genes (*COL4A3, COL4A4, COL4A5, CFHR5* and *FN1*) was developed and validated in 66 samples with known pathogenic variants

- Hierarchical filtering of variants accessing the pathogenic effect of each variant according to the ACMG criteria by:
  - searching genetic databases (**HGMD Professional, ARUP Alport, LOVD, Ensemble, ClinVar**)
  - *in silico* evaluation of pathogenicity through bioinformatic algorithms (**SIFT, PolyPhen-2, Mutation Taster, SNPs3D, Align-GVGD, Grantham score & REVEL score**)
- Genetic variants validated:
  - by Sanger DNA sequencing, PCR and RFLP and tested in at-risk family members, and in control samples including 368 CY WES genomes
  - in case of suspected copy-number imbalances conducting MLPA analysis and RT-PCR
- Additionally, an in-house variant database was established.

RESULTS

- We identified **21 pathogenic variants** in the *COL4A5* gene, of which **12 (57%) variants novel** and **5 (24%) probably *de novo*.**

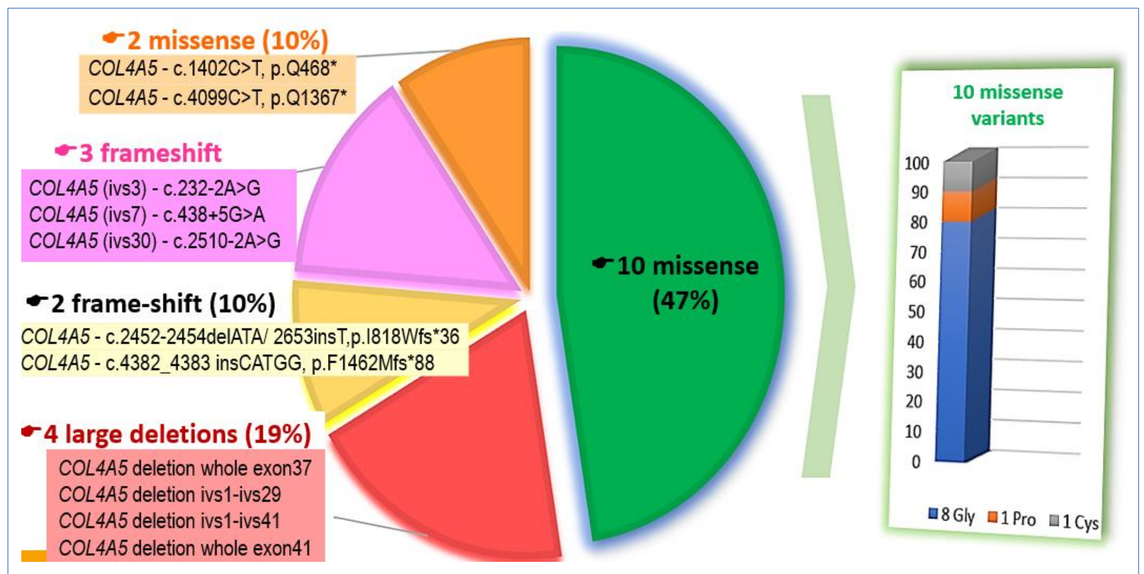

Figure 2. illustration of the categories of the 21 pathogenic variants in the *COL4A5* gene identified herein

- Notably in 6 families, we identified the hypomorphic ***COL4A5*-p.Gly624Asp** substitution, a founder pathogenic variant encountered all over Europe originating in the Middle Ages with mostly milder symptomatology.

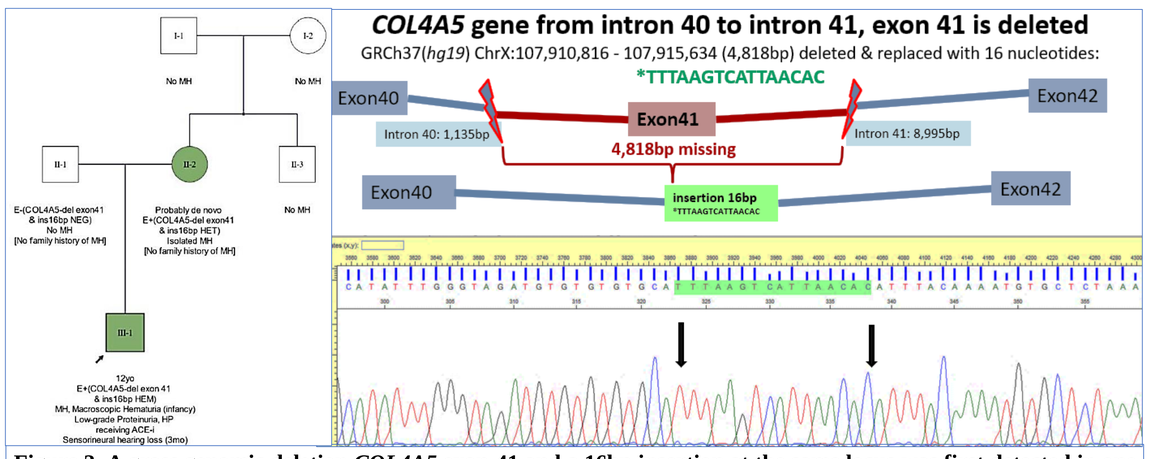

Figure 3. A gross genomic deletion *COL4A5* exon-41 and a 16bp insertion at the same locus was first detected in one Greek family. After MLPA analysis, several attempts with gap PCR using intronic primers flanking exon 41, followed by Sanger sequencing permitted the exact mapping of the deletion. The deletion spanned a sequence of 4,818bp [chrX:107,910,816-107,915,634 in genome assembly GRCh37(hg19)], which was replaced with a 16bp insertion (TTTAAGTCATTAACAC). The deletion extended from intron 40 to intron 41, including exon 41

CONCLUSIONS

- The genetic molecular investigation of X-linked Alport in families of the Greek population was achieved accurately successfully through NGS
- NGS technology:
  - is considered as the ideal and gold standard approach for parallel robust and rapid detection of pathogenic variants in large genes among multiple patients
  - achieves non-invasively the unambiguous diagnosis of AS, regardless of the patients' age or clinicopathological findings, avoiding long lasting investigations & unnecessary examinations
- Importantly, the indisputable and timely documentation of AS by genetic diagnosis
  - ☑ benefits the initiation of appropriate treatment as early as possible (like RAAS blockade)
  - ☑ avoids lengthy diagnostic investigations with the advantage of improving long-term disease outcomes and delaying effectively the progression to ESRD
  - ☑ reduces the possibility of incorrect medication (reclassifying patients as AS, terminated previous immunosuppressive/cyclosporine A therapy and switched to ACE inhibitors)
- Clarifying the causative disease of hematuria, proteinuria or chronic renal failure facilitates the appropriate family planning and/or selection of proper kidney donors.

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