University of Cyprus
Department of Biological Sciences

Ph.D. Thesis Defense

Student Presentation

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Building ΚΟΔ 7, Room 010, Panepistimioupoli Campus

This seminar is open to the public

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"Genetic Molecular Investigation of X-linked Alport in the Greek Population through Next Generation Sequencing"

This PhD thesis presents the application of next generation sequencing (NGS) as a laboratory diagnostic tool for the genetic molecular investigation of Alport Syndrome in the Greek population.

Alport syndrome (AS), the most common hereditary glomerulopathy, is characterized by clinical and genetic heterogeneity, with early onset of microscopic hematuria (MH), which, without therapeutic management results in ESRD. AS occurs from pathogenic variants in the collagen type IV genes, with the majority (85%) involving the X-linked *COL4A5* gene and less frequently the *COL4A3* and *COL4A4* autosomal genes (15%). Pathognomonic features in renal biopsy include alternating thickening and thinning of the glomerular basement membrane and podocyte foot process effacement.

In the context of this research thesis, we investigated clinically and molecularly 26 Greek families with a total of 98 patients (40 men / 58 women), with ages ranging from infancy to advanced adulthood, who were referred to the Center of Excellence in Biobanking and Biomedical Research, *CY*-Biobank (https://biobank.cy/), for genetic diagnosis of AS. Although clinical data was not always available, hearing loss was reported in 21 patients (18 men / 3 women) and ocular problems were reported in 5 men. Eleven patients were transplanted (10 men / 1 woman), 22 patients (13 men / 9 women) presented renal function impairment, while 15 (11 men / 4 women) reached ESRD, with the earliest age being 17 years.

NGS technology is considered to be the ideal and gold standard approach for the diagnosis of MH of glomerular origin, due to the large size of the genes involved and with the efficiency of quickly providing highly accurate results. We developed a cost-effective Ampliseq panel of 5 genes involved in glomerulopathies (*COL4A3*, *COL4A4*, *COL4A5*, *CFHR5* and *FN1*), that enables the robust and rapid detection of pathogenic variants in different genes simultaneously and in multiple patient samples, validating it successfully validated by performing sequencing of

samples with a known pathogenic variant. Hierarchical filtering of the vast amount of findings followed NGS sequencing, along with searching in genetic databases and in silico evaluation of the degree of pathogenicity using bioinformatic algorithms. The genetic diagnosis of the patient was feasible in less than 2 days, making NGS technology accessible to clinical routine practice.

DNA variants of great importance were verified in the proband through conventional molecular methods (Sanger DNA re-sequencing, RFLP or MLPA) then checked in the rest of the family members, along with investigation of the family clinicopathological data, while, where necessary, they were examined in the general population and in 368 WES genome data of Cypriots. In addition, we created an in-house database of pathogenic and non-pathogenic variants, facilitating the classification of findings and the investigation of their potential modifying role in the progression of renal disease. Also, this provided the potential for disease prognosis and categorizing even of asymptomatic patients, who may have a more severe course of renal disease rather than simply a mild course with isolated MH or low proteinuria.

The 26 Greek families under genetic investigation were successfully diagnosed with XLAS, as altogether, 21 pathogenic variants in the *COL4A5* gene were detected, of which 12 (57%) were identified for the first time (novel). In particular, 3 splicing, 2 nonsense, 2 frameshift and 10 missense variants were identified, of which 8 were Gly replacements (38%). Also 4 pathogenic variants (19%) involved large-scale deletions, while 5, a rather high percentage (24%), were probably de novo. Specifically, in 6 families, we identified the founder and hypomorphic, with pan-European presence, pathogenic variant *COL4A5*-p.G624D, which was previously reported in 3 other Greek families.

The correct, substantiated and unequivocally diagnosis of AS is achieved non-invasively through NGS technology, regardless of the age or clinical condition of the patient, avoiding long lasting investigations and unnecessary medical examinations. Additionally, it confers the important advantage of early diagnosis and initiation of appropriate treatment as early as possible, demonstrably and effectively delaying the progression to ESRD, prolonging the patients' quality of life. At the same time the correct genetic diagnosis clarifies the causative disease, reducing the possibility of administering incorrect medication. Specifically, in some families, genetic testing has reclassified patients as having AS, ending the previously erroneous immunosuppressive treatment. Finally, it becomes indisputably important to record pathogenic, even possibly pathogenic variants, in addition to investigating the affected members of a family, in order to predict the inheritance pattern, to choose members that are not carriers of a pathogenic variant as kidney donors or to follow appropriate family planning, with prenatal tests.