J Clin Exp Ophthalmol 2017, 8:1 (Suppl)
http://dx.doi.org/10.4172/2155-9570.C1.058

From the bench to the bedside: Infrared, Raman, NMR and Brewster spectroscopies, and the cause and cure for dry eye

Douglas Borchman
University of Louisville, USA

Dry eye affects over six million people in the United States. Tears become more unstable with age and meibomian gland dysfunction (MGD). Changes in the composition, and structure of a thin film of lipid on the surface of tears called the 'tear film lipid layer' (TFLL) may cause tears to become more unstable. In this study NMR spectroscopy was used to measure TFLL composition. Infrared, Raman and Brewster angle spectroscopies were used to measure the structure of the TFLL. Langmuir trough technology was used to measure TFLL rheology. Several abnormalities in the TFLL composition were identified that may contribute to TF instability including terpenoids, saturation, protein and cholesteryl esters. When terpenoid levels in TFLL are low as in MGD, the TF is unstable and patients have the signs and symptoms of dry eye. When terpenoids are restored with azithromycin treatment, TF stability is restored and patients no longer have the signs and symptoms of dry eye. A more saturated TFLL contributed to TF stability. The TFLL phase transition temperature and hydrocarbon chain decrease with increasing age. It is reasonable that stronger lipid-lipid interactions could stabilize the tear film since these interactions must be broken for tear breakup to occur. Meibum is fluid enough to be expressed from the meibomian glands and becomes more ordered (viscous) on the surface of the eye. A stiff ordered molecular arrangement results in a more elastic TFLL in which molecules are able to rearrange during the compression and expansion of a blink.

Copy number variation in the inherited retinal degenerations

Kinga M Bujakowska
Massachusetts Eye and Ear Infirmary- Harvard Medical School, USA

Statement of the Problem: Inherited retinal degenerations (IRDs) are an important cause of blindness affecting over two million people worldwide. Even though IRDs are mostly monogenic, they are genetically heterogeneous, with mutations in over 200 genes leading to disease. Despite substantial progress in sequencing and new disease gene discovery, current strategies can genetically solve only about 60% of IRD cases. The high number of unsolved cases can be attributed to the yet-unidentified genes, large insertion/deletions also called copy number variations (CNVs), and deep intronic mutations, which are not easily detected by targeted next-generation sequencing (NGS) approaches. This presentation will give an overview of the methodology and the challenges related to genetic diagnostic testing, based on the genetic results from a cohort of 500 IRD patients.

Methodology: The patients included in the study were recruited and clinically examined at the Massachusetts Eye and Ear Infirmary. Patients underwent a full ophthalmic examination and their DNA samples were studied by Genetic Eye Disease (GEDi) diagnostic testing, SNP genotyping array, quantitative real-time PCR (qRT-PCR), PCR and Sanger sequencing. The read-depth NGS analysis was performed with two methods (Exome-Depth and an in-house algorithm). The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. All subjects signed informed consent.

Findings: GEDi testing with the initial analysis of rare single nucleotide variants (SNVs) and small insertion/deletions solves 55% of IRD patients. Mutations in USH2A (10%), ABCA4 (3%), EYS (3%), RPGR (3%) and RHO (3%) were the most frequent cause of disease. Further analysis of NGS read-depth predicts likely causal CNVs in 11% of IRD patients, where deletions in USH2A were the most common (22%).

Conclusion & Significance: Analysis of a large IRD cohort indicates that CNVs are important contributors to the etiology of IRDs, being responsible for approximately 11% of genetic cases.

kinga_bujakowska@meei.harvard.edu