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Glycomics in tears: seeking for new biomarkers for ocular allergy diagnosis.

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An ocular allergy includes several clinical conditions that can be considered hypersensitivity disorders of the ocular surface ¹. No reliable test exists to differentiate these disorders, and no markers are available to monitor the course of ocular allergy. Protein concentration in tears is relatively high, reaching up to 10 micrograms per microliter of tears. Albumin and other proteins present in tears have long been recognized as key markers for allergic inflammation on the ocular surface. Compared to healthy individuals, patients with allergic conjunctivitis (AC) overexpress a number of proteins involved in inflammation pathways. However, studies of single proteins and the overall protein profile of human tears have been hindered by a lack of standardized and efficient techniques to collect tears. In addition, sampling conditions can have a more significant impact on, albumin levels than the assay technique². Damaging the conjunctiva during ocular fluid

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collection can stimulate the mucosa thereby resulting in tear dilution. A sufficient volume for mass spectrometric fluid analysis can be collected with a capillary glass tube directly from the external canthus (1 tear drop = 50 μ L) . This simple technique can be performed by any experienced physician and used even with children already positioned on a frame. The conjunctival imprint is now even easier to perform with techniques such as Eyeprim®, which allow collection of a greater number of cells from the ocular surface, and potentially adaptation of biomarkers assays by spectrometry ³.

In a previous publication Leonardi and co-workers analysed the protein profile of human tears from patients with AC and from healthy individuals using mass spectrometry and iTRAQ quantitative proteomics. ⁴In the tears of patients affected by vernal keratoconjunctivitis (VKC) they were able to identify several proteins which correlated with the severity of the disease. Similarly, Tomasi and co-workers documented significant differences in the proteomic profile in tears between allergic rhinoconjunctivitis patients and healthy controls ⁵.

Over 60% of proteins, including those present in biological fluids like tears, have long carbohydrate chains known as glycans, which can be harvested from a biological source. The glycome consists of all glycans (or carbohydrates) within a biological system and can modulate a wide range of biological functions, from protein folding to cellular communications. N-linked glycans are essential for glycoprotein secretion, folding, and stability, and also cell to cell interactions and signalling, and are potential new biomarkers for several disorders, including allergy. ⁶

In their study of glycans profiling in ocular fluid from patients with severe AC, Messina et al. used for the first time N-glycomic analysis of tears and compared the glycomic profile of ocular fluid in patients with VKC, atopic keratoconjunctivitis (AKC) and in healthy controls. ⁷ Searching for VKC and AKC biomarkers is essential since both diseases present a whole range of ocular symptoms, including severe symptoms such as photophobia, intense tearing, mucous discharge, and pain, which can result in visual disturbances and even permanent loss of visual acuity. The authors observed significant differences between N-glycome profiles of control, VKC, and AKC tear fluids. They described several specific glycoforms in the tears of control patients, mostly with a high degree of fucosylation. In VKC, dominant ions were assigned to bi-antennary and core-fucosylated glycans, whereas in AKC, profiles showed a significant increase in an unfucosylated glycoform. Glycomics results were validated by quantitative assay of tear serotransferrin, which was significantly higher in VKC than in control subjects and in AKC than

in VKC patients, suggesting a local production of serotransferrin and serum leakage as a response to the ocular inflammation. Peaks with increased intensities are referred to as glycans from serotransferrin and lactotransferrin, whereas peaks showing decreased intensity have been associated with immunoglobulins. Some ocular rosacea symptoms are shared by both VKC and AKC, and a reduced degree of N-glycans fucosylation has also been observed in tears from patients affected by rosacea. Messina et al. concluded that tear fluid N-Glycome could provide possible biomarkers and thus help in the diagnosis of VKC or AKC.

However, these findings have several limitations and should be considered with caution. The authors stated that this approach is unable to trace the proteins from which the identified glycans originated. At present therefore, identification of the protein sources remains a matter of speculation. The study included a relatively low number of patients (11 controls, 23 VKC, and 7 AKC patients) and so it was not possible to correlate the glycome data with for example the severity of the diseases. Furthermore, since age and gender are relevant variables associated in glycopeptide expression, they should also be considered in future studies of tear glycome in patients with ocular allergy. Further studies in a larger population of patients with ocular hypersensitivity disorders comparing proteomics in other types of AC (such as limbic and tarsal subtypes of VKC) would likely help to identify new biomarkers.

This paper sheds new light on the investigation of ocular allergy. Other omics could also be explored in ocular allergy, as they are in other allergic diseases. Proteomics assays in other biological fluids, such as nasal secretions, sputum, and exhaled breath condensate, have yielded new insights into the pathophysiology by defining subtypes of chronic rhinosinusitis or asthma phenotypes. Virkud et al.⁸ stated that metabolomics “provide the most integrated biological status profile reflecting the results of genetic and environmental interactions. Compared to other omics, the translation of quantified metabolites into the clinical setting is often very straightforward to diagnose and assess disease severity”. To date, no detailed report on the ocular surface has been published in this field. The ocular surface is exposed to many pollutants and allergens, as in allergic diseases of the mucosal airways, particularly in asthma. Hence the ocular surface could be seen as a promising milieu to investigate the exposome’s potential effects in allergic patients.

Using omics as a practical means to guide the diagnosis and management of ocular allergy needs further investigation to confirm the results obtained so far.

Conflict of Interest Statement: The authors have nothing to disclose.

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