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Understanding pathophysiology of rheumatic heart disease and coronary artery disease by mass spectrometry based protein identification

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Risk prediction of acute event of the subjects suffering from cardiovascular disease is an important strategy to save millions of lives. Thus, the detection, identification and characterization of variations in the proteome occurring during the course of heart disease will provide both insight into the underlying molecular mechanisms and potential cardiac specific biomarkers for regular, systematic observation and assessment of cardiac status. In the present study, we tried to identify the proteins in rheumatic heart disease and coronary artery disease in Indian subpopulation utilizing various proteomic tools. We utilized on-line label-free MS/MS using blood plasma as the source material. On-line LC-ESI-MS is the method of choice because the initial LC separation step decreases the amount of analytes that can be simultaneously ionized. Such label-free quantitative LC-MS approaches can compare innumerable samples. Therefore, they are ideal for biomarker discovery because experimental workflows normally compare a large number of specimens to validate the results from a statistical point of view. Consequently, we have identified 1500 proteins in plasma sample of acute coronary syndrome (heart failure) subjects by using Orbitrap LTQ (Thermo). During validation experiment one of the proteins ICB/CAD/1 is found to be significantly altered in the patient sample. The label-free quantitative LC-MS methods employed in this study helped to analyze the full potential of clinical plasma samples as a source of disease biomarkers in RHD and CAD; some of which might play an important role in the pathophysiology of RHD and CAD and improve the existing diagnostic strategies.

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The role of mass spectrometry in understanding and preventing food allergies

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Food allergies are adverse reactions to certain food proteins that occur in previously sensitized (IgE mediated) or genetically predisposed (not IgE mediated) subjects. For the severity of these reactions the European Commission approved the Directive 2007/68/EC, which lists all the allergenic foods that must be labelled in food products. Allergen detection methods can be basically divided into PCR-based methods and ELISA-based methods, but in the recent years also mass spectrometry is increasing its diffusion for allergen analysis. Identification of marker peptides allows an extremely specific quantification of allergenic proteins. This approach was used to quantify both single proteins, like the wheat allergens CM3, and complex protein mixtures, like gluten proteins. Mass spectrometry is also a useful technique to understand food allergy mechanism, for example by determining the resistance of different food allergens to gastrointestinal digestion. The higher allergenicity of peach LTP can be explained on the basis of its higher resistance to proteolysis (30% of intact protein at the end of the digestion) compared to apricot LTP (9% intact protein). Celiac disease related peptides were also identified and quantified using the isotopically labeled internal standard method, demonstrating that different wheat lines can produce a significantly lower amount of immunogenic peptides upon digestion. On the opposite, other wheat allergens, such as CM3, are more affected by environmental condition. Mass spectrometry has thus a fundamental role in the study of food allergens, both from the safety perspective (as a tool for allergen detection) and in understanding the molecular features of food allergies.

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