PA 53 - Subtyping of amyloidosis by direct proteomic analysis of fixed biopsy samples.

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ABSTRACT

Diagnosis and subtyping of amyloidosis are critical for prognostic and treatment. Subtyping has been recently demonstrated combining laser capture microdissection (LCM) and mass spectrometry. Here we show that ultrasonic treatment could help for the completion of enzymatic proteolysis followed by mass spectrometry analysis which is an emerging proteomic technique, directly on raw biopsy samples even without LCM and to get closer to the clinical routine application for amyloidosis.

INTRODUCTION

Amyloidosis is a disease where insoluble deposits of specific proteins occur in tissues. Different classes of amyloidosis have been reported and their diagnostic relies on the identification of the associated proteins: up to 10 proteins can be targeted using histochemical approaches. Currently, Congo red staining is the golden standard for the diagnosis of amyloidosis based on apple-green birefringence follow-up by subtyping using antibodies. However they can be inconclusive on certain cases due to the competence of the pathologist and the quality and availability of the dye, antibody and microscopy, leading to a lack of information about the underlying etiology. Subtyping has been recently demonstrated combining laser capture microdissection (LCM) and mass spectrometry (1). However, the LCM is not available in every clinical department, requires a specialist and can be time-consuming. Here we report a new sample treatment directly on paraformaldehyde-fixed slices using an ultrasonic probe to complete the enzymatic proteolysis in 60s instead of 15 hours incubation (2). Ultrasonic treatment combined to our data processing allows performing diagnosis and subtyping of different type of tissues: kidney, accessory gland salivary, lung, testicle, spleen.

METHOD

Paraformaldehyde-immobilized tissues (Bouin/AFA) from patients and controls were directly proteolyzed with an ultrasonic probe. Proteolytic peptide mixtures were analyzed by nanoLC-MS/MS (LTQ-FT Ultra, ThermoFisher equipped with TriVersa NanoMate chip-based nanosource, Advion) after nanoLC optimization (U3000 Dionex).

RESULTS

Ultrasonic tryptic treatment on fixed raw tissues allowed performing subtyping of amyloidosis. The results were compared with data obtained with immunohistopathology using the whole series of antibodies for amyloidosis diagnosis.

It is a non targeted approach. Abundance of the protein were evaluated according to (3) taking into account the area of the three most intense peptides. Only biopsies containing Apolipoprotein E (named ApoE) and Serum amyloid P component (named SAMP) which are common to all amyloid deposits, were considered as potential amyloid candidate. Finally amyloidosis were classified according to the relative intensity of amyloidogen protein.

Some significant examples from kidney tissues illustrate our results. The results are visualized with sector diagrams representing the relative intensities of amyloid proteins.

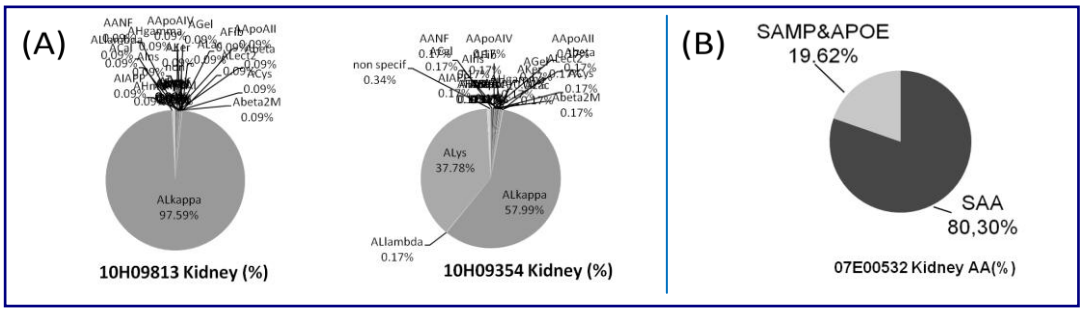

Figure 1. Biopsies of (A) negative controls, (A) AA amyloid patient.

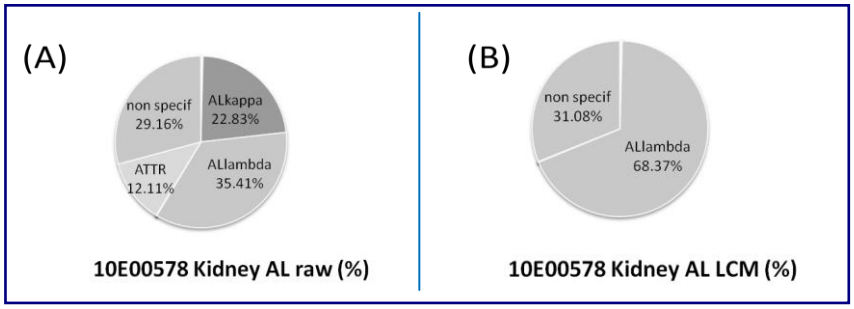

Figure 2. Biopsy of amyloid AL (A) raw tissue (50% amyloid), (B) Laser Capture Microdissected LCM tissue (95% amyloid)

No pure negative controls could be assayed because biopies come always from patients and are aimed to identify a pathology. Non amyloid diseased kidney does not present SAMP and ApoE (fibrils biomarkers), even if amyloidogen protein could be detected (Igκ and LysC) (1A). Their presence could be explained by inflammatory phenomenon or tissue dammage (eg. minor glomerular lesions) . The controls are non amyloid pathologies. As shown in this example when amyloid deposits represents more than 80% of the tissue the diagnostic and the classification are clear (Figure 1B). When the percentage goes below 50%, amyloid diagnostic is clear but classification lacks some robustness (Figures 2A) even if the amyloid type is correct: here we analyzed a raw biopsy without focalising on amyloid fibrils and without slicing. On the opposite LCM

treatment on the same sample could offer preparation above 95% where the classification is unambiguous (Figure 2B).

DISCUSSION

This strategy opens the way for a rapid and accurate amyloidosis subtyping directly from raw clinical samples and allows to avoid at the most the laser capture microdissection step which is highly time-consuming. This is of particular interest for classes that could not be distinguished by the classical histochemical analysis. Our next step will be to validate our approach to different amyloidoses and tissues with clinicians.

The whole analysis lasts 1 day for enzymatic treatment and roughly 10 slices are required and lasts 1 day for triplicate LC-MS/MS and roughly 1 slice of 10µm is required. In order to increase cohorts for a realistic clinical application we intend to automatize the first step. Miniaturization is required to decrease sample consumption of the first treatment (only 10% of the sample is actually analyzed).

We want to characterize specificaly the isofoms of protein involved. One example is ATTR addressed either by bottom-up (4) or top-down (5) strategies, where the transthyretin isoform can discriminate between senile systemic amyloidosis and familial thranthyretin amyloidosis.

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