**Identification of renal amyloid precursors by immunocytochemistry combining with proteomic analysis**

Li, D.[1], Xu, H.[2] and Wang, S.[1]

[1] Peking University First Hospital, China, [2] Chenggong Hospital, China

Accurate diagnosis and typing of amyloidosis are necessary for its therapy and prognosis. The commonly methods for identification of renal amyloid precursors are immunofluorescence or immunohistochemistry(IHC), which sometimes may come with diagnostic pitfalls. In this study, we illustrate the importance of combination with ultrastructural labeling, proteomics, and genetic analysis for establishing the accurate diagnosis of hereditary renal amyloidosis in two cases.

Case presentation: Both patients were middle-aged (P1: 40-year-old and P2: 47-year-old respectively) men, presented with proteinuria, hypertension and normal renal function, besides, one patient (P1) had liver, spleen and conjunctiva involvements. They all had a family history of renal diseases. The renal biopsies showed isolated glomerular amyloidosis, without interstitial or vascular amyloid deposition, in both patients. Electron microscopy demonstrated randomly fibrils in a diameter of 8-12 nm in glomerular mesangium. The IHC were performed by using a panel of antibodies to immunoglobulin light chains (kappa, lambda), AA, Lect-2, Fibrinogen A-alpha, ApoA-Ⅰ, Lysozyme, and Transthyretin. Then the ultrastructural labeling were performed by using the antibodies to Fibrinogen A-alpha and ApoA-Ⅰ, mass spectrometry (MS) on amyloid deposits captured by laser microdissection was investigated, and genetic study of gene mutations were performed.

Results: The IHC showed positive staining of both Fibrinogen A-alpha and ApoA-Ⅰ on glomerular deposits in two patients, while the intensity for Fibrinogen A-alpha was weaker compared with that for ApoA-Ⅰ in P1, but the equal intensity of staining for both of Fibrinogen A-alpha and ApoA-Ⅰ in P2. The MS analysis showed the high content of ApoA-Ⅰ, but not Fibrinogen A-alpha, detected in glomerular amyloid deposits of P1, and high content of Fibrinogen A-alpha in P2. The ultrastructural labeling showed specifically location of ApoA-Ⅰ or Fibrinogen A-alpha in the amyloid fibrils of P1, P2 respectively. Gene testing demonstrated P1 had a heterozygous mutation of p.Trp74Arg in apolipoprotein A-Ⅰ, and P2 had a heterozygous mutation of p.Arg547GlyfsTer21 in Fibrinogen A-alpha. Conclusion: the hereditary amyloidosis of ApoA-Ⅰ and Fibrinogen A-alpha were confirmed by combined ultrastructural labeling, MS analysis and genetic test, in which IHC was not sufficient to make the correct typing of amyloidosis.