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Comprehensive proteomic profiles of mouse AApoAII amyloid fibrils provide insights into the involvement of lipoproteins in the pathology of amyloidosis

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ABSTRACT

Amyloidosis is a disorder characterized by extracellular fibrillar deposits of misfolded proteins. The amyloid deposits commonly contain several non-fibrillar proteins as amyloid-associated proteins, but their roles in amyloidosis pathology are still unknown. In mouse senile amyloidosis, apolipoprotein A-II (ApoA-II) forms extracellular amyloid fibril (AApoAII) deposits with other proteins (AApoAII-associated proteins) in many organs. We previously reported that R1.P1-*Apoa2*^{c} mice provide a reproducible model of AApoAII amyloidosis. In order to investigate the sequential alterations of AApoAII-associated protein, we performed a proteomic analysis of amyloid fibrils extracted from mouse liver tissues that contained different levels of AApoAII deposition. We identified 6 AApoAII-associated proteins that constituted 20 of the top-ranked proteins in mice with severe AApoAII deposition. Although the amount of AApoAII-associated proteins increased with the progression of amyloidosis, the relative abundance of AApoAII-associated proteins changed little throughout the progression of amyloidosis. On the other hand, plasma levels of these proteins showed dramatic changes during the progression of amyloidosis. In addition, we confirmed that AApoAII-associated proteins were significantly associated with lipid metabolism based on functional enrichment analysis, and lipids were co-deposited with AApoAII fibrils from early stages of development of amyloidosis. Thus, these results demonstrate that lipoproteins are involved in AApoAII amyloidosis pathology.

Significance: This study presented proteomic profiles of AApoAII amyloidosis during disease progression and it revealed co-deposition of lipids with AApoAII deposits based on functional analyses. The relative abundance of AApoAII-associated proteins in the amyloid fibril fractions did not change over the course of development of AApoAII amyloidosis pathology. However, their concentrations in plasma changed dramatically with progression of the disease. Interestingly, several AApoAII-associated proteins have been found as constituents of lipid-rich lesions of other degenerative diseases, such as atherosclerosis and age-related macular degeneration. The common protein components among these diseases with lipid-rich deposits could be accounted for by a lipoprotein retention model.

1. Introduction

The amyloidoses constitute a group of disorders in which misfolded proteins lead to pathologic accumulation of extracellular amyloid fibrils in various tissues and organs. Currently, 36 amyloid proteins have been found in human and animal amyloidosis as represented by Alzheimer's disease (AD), familial amyloid polyneuropathy (FAP) and amyloid light chain (AL) amyloidosis [1]. Recent comprehensive studies of the molecular composition in amyloid deposits have shown that they contain a variety of proteins, glycosaminoglycans and lipids as amyloid-

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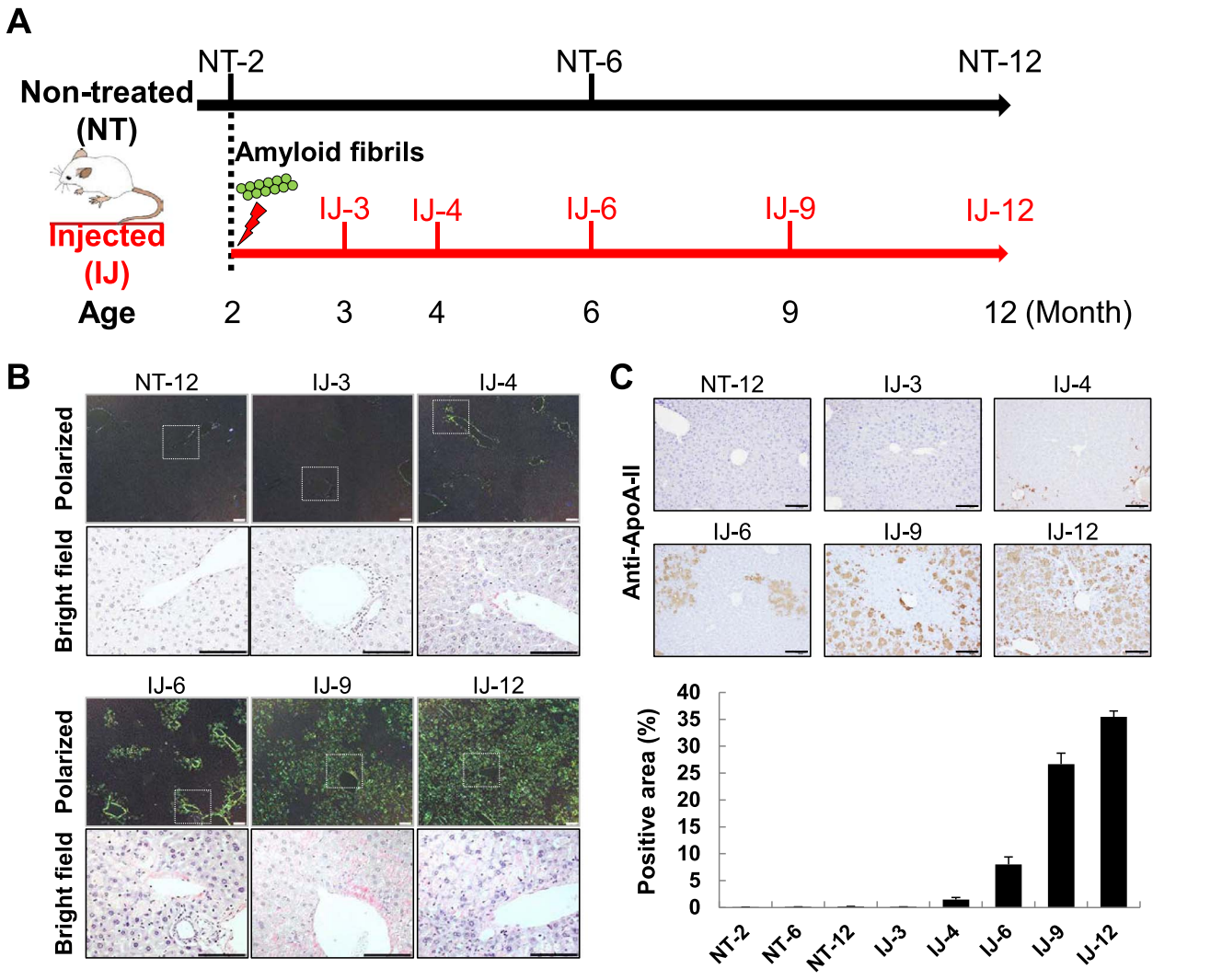

Fig. 1. Experimental design and AApoAII deposition in R1.P1-*Apoa2*^{c} mice after AApoAII fibril injection. (A) The induction of AApoAII amyloidosis was performed by intravenous injection of AApoAII fibrils into 2-month-old female R1.P1-*Apoa2*^{c} mice. (B) Amyloid deposition was confirmed by Congo red staining. Bright field photos represent enlarged views of polarized-light field (white squares). Scale bars represent 100 μm. (C) AApoAII deposition was evaluated by immunostaining of each mouse liver with ApoA-II antibody. Scale bars in photomicrographs represent 100 μm, and each bar in the graphic field represents the mean and ± S.D. (N = 3 (NT-12) and 5 (others)).

associated molecules [2,3]. Regardless of the differences between precursor proteins of amyloid fibrils and the tissues in which they are deposited, several amyloid-associated proteins, such as apolipoprotein E (ApoE), serum amyloid P-component (SAP), clusterin and heparin sulfate proteoglycans are common among various amyloidosis [2]. ApoE is identified as a primary genetic risk factor for late-onset AD from meta-analysis of genome-wide association studies [4] as well as isoform-specific effects on protofibril to fibril conversion of amyloid beta (Aβ) [5]. SAP and clusterin are known to act as extracellular chaperones that either increase solubility of extracellular insoluble aggregates and maintain protein homeostasis, or protect amyloid fibrils from degradation [6]. Many kinds of glycosaminoglycans and proteoglycans (including heparan sulfate) unfold amyloid proteins and catalyze their subsequent nucleus formation and stabilize the amyloid fibrils [7]. Thus, it is suggested that amyloid-associated proteins are involved in the pathogenesis of many kinds of amyloidoses. However, their role is still controversial.

Recent proteomic studies suggested that the proteins related to molecular transport, cell death or survival, extracellular matrix, lipid metabolism and mitochondrial functions may be involved in amyloidosis pathogenesis [8,9]. These proteomic studies analyzed the tissues from patients with severe amyloid deposition. Thus, the observed biological pathways reflected both constituents of amyloid deposits and cellular responses in tissues around the amyloid deposits. For that reason, the progressive changes in proteomic profiles of amyloidosis are not well understood.

In the present study, we analyzed sequential proteomic profiles using a reproducible mouse model of senile amyloidosis, which is an age-associated systemic amyloidosis characterized by apolipoprotein A-II (ApoA-II) amyloid fibril (AApoAII) deposition. ApoA-II is the second most abundant apolipoprotein of plasma high-density lipoprotein (HDL) and C-terminal extension of human ApoA-II derived from a mutation in the normal stop codon causes human AApoAII amyloidosis [10,11]. In mouse AApoAII amyloidosis, we previously reported that there are seven alleles of the ApoA-II gene (*Apoa2*) among inbred laboratory strains [12], and the mouse strain with the type C allele of *Apoa2* (*Apoa2*^{c}) frequently exhibits accelerated spontaneous AApoAII amyloidosis [13,14]. Importantly, a congenic strain of mice with the amyloidogenic *Apoa2*^{c} allele on the genetic background of the Senescence-Accelerated Resistant Mouse 1 (SAMR1), named R1.P1-*Apoa2*^{c} mice, rarely exhibits spontaneous AApoAII amyloidosis under specific pathogen-free condition. However, intravenous injection of AApoAII amyloid fibrils to R1.P1-*Apoa2*^{c} mice induces incremental AApoAII amyloidosis [15]. So, R1.P1-*Apoa2*^{c} mice are useful for analyzing the