PROF. MARK BRIAN PEPYS (Orcid ID : 0000-0003-2614-3248)

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Corresponding author mail id: m.pepys@ucl.ac.uk

Transthyretin amyloidosis: new answers but many questions

Professor Sir Mark Pepys FRS

Wolfson Drug Discovery Unit - UCL Royal Free Campus -

Rowland Hill Street, London NW3 2PF

United Kingdom

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Major recent advances in understanding of systemic transthyretin (ATTR) amyloidosis include the universal recognition that cardiac wild type ATTR amyloidosis is a prevalent cause of fatal heart failure with preserved ejection fraction (HFpEF) and/or fatal arrhythmia in the elderly, predominantly in men [1]. ATTR amyloid cardiomyopathy is strongly associated with carpal tunnel syndrome, caused by wild type ATTR amyloid deposition in the carpal ligaments. The very interesting study by Eldhagen *et al* [2] in this issue sheds further light on the association between cardiac and ligament ATTR amyloid but tantalisingly leaves several crucial questions unanswered.

Amyloid deposits, previously reported in resected ligament tissue from patients undergoing surgery for lumbar spinal stenosis, were confirmed to be very commonly present and to be of ATTR type in just over one third of cases. However, the identity of the even more prevalent non-ATTR deposits was not identified by immunohistochemistry, although the antibodies used are not disclosed. Another recent paper confirmed earlier reports that apoA-I amyloid is frequently present in the ligamentum flavum [3]. The tissues from the Eldhagen study [2] will therefore be fruitful material for further immunochemical and

proteomic mass spectrometry analysis, which could suggest just coincidental co-deposition or possible cross seeding of different fibril types.

Although the ostensible purpose of the Eldhagen study [2] was to assess association between ligament tissue amyloid deposition and cardiac amyloidosis, all patients with signs and symptoms of cardiovascular disease, including the hallmark manifestations of ATTR cardiomyopathy, were excluded. Among the remaining subjects, none with abundant ligamental amyloid, who were the only group to undergo cardiac investigation, had cardiac amyloidosis, demonstrating that either there is no association or that, as with carpal tunnel syndrome, ligamental amyloid deposition can precede cardiac involvement.

Leaving aside these intriguing questions, the curious conjunction of clinical manifestations of ATTR amyloid deposition in such diverse tissues as the heart and the ligaments was completely obscure until recently. Indeed, the determinants of the timing and localisation of systemic amyloid deposition are very long-standing issues on which only lately has a little light begun to be shed. In local amyloid deposits, confined to specific tissues or organs, the fibrils are often formed from locally produced proteins but the precursor proteins of systemic amyloid fibrils are overwhelmingly produced by the liver and are circulating plasma proteins. TTR is a case in point. Even though TTR is also synthesised by the choroid plexus, the first orthotopic liver transplantations performed for treatment of familial amyloid polyneuropathy (hereditary systemic ATTR amyloidosis) caused by the Val30Met TTR variant, showed that effectively all the circulating plasma TTR is produced by the liver [4]. Nevertheless, wild type ATTR amyloid is never deposited in the liver parenchyma itself, whilst variable deposition in the heart, ligaments and artery walls is commonly present, along with the spleen, kidneys, adrenal and eyes in patients with amyloid TTR variants. The underlying reasons for these different and varying distributions are mysterious but elucidation of the actual pathophysiological mechanism of TTR amyloid fibrillogenesis suggests a possible link between the heart, the ligaments and the arteries.

Nearly 30 years ago, Jeff Kelly pioneered the view that dissociation of the non-covalent homotetrameric assembly of the native TTR molecule is the critical first step towards its misfolding and eventual aggregation into amyloid fibrils. This phenomenon was modelled by prolonged *in vitro* exposure of isolated TTR to pH values around 4, emulating the lysosomes. Acid pH promotes dissociation of the protomers and produces insoluble aggregates of denatured material amongst which rare fibrillar forms are occasionally detectable. Using this model, Kelly innovatively designed small molecule ligands that are bound by the thyroxine binding site in native TTR, aiming to stabilise the protein against acid denaturation

just as it is stabilised by thyroxine binding. Kelly then developed his optimal ligand into the drug, tafamidis, for use as a stabiliser of TTR *in vivo*, to inhibit the amyloidogenic transformation of TTR. Although relatively modest, the apparently significant clinical benefit of tafamidis was a dramatic breakthrough.

Over the past 10 years, Vittorio Bellotti was inspired to re-examine the molecular mechanism of TTR amyloid fibrillogenesis, bringing together three seminal observations. Firstly, there is no extracellular body compartment, which could be relevant to amyloid fibril formation and deposition in the extracellular space of tissues and organs, in which TTR is exposed to pH ~4.0. The simple low pH model, which in any case produces remarkably few amyloid fibrils *in vitro*, therefore cannot be physiological. Secondly, analysis of *ex vivo* ATTR amyloid fibrils nearly always shows the presence of both the intact 127 residue TTR monomer and a proteolytic cleavage fragment comprising residue 49-127. The residue Lys48-Thr49 bond is a typical tryptic cleavage site. Thirdly, discovery of the first coding mutation in the human β_2-microglobulin gene, which produces the highly amyloidogenic Asp76Asn variant [5], led to Bellotti's observation that simple exposure of this variant to physiological scale mechanical forces was sufficient to induce its very efficient transformation into authentic amyloid fibrils under physiological conditions [6]. Applying these findings to studies of TTR, he showed that exposure of TTR to minimal tryptic activity in physiological solvent conditions together with physiological scale mechanical forces, produced swift and efficient conversion of TTR variants and wild type TTR into authentic TTR amyloid fibrils [7, 8].

The mechano-enzymatic mechanism has been elegantly and independently confirmed and is now generally accepted by those familiar with this aspect of the field [9, 10]. Indeed, a simple comparison of the quality and abundance of TTR amyloid fibrils produced by pH 4 exposure and by the mechano-enzymatic mechanism, demonstrates unequivocally which is more likely to be operative *in vivo*. However, the crucial pathogenetic protease obviously cannot be trypsin, since trypsin is not present in any relevant body compartment. The most likely enzyme turned out to be plasmin, as reported *in vitro* [11] and its activity was lately confirmed *in vivo* in a novel mouse model. Mice transgenic for the extremely aggressive amyloidogenic human Ser52Pro TTR variant, develop typical myocardial, lingual and other systemic human ATTR amyloidosis after they have been 'seeded' by intravenous administration of a homogenate of Ser52Pro TTR amyloidotic spleen tissue from a deceased patient with this disease. When these mice have been made deficient for murine anti-plasmin, by knocking out the corresponding gene, so that their endogenous plasmin activity is correspondingly increased, human ATTR amyloid deposition is markedly accelerated (Paul Simons *et al*, unpublished observations).

These compelling experimental findings are consistent with the clinical observations of the propensity of ATTR amyloid to deposit in myocardium and in ligaments, where TTR from the plasma is exposed to the physical forces that render the CD loop of the native structure, containing the critical residue 48-49 bond, susceptible to ubiquitous, constitutively activated plasmin. However, there is, as yet, no explanation of the predilection of most of the amyloidogenic TTR variants for the peripheral and autonomic nerves, in contrast to wild type TTR which never affects these nerves while the most common variant, Val122Ile, the polymorphic form of TTR that always involves the heart, very rarely affects the nerves. We thus have many more mysteries to ponder.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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