Commentary

A Novel Amyloidogenic Variant of Apolipoprotein AI *Implications for a Conformational Change Leading to Cardiomyopathy*

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Amyloidosis is a broad term for a group of diseases which have in common the extracellular deposition of pathological insoluble fibrillar proteins in organs and tissues.¹ Although unrelated in primary sequence and metabolic roles, almost all amyloid proteins exhibit β -pleated sheet secondary structure in their normal environments and green birefringence on polarization microscopy after Congo red staining, and deposit as nonbranching, insoluble, highly ordered fibrils.^{2,3} The protein in the fibril and the pathogenesis of the disease is unique for each type of amyloid.¹ The mechanism(s) by which such a diverse array of proteins with different metabolic roles become insoluble and deposit as pathological fibrils, damaging tissues and organs and ultimately causing death, are unknown.

Amyloidosis is either systemic (found throughout the body in organs and tissues) or localized in the brain, causing cerebral hemorrhage or neurodegeneration.^{4,5} During the past 25 years the systemic amyloidoses have been defined by specific clinical patterns and proteins, as well as by other factors associated with the fibrillar proteins in the deposits. Classification of type of amyloidosis is based on the nature of the plasma protein found in the fibril.⁴ The amyloidoses are, thus, commonly referred to as diseases of protein conformation or as protein-folding diseases.

In primary amyloidosis, the most common form found in the United States, the deposited protein is an overproduced immunoglobulin light chain or its fragments. In secondary reactive or inflammatory amyloidosis, elevated plasma concentrations of amyloid A protein are observed and also found in the deposited fibril. Systemic deposits of β_2 microglobulin in amyloid fibrils are a result of prolonged hemodialysis. Familial or hereditary amyloidosis is an autosomal dominant disease. Most affected persons are heterozygous for the disease; that is, they have both normal and variant forms of the protein and each

offspring has a 50% risk for the gene. Since familial amyloidosis is a late-onset disease in which the mutant allele of the gene and its gene product, the protein, have been present from birth, other unknown factors of aging may be involved in the time course of development of the disease.

A number of different proteins have been identified as having variant forms that result in familial amyloid. The major one is transthyretin, for which more than 50 variants have been identified.⁶ Clinically they are associated with midlife onset of peripheral and autonomic polyneuropathy, cardiomyopathy, and vitreous opacities. Normal transthyretin itself is amyloidogenic, causing systemic and cardiac amyloidosis in the elderly.^{7,8} Other proteins involved in familial amyloidosis are gelsolin, fibrinogen, lysozyme, and apolipoprotein AI (apoAI or apoLPAI). This Commentary will focus on familial amyloidosis and specifically on the form described as AapoAI, in which the variant or mutant protein in the fibrillar deposit is apoAI.

The paper by Asl et al⁹ is noteworthy because it identifies a kindred with a unique and previously unreported point mutation of thymine to cytosine at nucleotide 1389 in exon 4 of the apoAI gene. The predicted substitution of proline for leucine at position 90 (Leu90Pro) in the protein primary sequence is confirmed by sequence analysis of the protein isolated from amyloid fibrils from the cardiac amyloid deposits. Unlike other amyloidogenic apoAI variants, the mutation does not produce a change in charge from neutral to positive, but is a neutral-to-neutral substitution. Renal deposition is not observed and the major organ affected is the heart. The role apoAI plays in normal lipoprotein (LP) and lipid metabolism, its structure, and its amyloidogenic variants will be discussed. We will speculate on the conformation of this new variant, and why it may result in amyloidotic cardiomyopathy.

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Plasma LP are assemblies of lipids and proteins (called apoproteins or apolipoproteins (apoLP)) that function to transport lipids to and from various tissues and membranes. They are synthesized and assembled predominantly in the liver and intestine but also in other tissues and organs. ApoLP serve multiple roles, functioning to maintain the solubility of the lipids they transport, assuring the integrity of LP particles, acting as cofactors for enzymes involved in LP metabolism, and acting as ligands for cellular receptors involved in lipid uptake and catabolism.^{10,11} The LP which contain apoAI will be discussed briefly.

Chylomicrons (CM) and high density lipoproteins (HDL), the major LP-containing apoAI, undergo extensive remodeling during metabolism in which apoLP and surface and core lipids are altered through interaction with cell membranes or other LP classes or metabolized by enzymes. CM, containing both apoB48 and apoAI, are assembled and secreted from intestinal mucosal cells into the plasma. Nascent CM are remodeled by interactions with small phospholipid-cholesterol or phospholipid-cholesteryl ester-cholesterol-rich HDL containing varying amounts of apoAI, AII, E, and C proteins. The latter two apoLPs are transferred to CM while triglyceride is simultaneously undergoing lipolysis and its cholesterol and apoAI are transferred to HDL. CM, called remnants at this stage, are taken into cells via receptor-mediated endocytosis by interacting through apoE with the apoB,E receptor¹² on membranes to undergo lipid and apoLP recycling or catabolism.

HDL themselves are polymorphic, consisting of subclasses of particles varying in morphology (disk-like or spherical), lipid composition (phospholipid-cholesterol or phospholipid-cholesteryl ester-cholesterol), and apoprotein content (AI, AII, E).¹⁰ ApoE either remains associated with HDL or recycles between HDL and CM- or VLDL-remnants. HDL is significant because it participates in the processes of lipid remodeling and reverse cholesterol transport involving other LP classes and cellular membranes. High HDL levels with respect to total plasma cholesterol are associated with a reduced risk for the development of atherosclerosis and cardiovascular disease.¹³ In reverse cholesterol transport, for example, excess cholesteryl ester or cholesterol is taken up from LP or cells by these multicomponent HDL particles. This process is facilitated by the conformational adaptability of the apoprotein components. The lipid-enriched HDL are then transported to cellular sites for transfer of the needed cholesterol or for interaction with the apoB, E receptor¹² or scavenger receptors¹⁴ for catabolism. In receptor-mediated processing of HDL, the cholesterol and cholesteryl ester only are endocytosed, and apoAI and apoAII either remain associated with the phospholipid-rich HDL disks or circulate in a free, unbound form at transient, barely detectable levels before exchanging onto other HDL subclasses. The catabolism of normal apoAI *in vivo* is slow. It is recycled through the metabolic events involved in lipid and LP metabolism and reverse cholesterol transport with any transiently measurable free protein filtered by the glomerulus in the proximal tubule cells of the kidney.

The apoLP are described as exchangeable or nonexchangeable, depending on whether or not the specific LP moves readily between LP classes and the aqueous plasma compartment, where it may be present in a non-LP associated form. ApoAI is an exchangeable apoLP. The concentration of apoAI in fasting human plasma is 130 mg/dl, most of which is LP-associated and distributed in HDL subclasses; the lipid-free form¹¹ is at a very low level. The gene for apoAI encodes a 243-residue apoLP with a pre- and a pro- region.¹⁵ The pre-region is an 18-amino acid residue signal peptide, which is cleaved cotranslationally. ApoAI is secreted in the pro-apoAI form and includes a 6-residue N-terminal hydrophobic peptide which is later cleaved.¹⁵ Full-length normal apoAI contains 243 amino acids. Its primary sequence has been analyzed and found to contain segments of amino acids that are predicted to form amphipathic α -helices.¹⁶ Such an arrangement distributes non-polar and polar amino acids on opposite sides of the helix. Sequence variations within individual helices may strongly influence their ability to adopt this conformation.

Because apoAI plays important roles in several environments, one might predict that its biophysical properties would depend on environment since it is found on LP of varying classes and sub-classes, and also in the free form at low levels, in the aqueous plasma phase. ApoLP including apoAI, unlike other water-soluble proteins, exhibit low free energy of stabilization,¹⁷⁻¹⁹ suggesting that although these apoLP are composed of well-defined regions of secondary structure, their tertiary structure is less well stabilized and flexible or conformationally adaptable, increasing their ability to adapt by providing them greater freedom to alter their conformations.

ApoAI has been extensively studied in intact LP, in complex with defined lipids, and in aqueous buffers by a number of methods. Briefly, in aqueous solution spectroscopic and thermodynamic analyses suggest that apoAI exhibits a low free energy of stabilization,^{17,20} a non-two-state unfolding of low cooperativity whose enthalpy is associated with the melting or unfolding of α -helices comprising approximately 60% of its secondary structure, and noncoincident melting of the tertiary structural elements.¹⁸ Such behavior is typical of a molten-globule state of a protein with a compact folding intermediate, native secondary structure and a loosely organized tertiary structure. Gursky and Atkinson¹⁸ suggest that the molten-globule state is physiologically relevant for the appropriate lipid-binding interactions of native apoAI and its normal metabolic functions.

ApoAI has been predicted to contain a number of 22-residue tandem repeats²¹⁻²³ that form amphipathic α -helices divided into 2- to 11-residue subhelices interconnected by β -turns at Pro or Gly residues, and a number of flexible loops that allow adaptation of the conformation of apoAI to the varied LP surfaces and lipid environments it must accommodate. Although no three-dimensional structure for apoAI has been reported to date, two other apoLP structures have been described: apoLPIII²⁴ and the N-terminal domain of human apoE²⁵ from x-ray crystal data. Both of these x-ray structures strongly support the concept of the amphipathic α -helix

with long helices of 19–35 residues predicted in some regions. These helices are thought to be key mediators for lipid-binding and thus for normal LP metabolism.

Several groups have performed extensive secondary-structural analyses of human apoAI^{21–23,26} using statistical and theoretical approaches combined with analysis of primary sequence physical propensities, monoclonal antibody mapping, protease-cleavage, epitope expression,^{26,27} and direct biophysical measurements.²² Models have been developed^{21–23,26,27} and will be discussed with respect to the amyloidogenic apoAI variants, including the novel Leu90Pro variant presented by Asl et al.⁹

Since 1990 six amyloidogenic variants of apoAI have been reported.^{9,28–35} All mutations are located in the N-terminal region of apoAI. Four are single residue changes resulting from point mutations in the gene.^{9,28–32} One produces a deletion/insertion³³ and deletes three amino acids.³⁴ Briefly, the first amyloidogenic variant of apoAI was described in 1990 by Nichols et al²⁸ and later identified in two other families.^{29,30} It is a point mutation of guanine to cytosine at the position corresponding to the first base of codon 26, resulting in a single amino acid change of glycine to arginine at residue 26, or Gly26Arg. This variant is also known as apoAI_{IOWA}. This single residue change produces a +1 alteration in charge for the variant. Clinically, patients develop amyloid in the kidneys and severe gastric ulcer disease. Serum samples show both normal and variant forms of apoAI. Thus, these individuals are heterozygous for this mutation. Residues 1–83 of the variant have been identified in samples from deposited fibrils, whereas normal apoAI is not found.

Metabolic studies by Rader et al³⁵ using radiolabeled normal and variant apoAI show that the presence of apoAI_{IOWA} affects the catabolism of normal apoAI, reducing the overall level of circulating HDL, altering their subclass characteristics in comparison to a normal individual, which leads to extravascular deposition of amyloid fibrils containing the N-terminal fragment of variant apoAI. To date this is the sole study examining the effect of an amyloidogenic apoAI variant on LP metabolism and catabolism.

Two other single residue variants resulting from point mutations in the gene, Leu60Arg and Trp50Arg, have also been identified.^{31,32} Both mutations also result in a charge change of +1 for the variant protein and, clinically, renal involvement. Individuals are heterozygous for the mutant gene with fibril deposits containing fragments of predominantly the variant (with only a trace of normal apoAI, if any) from 1–88, 92, 93, or 94 amino acids.

Two variants involve deletions^{33,34} from the gene in exon 4 that produce a variant protein with either a deletion and insertion of two amino acids or a deletion only. In the deletion/insertion variant³³ residues 60 to 71 have been deleted and two residues, valine and threonine, have been inserted. These mutations in the gene produce a variant protein with a +1 change in charge. Individuals are heterozygous for the mutation and developed hepatic amyloid for which liver transplantation was performed. Fibrils isolated from amyloid deposits contained amino acids 1–83 or 1–92 of the normal apoAI sequence. The second deletion variant³⁴ results from a novel 9-bp in-frame deletion in exon 4 of the apoAI gene.

Three residues (glutamine 70, phenylalanine 71, and tryptophan 72) are deleted, producing a +1 change in charge. Individuals who are heterozygous for the mutation have renal and cardiac involvement and, eventually, organ failure requiring transplant(s). Fibrils deposited in the liver contained variant apoAI.

All of the above-described amyloidogenic apoAI variants carry an extra +1 charge with respect to normal apoAI and all have their mutation in the N-terminal region. Based on these characteristics, it has been hypothesized that the charge or electrostatic alteration might be one of the key features involved in the amyloidogenicity of apoAI variants, because their more cationic nature might increase deposition into fibrils and interaction with negatively charged glycosaminoglycans or other factors commonly associated with amyloid. The current report of Asl et al,⁹ described earlier, identifies an amyloidogenic apoAI variant, Leu90Pro, that does not confer a change in charge but does result in a unique clinical presentation of cutaneous amyloid deposition and restrictive cardiomyopathy with N-terminal fragments 1–94 isolated from fibrils. The authors speculate that, as was reported for Gly26Arg by Rader et al,³⁵ the normal metabolism/catabolism may be altered in this variant, leading to significant changes in LP composition and subclasses and producing the amyloid deposition and resulting cardiomyopathy. For example, HDL subclasses might well be altered with respect to their lipid components, particle morphology, or apoAI/apoAII ratios, producing altered metabolism or even a significant amount of free apoAI in plasma. Such free apoAI variant might have an increased tendency to self-associate, aggregate, and deposit as fibrils, due to the thermodynamic and structural considerations presented earlier.^{17,18,20}

As mentioned above, several groups^{16,21–23,26,27} have presented secondary structural models for apoAI associated with phospholipid. Focusing on the N-terminal portion, which thus far is the region in which all amyloidogenic mutations have been identified for the apoAI protein, all groups agree that this region contains varied types of structure including amphipathic helices, flexible turn-containing loops, and strands, and thus a complex series of epitopes. Using different predictive or experimental methods, residues 60 through 120 (or higher) are suggested to be helical. Marçel et al^{23,26} describe epitopes in the N-terminal that are distinct and complex and others that are tertiary-structural and discontinuous. Alteration of a single residue in such epitopes might well significantly alter their conformation and normal interactions. Nolte and Atkinson²² suggest that helices composed of multiple repeats may fold back on each other in an antiparallel manner, providing longitudinal separation of charge in the amphipathic helices, and speculate that oppositely charged regions may interact when helices align, producing stabilization of helical packing. A single proline residue, such as was introduced by the mutation identified for the variant of the current report,⁹ can be accommodated in a long helix²² by distorting the helical geometry locally. Such a local distortion, however, may well alter other features, which could change lipid-apoAI interactions and LP classes and metabolism or expose sites for proteolytic cleavage. At this time it is not known

whether cleavage of the N-terminal fragments of the variants of apoAI occurs while the variant is still bound to lipid on LP or after deposition in the fibril. It will be interesting and important to use extraordinarily well-characterized monoclonal antibodies to apoAI^{23,26,27} in epitope-mapping studies with amyloidogenic apoAI variants complexed with lipid, to perform metabolism/catabolism studies with variants in addition to Gly26Arg, to examine protease-cleavage sensitivity, and to investigate the stability of apoAI variants to attempt to understand the mechanism of amyloid fibril formation.

Clearly, apoAI performs roles in numerous metabolically distinct environments in which its participation is critical and for which the adaptability of its conformation is essential.³⁶ Alteration in any component of HDL—its lipid composition and content, apoprotein content, or mutation in any of the proteins—might thus be expected to perturb the delicate molecular equilibrium of this complex process and produce a pathological situation related to lipid, lipoprotein, or apoprotein metabolism or catabolism. Additional genetic or environmental factors may determine the location and clinical effects of amyloid deposition. The current paper of Asl et al⁹ and the novel variant it describes with involvement in cardiomyopathy emphasizes the value of the apoAI model for the investigation of the molecular mechanism of fibrillogenesis.³⁷

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