

# The scope and limitations of *in vivo* and *in silico* models of cardiac amyloidosis

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**Abstract:** Amyloidosis is a systemic disease, leading into disfunction of many organs. There are several clinical and morphological forms of amyloidosis based on the organ-specific nature of the amyloid fibril deposition, which are found in the heart, brain, kidneys, spleen, liver, pancreas and thyroid glands, bone marrow, intestines. The nature of organ damage correlates with the types of amyloid fibrils. Thus, damage to the tissues of the heart and kidneys are the most significant factors affecting mortality. The complexity of drug molecules discovery against amyloidosis is connected with the fact that more than 30 proteins are involved in the fibril formation. The fact that only two small molecules, namely diflunisal and tafamidis are clinically used nowadays underlines the complexity in this field of research. The mechanism of action for both of drugs include the stabilization of the tetrameric form of transthyretin. The crucial approach for the discovery of drug molecules against cardiac amyloidosis needs the predictive models. The main restrictions of most developed *in vivo* models related to their reproducibility and cost. Therefore, *in silico* approach may be quite effective procedure to minimize time and difficulties during drug discovery process. In this paper we collected main knowledge which highlights scope and limitations considered during *in silico* approach development.

**Keywords:** Cardiac amyloidosis; Amyloid fibrils; Models; *in vitro*; *in vivo*; *in silico*

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## 1. Introduction

Amyloid deposition in the heart tissues leads to cardiac failure having such symptoms as breathlessness and fatigue, caused by the progressive loss of elasticity of the myocardium [1].

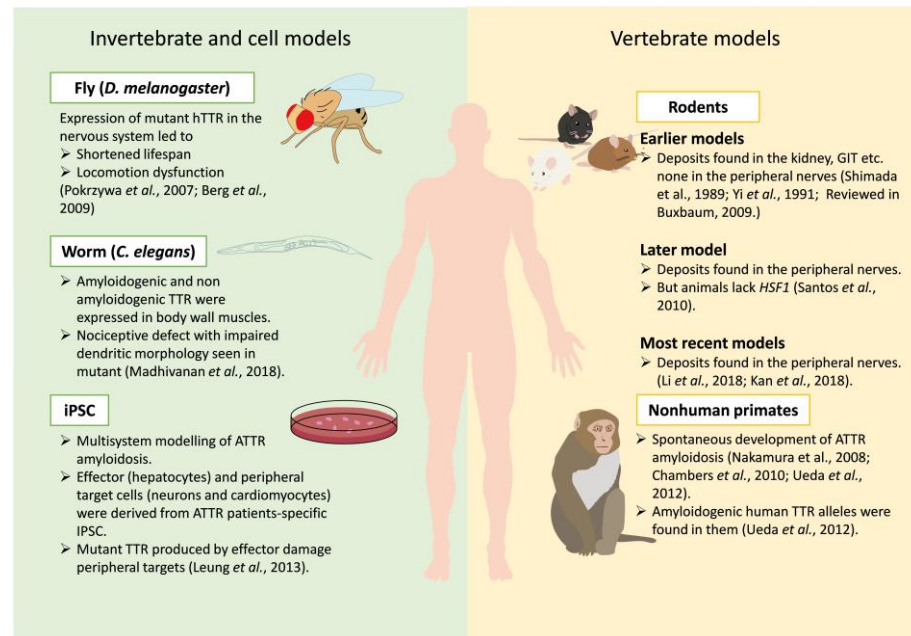
The most known forms of cardiac amyloidosis are as transthyretin-related (ATTR) and immunoglobulin light chain (AL) amyloidosis. In the case of AL type the median survival of patients is half year from the heart failure beginning [2]. There are more than 30 proteins involved in the cardiac amyloidosis development, that make the *in vitro* and *in vivo* models' development quite difficult. Molecular mechanisms of cardiac amyloidosis are still not clear, and the last knowledge about its mechanisms is discussed in the recent review [3].

To have the markers of disease development and progression the quite useful tool is represented by *in silico* models, which also have great potential for drug discovery opportunity. The main basis for *in silico* models creation includes the experimental data collection describing the main indicators and possible mechanisms.

In this review we overview modern models developed for the cardiac amyloidosis, and consider their scope and limitations, especially *in silico* models.

## 2. *In vivo* models

Most of known animal and cell models are discussed in the recent review [4]. Authors focused on ATTR amyloidosis. Main current models available for studying ATTR amyloidosis are presented in Figure 1.

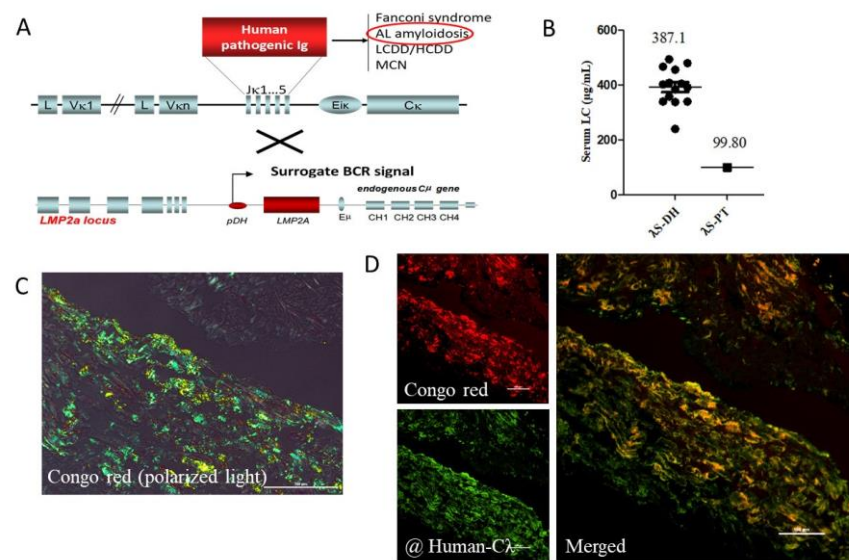


**Figure 1.** Current models available for the study of ATTR amyloidosis. Various models for ATTR amyloidosis include invertebrate, cell and vertebrate models. Key phenotypes and findings from these models are indicated with proper references. Reproduced from [4], with the permission from Frontiers Media S.A., 2023.

This important review very well demonstrates that amyloidosis is systemic diseases which affect several organs, because unfolded TTR aggregates are found in the heart, peripheral nerves and other organs, that is the reason of model development diseases difficulties, especially, selective for cardiac amyloidosis. This is well illustrated by the data cited in this review, majority of the models are related to amyloid polyneuropathy. Only spontaneous development of ATTR cardiac amyloidosis in several vervet monkeys has been indicated in this review.

Among *in vivo* models, the article about the first transgenic mouse model of cardiac AL amyloidosis based on the insertion of human pathogenic LC gene in the endogenous mouse kappa locus has been published [5]. Transgenic strategy includes the insertion of the human Ig gene in the endogenous murine kappa locus (Figure 2).

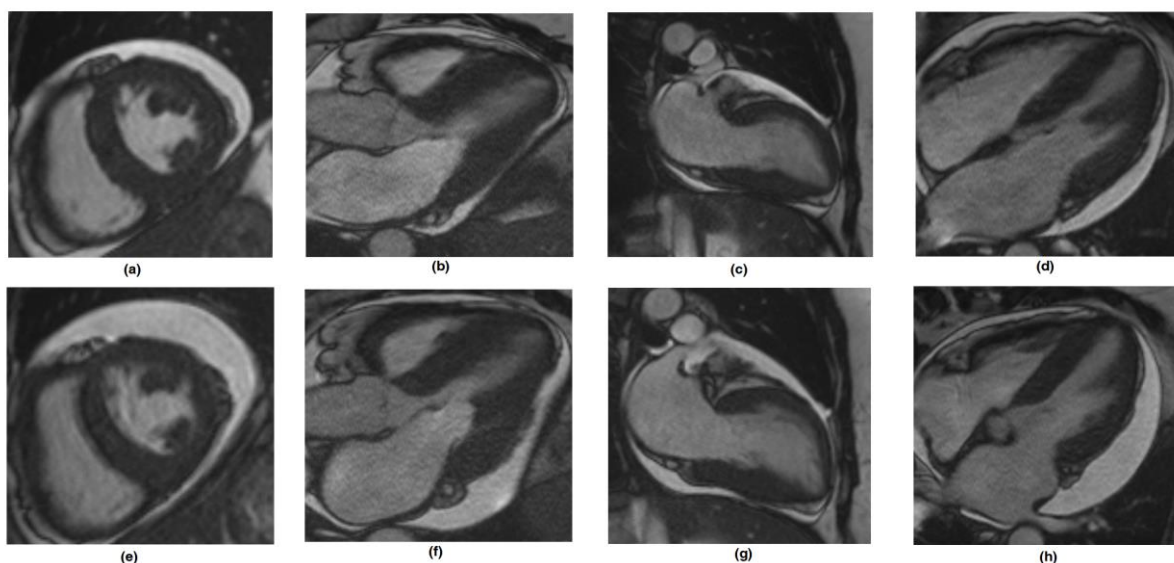
Authors underline that AL amyloidosis was not developed under strong LC production, because only the variable domain (IGLV6) was able to form fibrils while full length LC showed resistance for amyloid formation. After the single injection fibrils were found in the spleen, liver, the kidney and mainly in heart.



**Figure 2.** AL amyloidosis model. A: Transgenic strategy: insertion of the human Ig gene in the endogenous murine kappa locus, such as the naturally Ig-producing B and plasma cells produce the human pathogenic Ig in high amount. To further increase the production of free LC, normally observed in AL amyloidosis patients, it backcrossed these mice with another transgenic strain, DH-LMP2A mice, characterized by a high number of plasma cells devoid of endogenous HC. This strategy avoids the association of human LCs with endogenous murine HCs, leading to a quasi-monoclonal expression of the free LC. B: serum free LC levels compared to the corresponding patient. C: Congo red staining (polarized light) on heart section in AL transgenic mice. D: Same section than in C showing the colocalization of amyloid deposits with anti-human I LC antibody (recognizing the constant domain). Adapted from [5], with the permission from Elsevier, 2023.

### 3. *In silico* models

It is important to have an indicators of cardiac tissue function which is quite important for the treatment. Li et al use mathematical models of the left ventricle derived from routine clinical magnetic resonance imaging to find new markers, and demonstrated the agreements with clinical symptoms (double-blind test in six out of the seven sample cases). The next factors were evaluated - the strains, stresses, p-V curve, LV shape, and volume of a group of amyloidosis patients before and after the treatment [6].



**Figure 3.** The CMR images for the reconstruction LV model in diastole. (a)–(d) are cine images at short-axis and three long-axis planes at the baseline scan, (e)–(h) are corresponding cine images at the follow-up scan from the same patient. Reproduced from [6] (Supplementary Material), with the permission from Frontiers Media S.A., 2023 .

Authors underline that the results should be interpreted carefully, because many factors have to be considered, and no single biomarker is able to give prediction due to complexity of the processes in the heart.

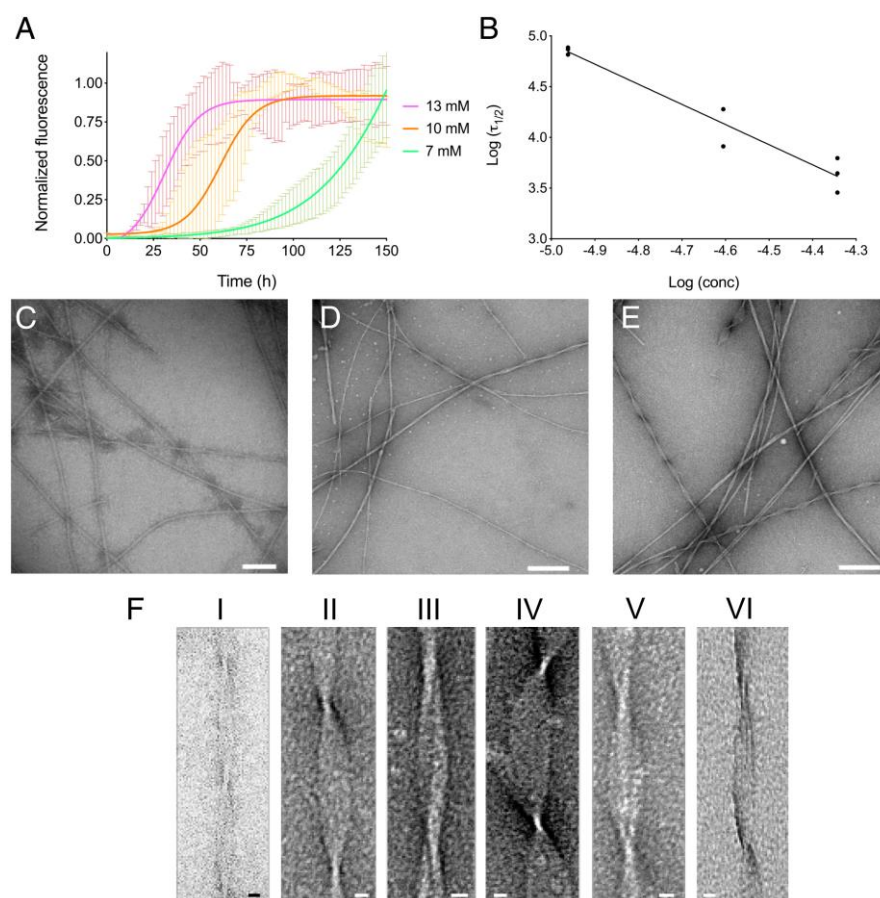
A random forest machine learning model has been developed, and it was demonstrated that medical claims data well identify patients with wild-type transthyretin amyloid cardiomyopathy. The model was validated in three nationally representative cohorts (9412 cases, 9412 matched controls), and a single-center electronic health record-based cohort (261 cases, 39393 controls) [7].

Based on combined factors such as age, gender, carpal tunnel syndrome, interventricular septum in diastole thickness, and low QRS interval voltages, with an area under the curve (AUC) of 0.92 the model for ATTR-CA diagnosis has been developed (the score had an AUC of 0.86). In all 3 clinical validation cohorts: 1) hypertensive cardiomyopathy (n = 327); 2) severe aortic stenosis (n = 105); and 3) heart failure with preserved ejection fraction (n = 604) the model demonstrated good diagnostic accuracy [8].

Model based on the evaluation of circulating retinol-binding protein 4 (RBP4) concentration has been developed for the identification of ATTR V122I amyloidosis in elderly African American patients [9]. Authors also noted, that RBP4 concentration may be considered as a predictor marker of disease progression.

The number of diseases, which is associated with amyloid fibrils formation is more than 50.

Hybrid structure-based model (molecular dynamics simulations), describing the conformational dynamics of monomers as well as structure of fibrils has been developed and named multi-eGO. This model considers structural and kinetics of protein aggregation, including aggregation of thousands of monomers. Data about concentration dependence and structural features of the fibrils formed are in good agreement with *in vitro* and *in vivo* experimental data for Transthyretin (Figure 4). This model may be quite useful for the development of drugs against cardiac amyloidosis [10].



**Figure 4.** TTR peptide aggregation kinetics in vitro. (A) Aggregation kinetics of the TTR105-115 peptide at 13 mM, 10 mM and 7 mM are shown in magenta, orange and green, respectively. TTR peptide at 37 °C were obtained by monitoring of ThT fluorescence. The mean value of three independent experiments analyzed by linear regression using Boltzmann sigmoidal equation is reported. (B) Log-log plot of the in vitro half times,  $\tau_{1/2}$ , as a function of the initial monomer concentration. (C-E) Electron micrographs of fibrils formed by TTR105-115 peptide incubated at 13 mM (C), 10 mM (D) or 7 mM (E) at 37 °C for 150 h. Scale bars correspond to 100 (C) or 200 (D and E) nm. (F) Representative TEM images of the six main fibrillar morphologies. Reproduced from [10], with the permission from National Academy of Science, 2023.

The several approaches like as artificial intelligence for the cardiac amyloidosis predictions are overviewed very recently [11].

#### 4. Conclusion

The *in silico* models development for the understanding of cardiac amyloidosis mechanisms, pathology as well as for drug target and biomarkers discovery, meet many challenges, because for these models did not recapitulate all symptoms, especially neurological presentation. Nevertheless, several computer-based models are in good correlation with clinical symptoms. In most cases, the predictive models were tested on a small cohort of patients, and external validation in a larger, independent patient population is required. Taking into account the complexity of disease mechanisms, a multi-target drug design is required.

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