engineering proceedings

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Proceeding Paper

The Scope and Limitations of In Vivo and In Silico Models of Cardiac Amyloidosis †

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† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

Abstract: Amyloidosis is a systemic disease, leading to the disfunction of many organs. There are several clinical and morphological forms of amyloidosis based on the organ-specific nature of amyloid fibril deposition, which is found in the heart, brain, kidneys, spleen, liver, pancreas, thyroid glands, bone marrow and 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 molecule discovery against amyloidosis is connected with the fact that more than 30 proteins are involved in 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 drugs include the stabilization of the tetrameric form of transthyretin. The crucial approach for the discovery of drug molecules against cardiac amyloidosis requires the use of predictive models. The main restrictions of most developed in vivo models, however, are related to their reproducibility and cost. Therefore, an in silico approach may be a relatively effective procedure to minimize time and difficulty during the drug discovery process. In this paper, we collected key information which highlights the scope and limitations of the development of an in silico approach.

Keywords: cardiac amyloidosis; amyloid fibrils; models; in vitro; in vivo; in silico

Citation: Morozkina, S.; Snetkov, P.; Uspenskaya, M. The Scope and Limitations of In Vivo and In Silico Models of Cardiac Amyloidosis. *Eng. Proc.* **2023**, *56*, 239. https://doi.org/10.3390/ASEC2023-15869

Academic Editor: Gianrico Spagnuolo

Published: 7 November 2023

1. Introduction

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

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

To obtain the markers of disease development and progression, a rather useful tool is through the use of in silico models, which also have great potential for drug discovery opportunity. The main basis for in silico model creation includes a collection of experimental data describing the main indicators and possible mechanisms of the disease development.

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

2. In Vivo Models

Most of the known animal and cell models are discussed in a recent review [4], wherein the authors focused on ATTR amyloidosis. A summary of the main current models available for studying ATTR amyloidosis is presented in Figure 1.

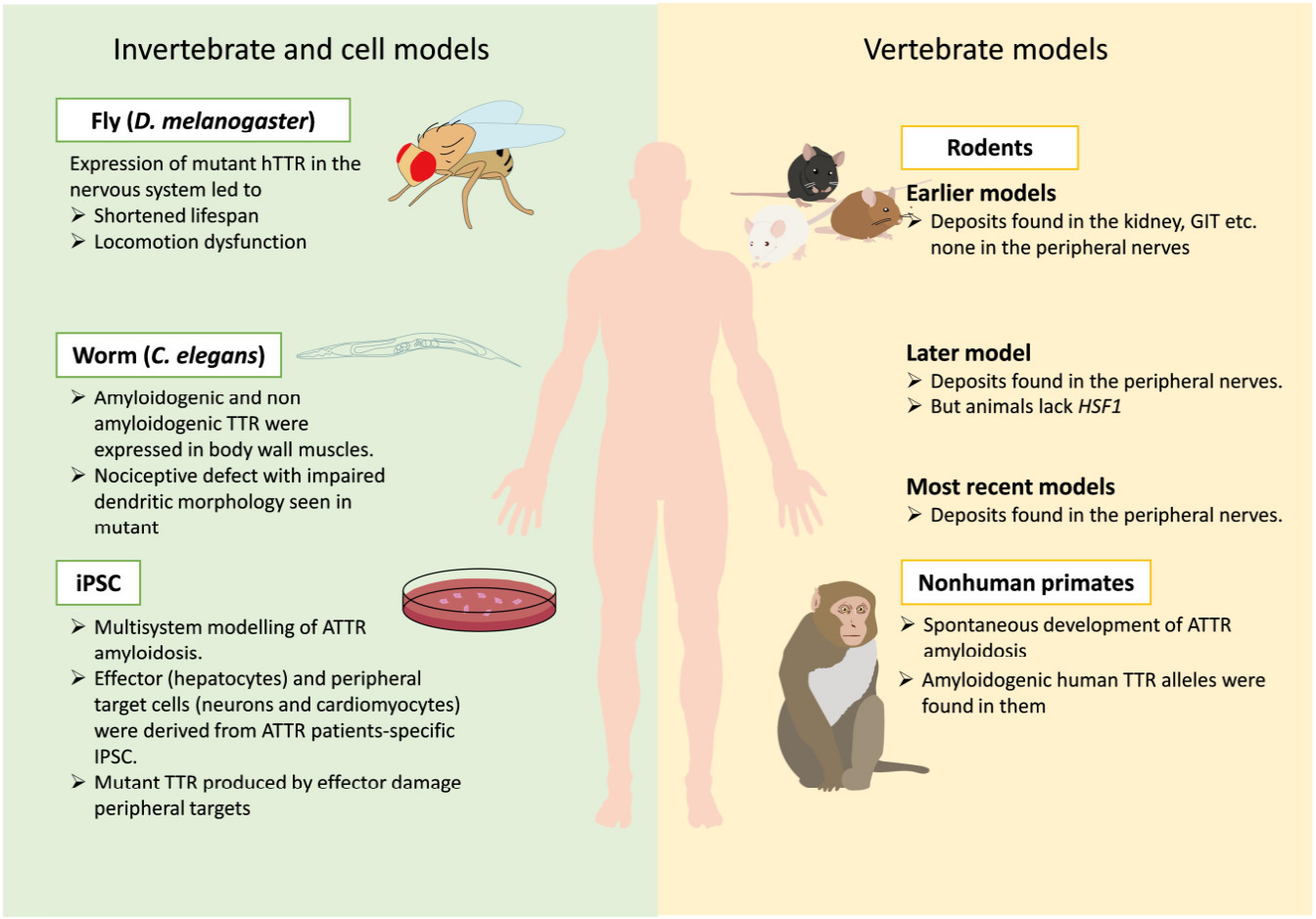

Figure 1. Current models available for the study of ATTR amyloidosis. Various models for ATTR amyloidosis include invertebrate, cell and vertebrate models. Key phenotypes and findings from these models are indicated with proper references. Adapted from [4], with the permission from Frontiers Media S.A.,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

This important review very well demonstrates that amyloidosis is a systemic disease which affects several organs because unfolded TTR aggregates are found in the heart, peripheral nerves and other organs, which results in difficulties in modeling the development of diseases, especially cardiac amyloidosis. This is well illustrated by the data cited in this review, which demonstrate that a majority of the models are related to amyloid polyneuropathy. The only example of a spontaneous development of ATTR cardiac amyloidosis is that which was seen in several vervet monkeys, as indicated in this review.

Among the in vivo models, an article about the first transgenic mouse model of cardiac AL amyloidosis, based on the insertion of the human pathogenic LC gene in the endogenous mouse kappa locus, was previously published [5]. The transgenic strategy includes the insertion of the human lg gene in the endogenous murine kappa locus (Figure 2).

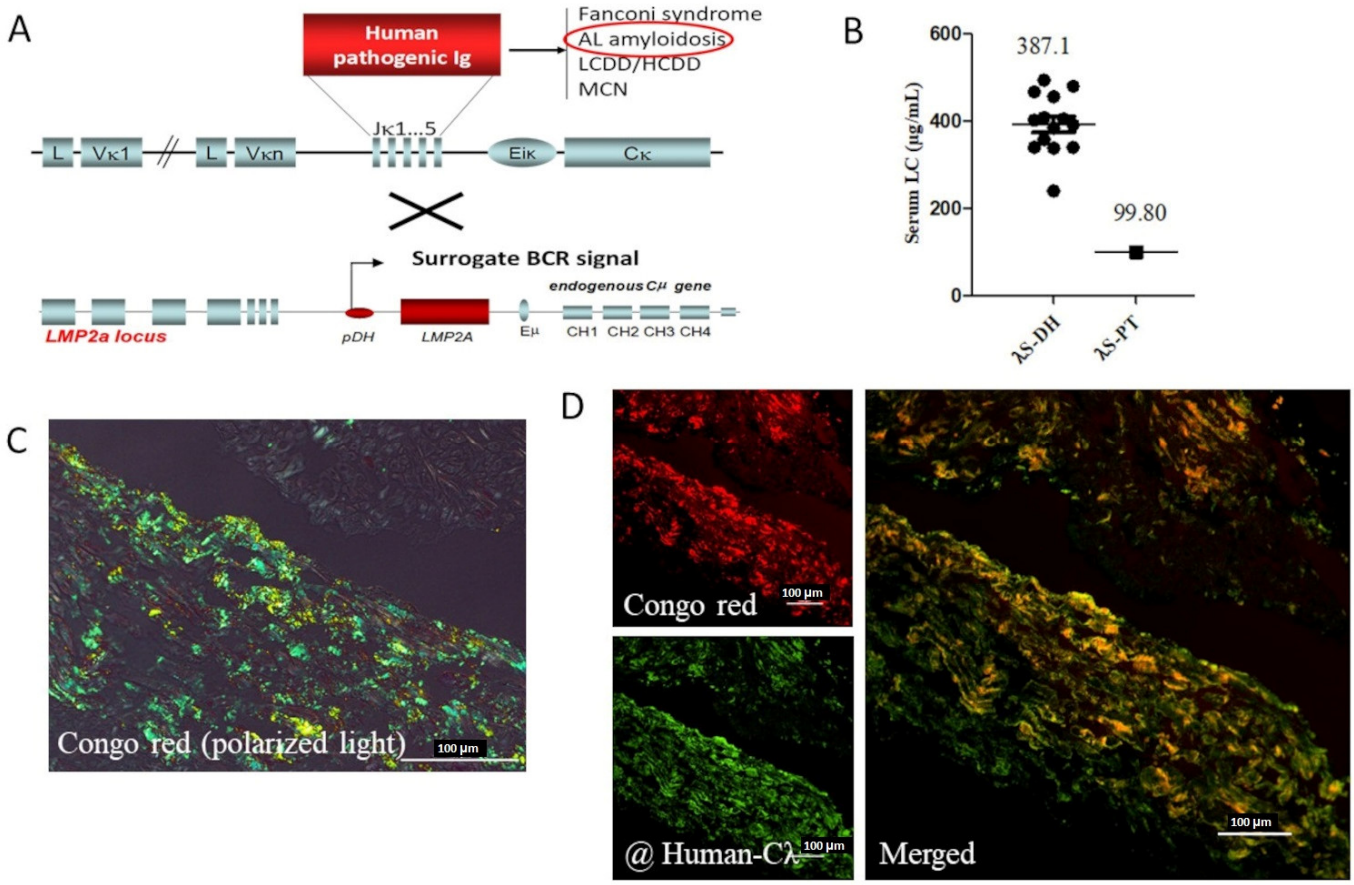

Figure 2. AL amyloidosis model. (**A**) Transgenic strategy: The insertion of the human lg gene in the endogenous murine kappa locus, such as the naturally lg-producing (**B**) and plasma cells that produce the human pathogenic lg in high amounts. To further increase the production of free LC, normally observed in AL amyloidosis patients, these mice were backcrossed 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**) The same section as in (**C**), showing the colocalization of amyloid deposits with anti-human l LC antibody (recognizing the constant domain). Adapted from [5], with permission from Elsevier, 2023.

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

3. In Silico Models

It is important to have an indicator of cardiac tissue function, which is important for treatment. Li et al. used 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-blinded test in six out of the seven sample cases). The following factors were evaluated in a group of amyloidosis patients before and after treatment: the strains, stresses, p–V curve, LV shape and volume (Figure 3) [6].

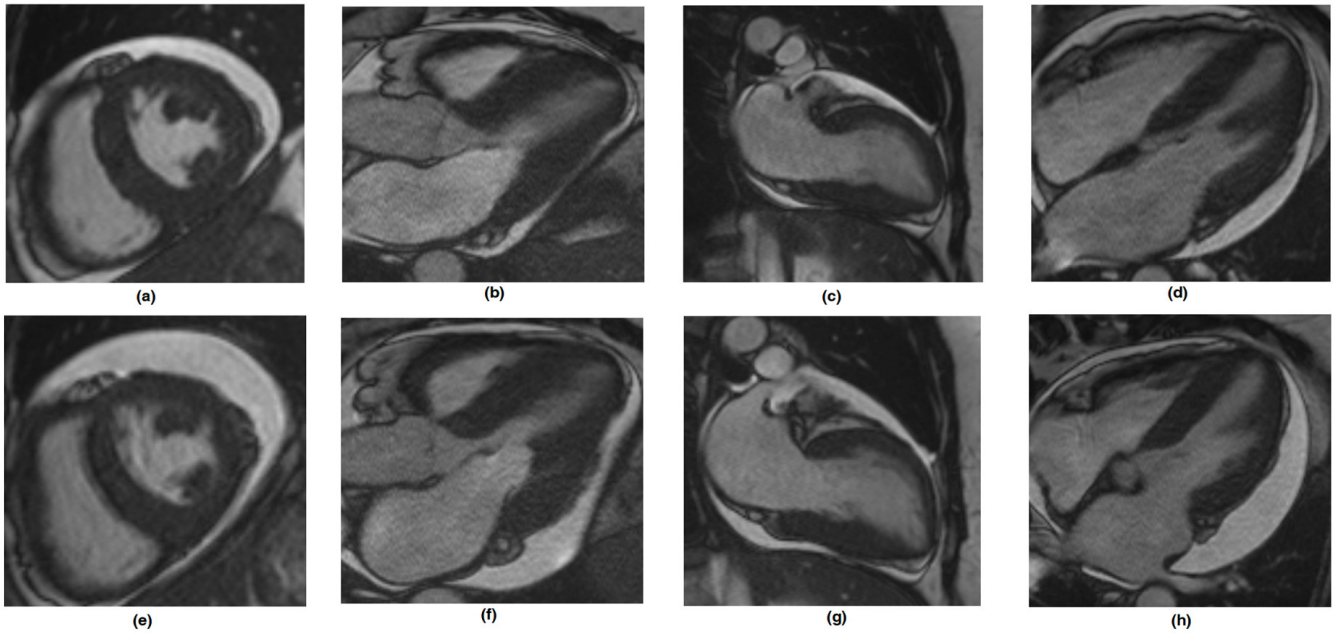

Figure 3. The CMR images for the reconstruction LV model in diastole. (**a**–**d**) are cine images at the short-axis and three long-axis planes at the baseline scan, (**e**–**h**) are corresponding cine images at the follow-up scan from the same patient. Reproduced from [6], with permission from Frontiers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

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

A random forest machine learning model was developed, and it was demonstrated that the data of medical claims 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, 39,393 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 three of the following clinical validation cohorts, the model demonstrated good diagnostic accuracy [8]: (1) hypertensive cardiomyopathy (n = 327); (2) severe aortic stenosis (n = 105); and (3) heart failure with preserved ejection fraction (n = 604).

A model based on the evaluation of circulating retinol-binding protein 4 (RBP4) concentration was developed for the identification of ATTR V122I amyloidosis in elderly African American patients [9]. The authors 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.

A hybrid structure-based model (molecular dynamics simulations), describing the conformational dynamics of monomers as well as the structure of fibrils, was developed and named multi-eGO. This model considers the structure and kinetics of protein aggregation, including the 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].

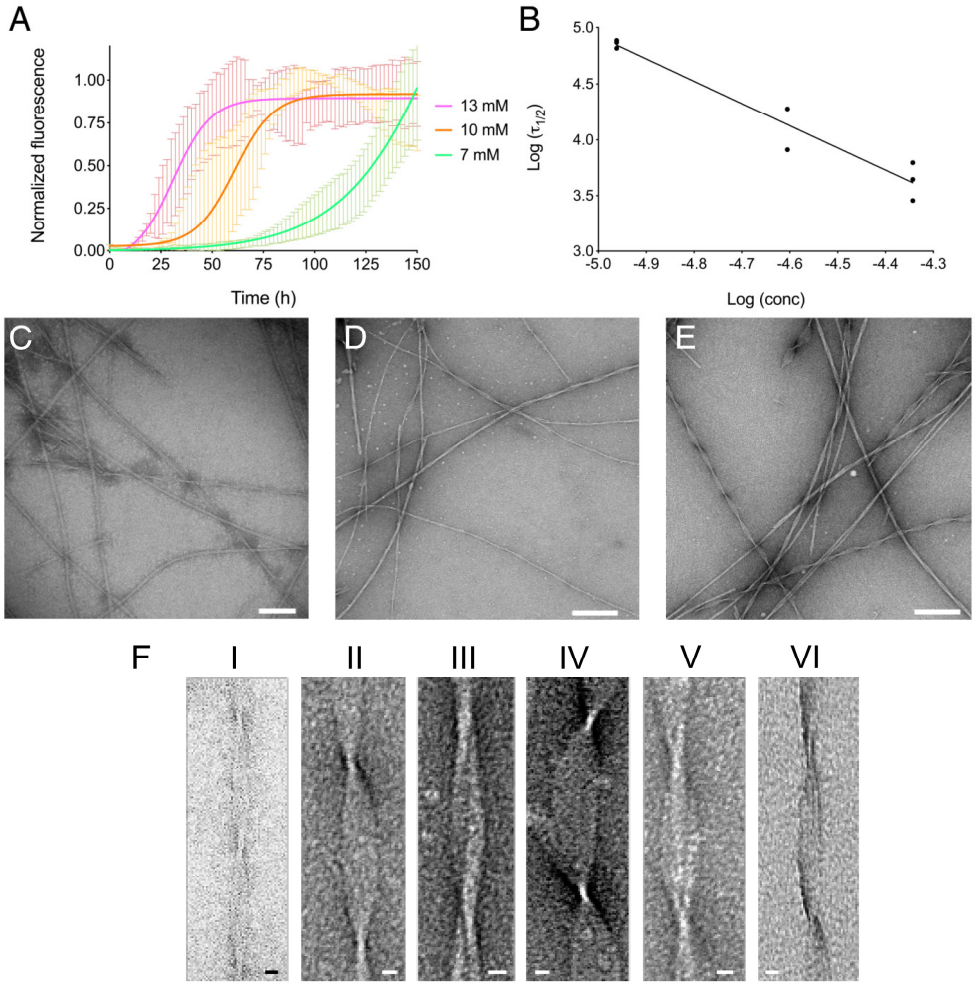

Figure 4. TTR peptide aggregation kinetics in vitro. (**A**) Aggregation kinetics of the TTR105-115 peptide at 13 mM, 10 mM and 7 mM are shown in magenta, orange and green, respectively. TTR peptides at 37 °C were obtained via monitoring of ThT fluorescence. The mean value of three independent experiments analyzed via 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**,**E**) nm. (**F**) Representative TEM images of the six main fibrillar morphologies. Reproduced from [10], with the permission from National Academy of Science, 2023.

Several other approaches such as the use of artificial intelligence for conducting cardiac amyloidosis predictions were very recently reviewed [11].

4. Conclusions

The development of in silico models for the understanding of cardiac amyloidosis mechanisms and pathology, as well as for drug target and biomarker discovery, face many challenges, because these models do 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.

Author Contributions: Conceptualization, P.S. and S.M.; methodology, P.S.; validation, S.M.; formal analysis, P.S. and S.M.; resources, P.S., S.M. and M.U.; writing—original draft preparation, P.S. and S.M.; writing—review and editing, S.M.; visualization, P.S.; supervision, S.M.; project administration, S.M. and M.U. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation, project number 21-74-20093. Link to information about the project: https://rscf.ru/en/project/21-74-20093/, (accessed on 28 September 2023).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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