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TTRMDB: A database for structural and functional analysis on the impact of SNPs over transthyretin (TTR) using bioinformatic tools

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ABSTRACT

Hereditary Transthyretin-associated amyloidosis (ATTR) is an autosomal dominant protein-folding disorder with adult-onset caused by mutation of transthyretin (TTR). TTR is characterized by extracellular deposition of amyloid, leading to loss of autonomy and finally, death. More than 100 distinct mutations in TTR *gene* have been reported from variable age of onset, clinical expression and penetrance data. Besides, the cure for the disease remains still obscure. Further, the prioritizing of mutations concerning the characteristic features governing the stability and pathogenicity of TTR mutant proteins remains unanswered, to date and thus, a complex state of study for researchers. Herein, we provide a full report encompassing the effects of every reported mutant model of TTR protein about the stability, functionality and pathogenicity using various computational tools. In addition, the results obtained from our study were used to create TTRMDB (Transthyretin mutant database), which could be easy access to researchers at http://vit.ac.in/ttrmdb.

1. Introduction

TTR gene is located on chromosome 18 in humans which contains 4 exons and 5 introns. It encodes 127 amino acid long homotetrameric carrier protein, Transthyretin. Transthyretin is a highly conserved protein that functions as a thyroid hormone-binding protein and transports thyroxine in the plasma to cerebrospinal fluid and brain. It also plays a role in retinol (vitamin A) transport through its association with retinol-binding protein (Buxbaum and Reixach, 2009). Native TTR has a globular shape with eight antiparallel β-strands that make up each monomer. Two four-stranded β-sheets (DAGH and CBEF) form the antiparallel strands. Further, the β-strand E has a short α-helix. Hydrogen interaction of the β-strands, F and H of each subunit results in the formation of a dimer. Also, the interaction of the residues of the loops that join β-strands, G to H and A to B lead to the formation of tetramer. Synthesized by the liver, kidney, pancreas and choroid plexus, transthyretin has emerged to be involved in several functions apart from being a carrier protein (Vieira and Saraiva, 2014). Liver and choroid plexus are the well- known major sites in TTR synthesis (Vieira and Saraiva, 2014; Herbert et al., 1986). Additionally, TTR is also synthesized by cellular components of the peripheral nerve(axons), vascular smooth muscle cells, Schwann cells and neurons (Murakami et al., 2010; Gonçalves et al., 2017).

Mutations in TTR gene generally result in amyloidosis. TTR amyloidosis is characterized by the deposition of amyloid fibrils usually in the peripheral nerves or the heart and also, present in the retinal epithelium, leptomeningeal epithelium of the eye and choroid plexus of the brain (Herbert et al., 1986; Liepnieks et al., 2006). The process of amyloidogenesis involves the dissociation of tetrameric TTR, misfolding of the protein, followed by aggregation into amyloid fibrils (Ando et al., 2013). Thermodynamic studies have concluded that the dissociation of tetramer is the rate-limiting step (Kelly, 2000). Heritable mutations of TTR are inherited in an autosomal dominant manner (Ando et al., 2005). Familial amyloid cardiomyopathy (FAC) or familial amyloidotic polyneuropathy (FAP) occur due to these mutations in TTR protein. However, TTR amyloidosis can also result from the misaggregation of wild-type TTR, causing senile systemic amyloidosis (SSA), a sporadic non-inheritable disease (Ruberg and Berk, 2012).

ATTR has been found to be the most common form of autosomal dominant hereditary neuropathy (Shin and Robinson-Papp, 2012). It is an irreversible sensorimotor and autonomic neuropathy. Specifically, the three stages manifested by TTR-FAP includes sensory polyneuropathy, progressive walking disability and wheelchair bound or bedridden. It is a rapidly progressive disease with a life expectancy of 7.3–11 years from onset (Parman et al., 2016). The current therapeutic approaches to polyneuropathy include prevention of amyloids followed by symptomatic therapy for the affected areas and finally, the treatment of end-stage organ failure (Adams, 2013).

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Though liver transplantation remains the 'gold standard' for the treatment of FAP, various drugs are being developed to slow or halt the progress of the disease. Tafamidis is a drug that prevents the dissociation of transthyretin tetramer and binds to one of the two thyroxine binding sites of tetramer to stabilize the correctly folded form, which is used as a treatment for FAP and FAC (Maurer et al., 2018). The drugs, Patisiran and Inotersen, both inhibit the synthesis of transthyretin in the liver. While Patisiran acts as an RNA interference therapeutic agent and Inotersen is a 2′-O-methoxyethyl-modified antisense oligonucleotide respectively (Mathew and Wang, 2019; Adams et al., 2018). However, for the specific therapy, the familial screening of individuals affected by TTR mutation, as well as the nature of these mutations, how they affect the individual and the stability of the protein produced, need to be studied (Adams, 2013). On the other hand, Antisense Oligonucleotides (ASO) and small interfering RNA (siRNA) are used as therapeutic oligonucleotides. These oligonucleotides degrade and block the mutated mRNA, help to treat various types of TTR mutations. Currently, this process of RNA interference (RNAi) that inhibits the formation of protein aggregation plays an vital role in the treatment of TTR (Mathew and Wang, 2019).

More than 100 mutations of TTR have been identified from different ethnic groups that lead to different phenotypes (Rowczenio et al., 2014). It has been found that when mutants are more destabilizing, the onset of amyloidosis is earlier. However, two mutations D18G and A25T, though found to be the most destabilizing, are not the most pathogenic, which attributed to the fact that factors like cellular secretion efficiency of the protein, along with instability, also influence pathogenicity (Johnson et al., 2012a). The most common amyloidogenic mutation is V30M. This mutation is found to have different clinical manifestations for patients from different geographic areas. Studies conducted on affected individuals conclude that there could be a common founder for this mutation of patients from Japan and Portugal (Ohmori, 2004). Moreover, the reports from the clinical data have suggested that V30 M mutations are also endemic in other countries such as Sweden, French and Brazil (Norgren et al., 2012; Zaros et al., 2008). Other common mutations include T60A and V122I. T60A is implicated in both, cardiomyopathy and neuropathy which is found in the affected population in north-west Ireland (Reilly et al., 1995). V122I is a predominant mutation found in patients affected by cardiomyopathy. It is especially prevalent among African-American patients (Buxbaum et al., 2010).

Most studies use direct DNA sequencing to identify such mutations in specific patients. These mutations need to be studied, not only to assess their pathogenic impact, but also to check how they affect the stability of the protein. In the biochemistry perspective, the stability of a protein is a key characteristic feature that affects function, regulation and activity of the protein. Thus, studying the impact of these mutations helps gain a better understanding of the affected protein in finding therapeutic design solutions for the disease-causing variants. However, performing extensive wet-lab experiments to study the pathogenicity and functional impacts of each would be a labor-intensive and cumbersome task. Thus, the use of the bioinformatics tools could provide a secure and better understanding of the stability and pathogenicity of the protein upon various notable disease-causing missense mutations. Generally, homologous and distantly related sequences are aligned with amino acid sequences to obtain information on conservation. Moreover, the conservation across various species, the physicochemical properties of these amino acids, the potential protein structural changes and the database annotations are the most common criteria considered in many bioinformatics programs for predicting the functional effect of an amino acid substitution (Seifi and Walter, 2018). In this study, the effects of different mutations on the stability and the pathogenic effects of transthyretin were studied using different tools, such as, iMutant 3.0, STRUM, Provean, PredictSNP, PhD-SNP, PolyPhen-2, SIFT, FATHMM, iStable, mCSM, SDM, DUET, DynaMut, FoldX, ENCoM, CUPSAT, TANGO, WALTZ and LIMBO.

2. Materials and methods

2.1. Data collection

Initially, the sequence and the structure of Human Transthyretin were retrieved from RCSB PDB (id: 3A4D). In our study, the first 20 sequence of the amino acids were neglected in order to maintain the consistency with the experimental studies. Further, the list of mutations of TTR was obtained from the database, 'Mutations in Hereditary Amyloidosis' (Rowczenio et al., 2014).

2.2. Identification and selection of tools

Various bioinformatics tools were chosen based on their relevance and the reported accuracy of their predictions. Subsequently, the tools were segregated based on whether they provide information on sequence, structure or aggregation properties of the protein. For sequence studies, the tools used were iMutant 3.0, STRUM, Provean, PredictSNP, PhD-SNP, PolyPhen-2, SIFT, FATHMM and iStable. The tools mCSM, SDM, DUET, DynaMut, FOLDX, ENCoM and CUPSAT were used for structural studies, and the aggregation properties were studied, using TANGO, WALTZ and LIMBO for every reported mutant.

2.3. Tools used

Generally, the changes in protein stability are measured by the difference in free energies of wild-type and mutant protein (ddG). Sequence-based methods are commonly based on support vector machines, neural networks or decision trees. Herein, the input was the sequence of Chain A of Transthyretin. As the protein contains 2 identical chains, the predictions on the effect of SNPs on one chain can be correlated for the entire protein. Distinctly, iMutant predicts the change in the stability of a protein upon mutation. It is Support Vector Machine (SVM) based program that was trained using a data set derived from ProTherm, a database of changes in free energy of protein due to change in stability, upon mutation, obtained from thermodynamic studies (Capriotti et al., 2005). STRUM is a structure-based prediction tool that takes protein sequences, constructs 3D models using I-TASSER simulations and predicts the effect of SNPs on the stability of protein structure (Quan et al., 2016). iStable is an integrated predictor that uses sequence information and prediction results from different element predictors. It was found to have a better performance than all the element predictors after training and cross-validation. The tool was trained using several data sets and SVM was used as an integrator for the several evaluated machine learning methods (Chen et al., 2013). PROVEAN (Protein Variation Analyzer) uses an alignment-based score approach to predict the functional effect of single or multiple amino acid substitutions, insertions and deletions, unlike most existing tools that generate predictions only for single amino acid substitutions (Choi and Chan, 2015). PredictSNP is a consensus server. It uses three independent data sets and evaluates eight established prediction tools in an unbiased manner. Wherein, the tools include MAPP, nsSNPAnalyzer, PANTHER, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT and SNAP. PredictSNP combines the six best performing tools, which has an improved prediction performance. It also returns the results from all the eight servers (Bendl et al., 2014). PhD-SNP is also an SVM-based tool that uses protein sequence information to predict whether an nsSNP is related to a genetic disease in humans. The predictor has shown to reach more than 74% accuracy in predicting whether an SNP is disease-related or not (Capriotti et al., 2006). PolyPhen-2 uses an iterative greedy algorithm to select eight sequences and three structure-based predictive features. Most of these features involve the comparison of the wild-type and mutant allele to obtain information on various parameters. Naive Bayes classifier is then used to predict the functional significance of an allele replacement (Adzhubei et al., 2010). SIFT (Sorting Intolerant from Tolerant) is an algorithm used to predict the effects of coding

Fig. 1. Home page of the TTRM Database.

variants on protein function by the use of sequence homology. It assumes that the amino acid substitutions or insertions/deletions in evolutionarily conserved regions affect the function of the protein as these regions are generally less tolerant of mutations (Sim et al., 2012). FATHMM (Functional Analysis Through Hidden Markov Models) is a server that predicts the functional effects of missense variants. It employs the powerful probabilistic models, 'Hidden Markov Models', that, unlike the position invariant BLAST scoring matrices, can obtain position-specific information within a multiple sequence alignment of homologous sequences (Shihab et al., 2013).

Predicting mutation effects via structure-based methods generally employs machine learning or potential energy-based approaches. Statistical scoring functions along with machine learning approaches have also been used by multi-agent prediction. Particularly, mCSM (mutation Cutoff Scanning Matrix) predicts the impact of SNPs on protein stability by employing graph-based structural signatures (Pires et al., 2014a). SDM is a structure-based machine tool that calculates the difference in stability scores between the wild type and mutant protein structures using environment-specific substitution tables (Pandurangan et al., 2017). DUET is a consensus server that employs Support Vector Machines to optimize the prediction results of mCSM and SDM approaches. It was shown that DUET is an improved prediction accuracy in comparison with both mCSM and SDM (Pires et al., 2014b). DynaMut is also a consensus server that implements Normal Mode Analysis (NMA) and graph-based signatures to predict the change in stability of a protein upon mutation. NMA is implemented through Bio3D and ENCoM, which provide valuable information about protein motions (Rodrigues et al., 2018). CUPSAT (Cologne University Protein Stability Analysis Tool) is a tool that predicts the difference in free energy between wild-type and mutant proteins by using structural environment-specific atom potentials and torsion angle potentials. It was found to give > 80% prediction accuracy for most of the several validation tests carried out (Parthiban et al., 2006). ENCoM is an NMA-based tool that predicts the effects of mutations on protein stability and function. It accounts for the nature of amino acids which has been shown to result in improved conformational space sampling and enables a coarse-grained NMA method to predict the effect of SNPs on protein dynamics and stability due to changes in vibrational entropy (Frappier et al., 2015). FoldX is a method based on empirical effective energy functions (EEEF). It is used to accurately estimate the free energy changes on the stability of a protein, upon mutation. It calculates the free energy of a macromolecule based on its high-resolution 3D structure (Schymkowitz et al., 2005).

SNPeffect is a web server that gives information on the aggregation, amyloid propensity, chaperone binding property and protein stability of a protein upon mutation. It makes use of the tools- TANGO, WALTZ, LIMBO and FoldX (Reumers, 2004). TANGO is a statistical mechanics algorithm used to predict protein aggregation. It can also be employed to predict pathogenic as well as protective mutations of various diseases (Fernandez-Escamilla et al., 2004). LIMBO is an algorithm that uses peptide binding experiments to obtain sequence information and homology modelling for structure parameters. LIMBO was shown to have improved performance compared to both the single approaches (Van Durme et al., 2009). WALTZ is a tool that identifies amyloid-forming sequences in a protein using a position-specific scoring matrix. It is developed by extrapolating data from amyloid hexapeptide sequence space (Morris et al., 2013).

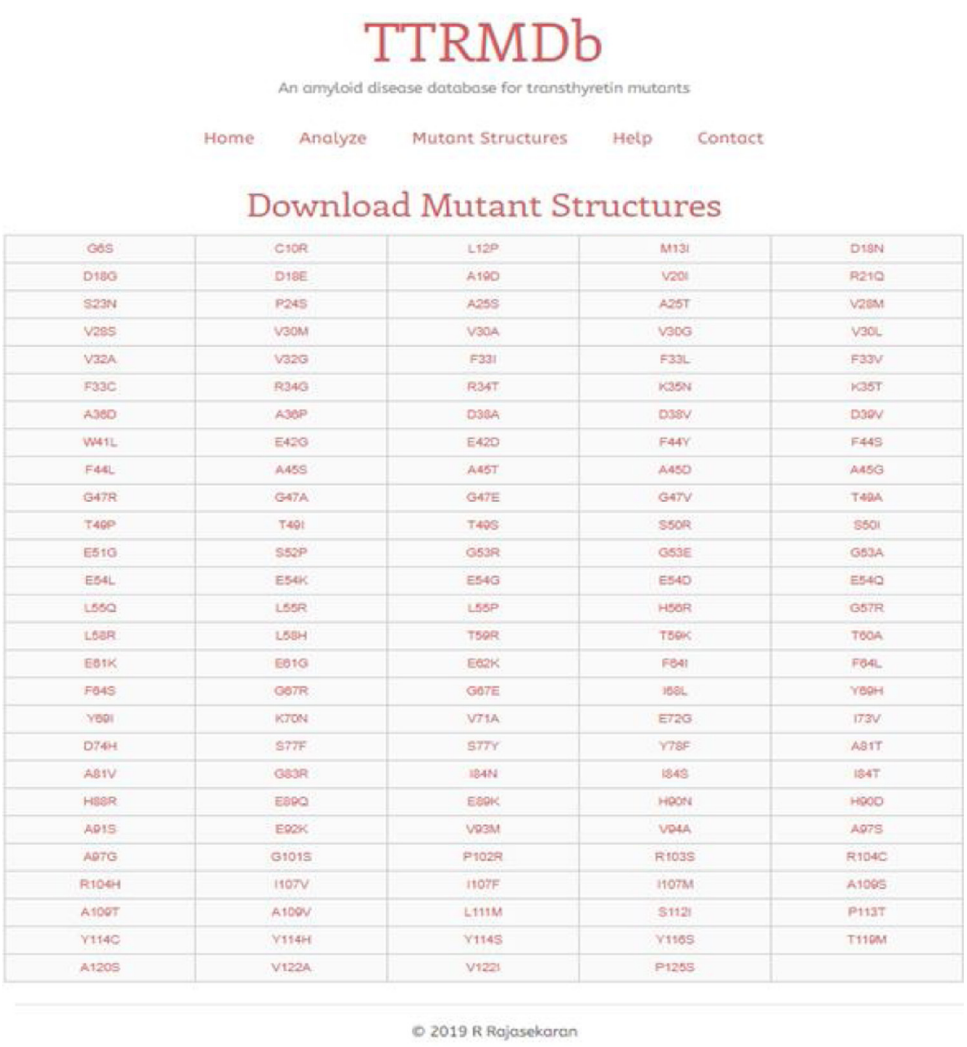

Fig. 2. Mutant Structures page of the TTRM database.

2.4. TTRMDB score

We deployed the predicted results from the tools used in our study in order to rank the mutations considering the stabilizing or destabilizing factor, using neural network prediction. The predictions were performed using MATLAB and the outputs were plotted from the error histogram and confusion matrix.

2.5. Creation of database

TTRM database was created using HTML5, CSS3 and Python version 3.7. Accordingly, the Flask microframework of Python was used to build the webserver. Subsequently, the mutant structures available for download were generated using the DUET server and the structures were energy minimized using the Yasara program. Further, the images were created in UCSF Chimera 1. 13. 1.

3. Results

3.1. Overview of database

TTRM database provides information on TTR, its mutations, the tools used to predict the impact of mutations and the prediction results for each mutation. Specifically, the database was created to study how changes in the sequence of TTR affect the structural and functional properties of transthyretin.

3.2. Home

TTRM database was created to provide a better understanding of how a mutation in the sequence of TTR gene can affect the structure and function of protein. The 'Home' page provides necessary information about the database, the transthyretin protein and its mutations (Fig.1). It forms the homepage of the web server which can be used to navigate to all the other pages. It also displays the structure of transthyretin in various representations.

3.3. Mutant structures

Distinctly, the mutant structures were obtained from DynaMut server which uses DUET to generate mutant structures. DUET utilizes the ANDANTE program to create mutant structures (Pires et al., 2014b). Further, the mutant structures were energy minimized, using YASARA program with AMBER forcefield. Each mutant can be selected to download the PDB file (Fig.2).

3.4. Analyse

Overall, we have reported 134 mutations in a tabular form, which can be selected to obtain the results of all the computational tools used to predict the effect of that mutation on the protein (Fig.3). Moreover, the prediction results of each mutation are provided on a separate page categorized based on whether they provide sequence, structure or

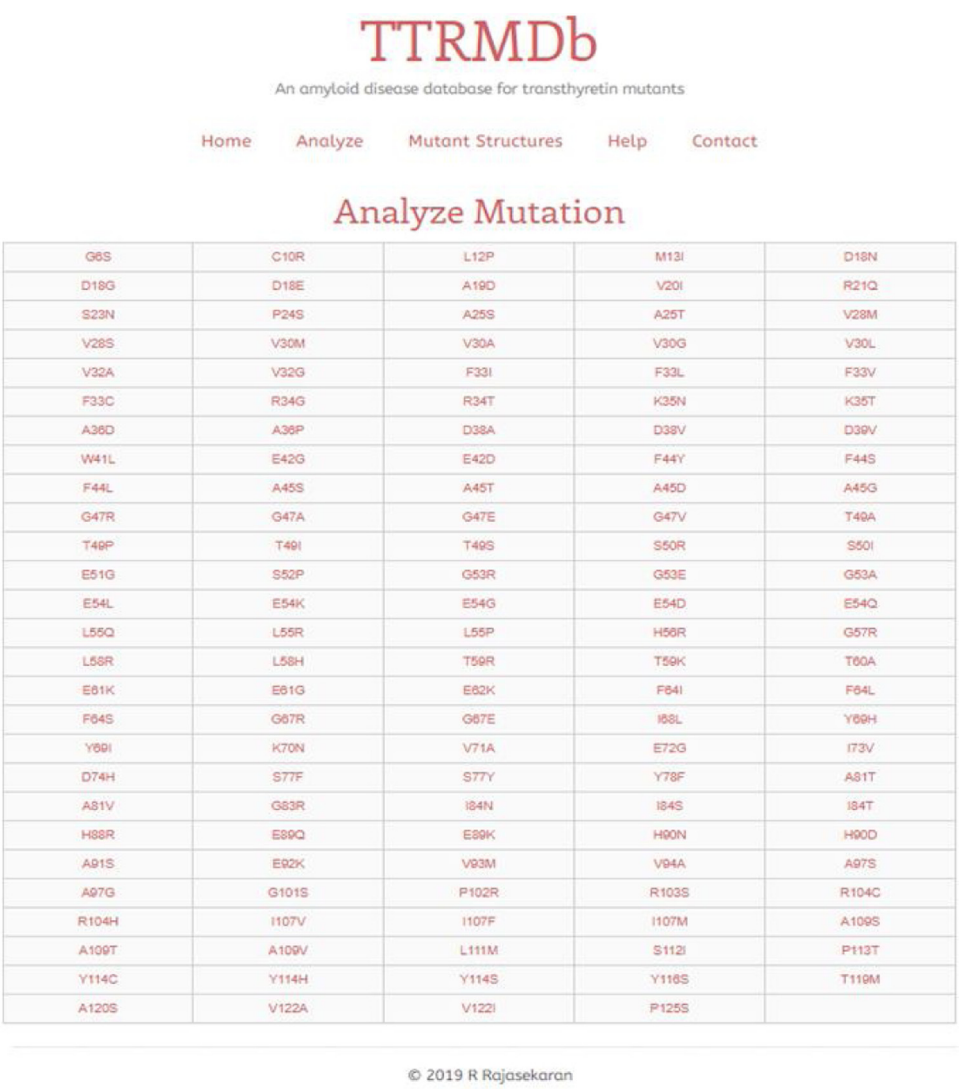

Fig. 3. Analyze page of the TTRM database.

aggregation related data (Fig.4). Further information on reported articles, the strategy for therapy and PDB IDs for mutant structure are also provided, if available.

3.5. Sequence studies

About 6 tools were used to study whether the mutations were deleterious or neutral and about 3 tools were used to study whether the mutation destabilizes the protein. Of the 134 mutations studied, Provean found 83 to be deleterious and 51 to be neutral; PredictSNP found 83 mutations to be deleterious and 51 neutral; PhD-SNP found 80 to be deleterious and 54 to be neutral. About 92 mutations were found to be deleterious by PolyPhen-2 and the rest neutral; SIFT found 93 mutations to be deleterious and 41 to be neutral. FATHMM found every mutation to be damaging. Notably, the number of mutations found to destabilize the protein were 109 by iMutant 3.0, 115 by STRUM and 87 by iStable. Almost 72 mutations were found to be destabilizing by all tools (Fig.5). Based on sequence studies, the mutations that were found to be destabilizing and deleterious by all the tools used were C10R, L12P, D18N, D18G, A25T, V28S, V30A, V32A, V32G, F33I, F33L, G47V, L55Q, L55R, L55P, L58R, L58H, F64I, Y69H, Y69I, K70N, D74H, E92K, V93M, V94A, Y114H, Y114S and Y116S.

3.6. Structure studies

About 7 tools were used to study whether the mutation destabilizes the protein. Significantly, the number of mutations found to destabilize the protein were 125 by mCSM, 99 by SDM, 116 by DUET, 91 by DynaMut, 91 by CUPSAT and 128 by ENCoM. FoldX predicted the stability of the protein to reduce in varying degrees for 91 mutations (Fig.6). Distinctly, the mutations that were found to be destabilizing by all tools used in structure studies were L12P, D18G, A25S, A25T, V28S, V30M, V30A, V30G, V32A, V32G, R34T, W41L, F44S, F44L, A45S, T49S, L55Q, L55R, L55P, L58R, L58H, E61G, F64I, F64S, G67E, Y69H, V71A, I73V, A81V, I84N, I84S, I84T, E89Q, H90D, V94A, A97S, A97G, G101S, R104C, I107V, A109S, V122I and P125S.

Since the changes in sequence affect the structure of protein, which in turn affects the function of protein, the mutant structures provided in the database can also be used to study other functional impacts of the mutation like its interaction with its environment or other proteins. Furthermore, the mutant structures can also be used to screen therapeutic strategies for that mutation.

3.7. Aggregation studies

SNP effect tool was used to study the effect of mutations on the aggregation properties of protein. Consequently, the aggregation

TTRMDb

An amyloid disease database for transthyretin mutants

Home Analyze Mutant Structures Help Contact

Sequence

Analysis Tool	Result
iMutant 3.0	Destabilizing
STRUM	Stabilizing
I-Stable	Destabilizing
Provean	Deleterious
PredictSNP	Neutral
PhD-SNP	Deleterious
PolyPhen-2	Neutral
SIFT	Neutral
FATHMM	Damaging

Structure

Analysis Tool	Result
mCSM	Destabilizing
SDM	Destabilizing
DUET	Destabilizing
DynaMut	Destabilizing
CUPSAT	Stabilizing
ENCoM	Destabilizing
FoldX (Protein Stability)	No effect

Aggregation

Analysis Tool	Result
TANGO (Aggregation Tendency)	No effect
WALTZ (Amyloid Propensity)	No effect
LIMBO (Chaperone Binding Tendency)	No effect

Reported Articles

- Altland K, Benson MD, Costello CE, Fortini A, Hazenberg BPC, Hund E, et al. Genetic microheterogeneity of human transthyretin detected by IEF. ELECTROPHORESIS. 2007;28: 2053–2064. doi:10.1002/elps.200600840
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- Benson MD, Wallace MR, Tejada E, Baumann H, and Page B. (1987). Description of a new American kindred with late onset cardiomyopathy. Arthritis Rheum. 30:195-200.
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Studies on Therapeutic Strategy*

- Tojo K, Sekijima Y, Kelly JW, Ikeda S. Diflunisal stabilizes familial amyloid polyneuropathy-associated transthyretin variant tetramers in serum against dissociation required for amyloidogenesis. Neuroscience Research. 2006;56: 441–449. doi:10.1016/j.neures.2006.08.014
- Merlini G, Planté-Bordeneuve V, Judge DP, Schmidt H, Obici L, Perlini S, et al. Effects of Tafamidis on Transthyretin Stabilization and Clinical Outcomes in Patients with Non-Val30Met Transthyretin Amyloidosis. J Cardiovasc Transl Res. 2013;6: 1011–1020. doi:10.1007/s12265-013-9512-x
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- Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang C-C, Ueda M, Kristen AV, et al. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018;379: 11–21. doi:10.1056/NEJMoa1716153

*Includes studies on model organisms and commercially unavailable drugs

Available PDB Structures

- 3DO4

Fig. 4. Prediction results of computational tools and further information for the mutation T60A.

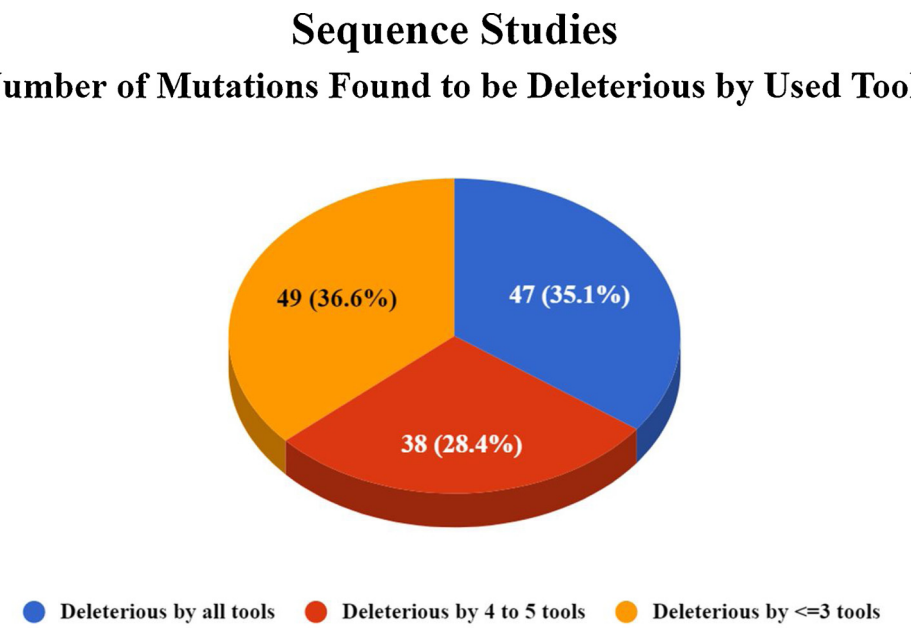

Fig. 5. Number of mutations found to be deleterious by the different computational tools used in sequence studies.

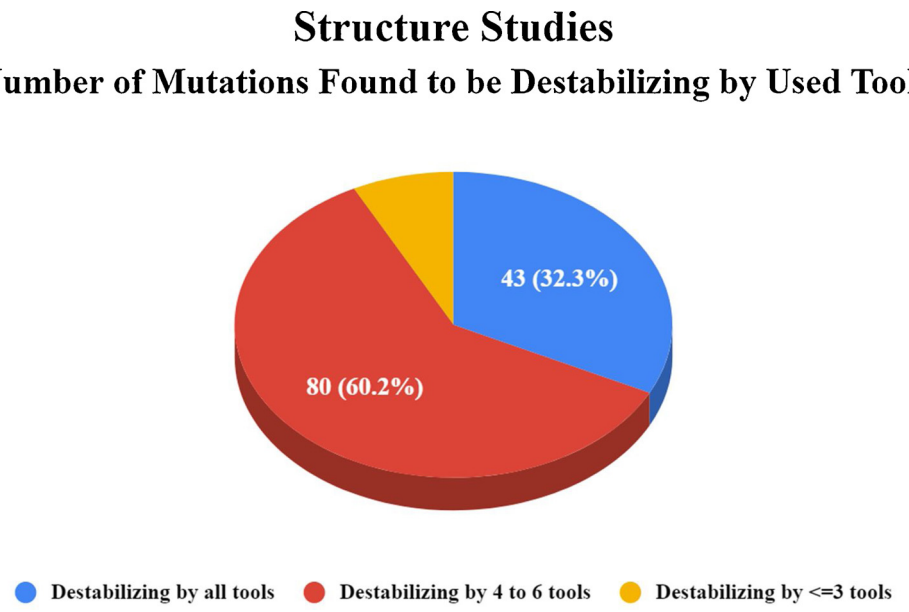

Fig. 6. Number of mutations found to be destabilizing by the different computational tools used in structure studies.

tendency predicted by TANGO was found to be increasing for the mutations- M13I, E92K, R104C, A109V and P113T; and decreasing for the mutations I107M, A109S, L111M and V122A. Further, the amyloid propensity predicted by WALTZ was found to be in the region around 91 – 97. LIMBO predicted the chaperone binding tendency to increase for the mutations- M13I, D18N, D18G, D18E, V20I, V32A, V32G, F33V, F33C, R34G, K35N and A36D; and decrease for mutations- A19D, V30L, F33I, F33L, R34T, A36P, W41L, G57R, G67R and G67E.

Protein aggregation results from the (partial) unfolding of the protein, which exposes short stretches that mediate beta aggregation. However, the intrinsic aggregation property and stability of the protein are both considered when analyzing the effect of mutations on the protein. When the stability of the protein changes, the aggregation-prone regions may get exposed. Therefore, the presence of TANGO, WALTZ or LIMBO regions does not necessarily imply that the protein readily forms aggregates, amyloid or exposes chaperone binding sites, respectively. Conversely, the regions which are normally buried in the core of the protein may become exposed due to factors like destabilizing mutations which can become more prominent (Reumers, 2004).

3.8. Help

This tab provides information about all the computational tools used to predict the effects of SNPs on the TTR gene used in this study (Fig.7).

3.9. Discussion

Thus, the results of bioinformatic tools can play an essential role in analyzing the variants and help in their prioritization for experimental characterization. According to sequence studies, about 72 mutations were found to be destabilizing by all tools used. Nearly, 42 mutations

Fig. 7. Help page of the TTRM database.

were found to be destabilizing by all tools used in structure studies. About 28 mutations were found to be destabilizing by all tools used in combined, the sequence and structure studies; and 17 mutations were found to be destabilizing by all tools, except one (Fig.8).

Significantly, the mutations that were found to be destabilizing by all tools used were L12P, D18G, A25T, V28S, V30M, V30A, V30G, V32A, V32G, A45S, L55Q, L55R, L55P, L58R, L58H, F64I, Y69H, V71A, I73V, I84N, I84S, I84T, E89Q, V94A, G101S, R104C, I107V, A109S and V122A. Also, the mutations found to be destabilizing and deleterious by both sequence and structure tools are L12P, G18G, A25T, V28S, V30A, V32A, V32G, L55Q, L55R, L55P, L58R, L58H, Y64I, Y69H and V94A (Table 1).

In general, the difference between the predictions of the sequence and structure studies can be attributed to the difference in factors considered by each set of tools while predicting the change in the stability of the protein upon mutations. Sequence studies consider factors like hydrophobicity, charge, polarity, bulkiness, etc. and compare these properties of the wild-type amino acid with the mutated residue. Some tools also make use of evolutionary information of conserved sequences, whereas structure-based tools take into account the structural environment of the mutation (Capriotti and Altman, 2011).

Experimental studies have been conducted on many of these mutations to study their penetrance, clinical manifestations and diagnostic purposes. Extensively, the SNP V30 M, has been studied, not only in the Portuguese population but in other populations as well (Arvidsson et al., 2015; Solovyov et al., 2011). A study was also conducted to explore siRNA based therapeutic approaches to V30 M mutated animal models (Gonçalves et al., 2016). For the mutation D18G, a study proposed selected small-molecule stabilizers that can act as effective therapeutic strategies (Hammarström et al., 2003). Also, the effects of the drugs such as Intorsen, Patisiran and Tafamidis have also been studied in various mutations (Coelho et al., 2016; Madhivanan et al.,

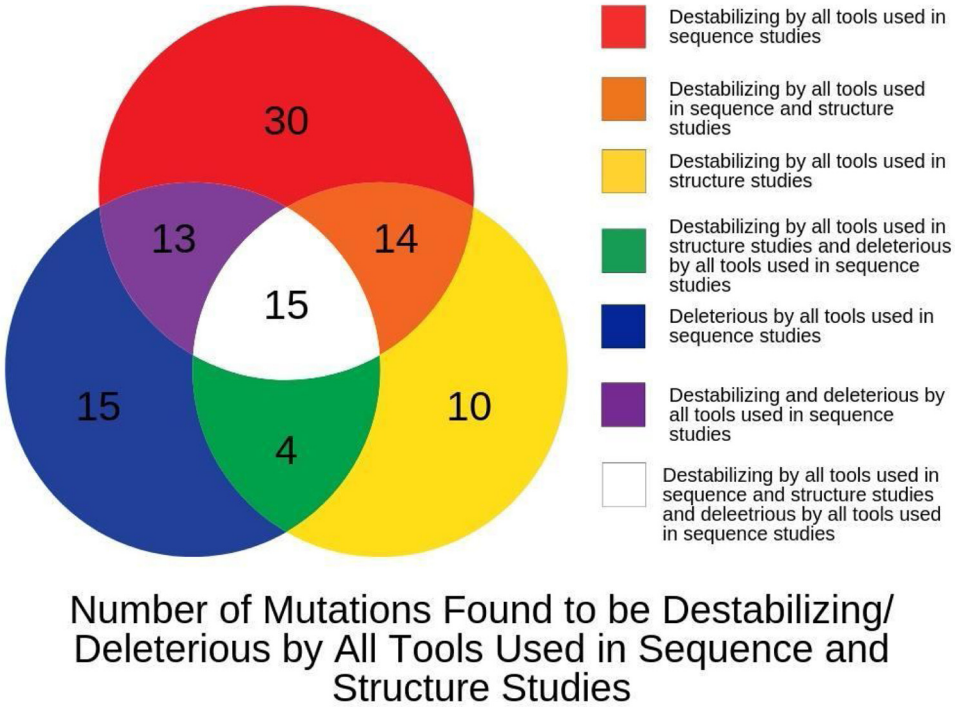

Fig. 8. Venn diagram showing the number of mutations that were found to be destabilizing or deleterious by all tools used in sequence and structure studies.

Table 1
Summary of results obtained from the Venn diagram.

Prediction Effect	Number of Mutations	Mutations
Deleterious by all tools used in sequence studies Destabilizing by all tools used in sequence studies Destabilizing by all tools used in structure studies	15	L12P, D18G, A25T, V28S, V30A, V32A, V32G, L55P, L55Q, L55R, L58H, L58R, F64I, Y69H, V94A
Deleterious by all tools used in sequence studies Destabilizing by all tools used in sequence studies	13	C10R, D18N, F33I, F33L, G47V, Y69I, K70N, D74H, E92K, V93M, Y114H, Y114S, Y116S
Destabilizing by all tools used in sequence studies Destabilizing by all tools used in structure studies	14	V30G, V30M, A45S, V71A, I73V, I84N, I84S, I84T, E89Q, G101S, R104C, I107V, A109S, V122A
Deleterious by all tools used in sequence studies Destabilizing by all tools used in structure studies	4	A25S, W41L, G67E, A97G
Destabilizing by all tools used in sequence studies	30	G6S, M13I, V20I, V28M, V30L, F33C, F33V, A36P, D38A, E42D, A45G, A45T, E54D, E54G, E54K, E54Q, E62K, F64L, E72G, A81T, E89K, A91S, P102R, R104H, I107F, A109T, L111M, P113T, T119M, A120S
Deleterious by all tools used in sequence studies	15	A19D, P24S, G47A, G47R, T49A, T49P, G53A, G53E, G53R, E54L, G67R, S77F, S77Y, G83R, H88R
Destabilizing by all tools used in structure studies	10	R34T, F44L, F44S, T49S, E61G, F64S, A81V, H90D, A97S, P125S

2018; Johnson et al., 2012b; Monteiro et al., 2019). Among the mutations that were found to be destabilizing by all tools used, the experimental studies have been conducted on L12P, D18G, V30M, V30A, V32A, L55P, L58H, Y69H, I84S, A25T and G101S to study various aspects like their characterization, pathogenic mechanisms or effects or strategies for therapy (Batista et al., 2013; Keetch et al., 2005; Zhang et al., 2013; Mariani et al., 2015; Lashuel et al., 1999; Takeuchi et al., 2006; Zanotti et al., 2013; Wakita et al., 2018; Ziskin et al., 2015; Sekijima et al., 2003; Pica et al., 2005; Cazzato et al., 2019). On the other hand, the structural changes due to mutations such as L55P, V30M, T119M, V122I, T60A, A25T in TTR results in the form of toxic aggregates (Schonhoft et al., 2017). Therefore, the reduction of TTR amyloidoses suggested that the development of structure-based, RNA interference and kinetic stabilization drug design is found to be an effective therapy in TTR (Coelho et al., 2016; Madhivanan et al., 2018; Johnson et al., 2012b; Monteiro et al., 2019). Herbal compounds such as Genistein also prevents TTR aggregation (Green et al., 2005). Currently, the use of peptide probes are vital in the diagnostics of TTR amyloidoses, as they bind to the misfolded oligomeric TTR (Schonhoft et al., 2017). Moreover, the reports from the David's Lab have designed the small peptide inhibitors that binds to the F and H beta sheets and inhibits the protein aggregation. These strands plays an vital role in the

formation of TTR structure, as they are arranged in the steric zippers that supports the amyloid structure (Saelices et al., 2018; Saelices et al., 2015). Whereas, the other mutations identified have extensively not been studied. Out of the remaining mutations, the ones that were also found to be pathogenic by all tools were V28S (Okada et al., 2017), V32G (Plante-Bordeneuve et al., 2003), L55Q (Yazaki et al., 2002), L55R (Altland et al., 1999), L58R (Saeki et al., 1991), F64I (Tarquini et al., 2007) and V94A (Kristen et al., 2007). Therefore, with the results of our study, we could infer that these mutations could be prioritized for future studies.

3.10. TTRMDB score

In order to quantify the results from our study, we used the neural network prediction, using MATLAB to further determine the effect of mutation in TTR protein. The results obtained from the prediction were tabulated in Supp. Table 1. From the Table 1, we could infer that the mutations namely., F64S, F44S/L, V30G/A/M, V71A, I84S, I73V, A25S, L12P, K70N, R34/G/T, V94A, V32G/A and E61G exhibited greater destabilizing effect as compared to that of all mutations. Thus, from the overall results, we could infer that the mutations positioned at 28, 30, 32, 34, 44, 55, 64, 94 bearing multiple mutations are to be provided with utmost importance in future studies (Supp. Fig.1). Moreover, the results from our study also suggested that the mutations reported were positioned in the hotspot regions of TTR protein. Therefore, our study could help the experimental biologists and clinicians to better understand these mutations, while performing the experimental characterization, thus providing a prominent importance in understanding the structural stability of TTR protein relating towards the disease pathogenicity.

4. Conclusion

Due to advances in sequencing technology, SNPs are being identified at a high rate, but because carrying out wet-lab studies for each of these mutations is a cumbersome task, various bioinformatics tools have also been developed to predict the effect of these mutations on the affected protein. However, the accuracy of prediction varies for each tool and thus, many tools are used in combination to increase the accuracy of the prediction. In this study, the structural and functional impact of SNPs on TTR were computed using various tools. Further, we identified and ranked various mutations using the predicted TTRMDB score that could be used for further characterization in upcoming research studies. TTRM database gives information on TTR and the results of the predicted effects of mutations using various computational tools and also provides further information available for each mutant, thus enabling the researchers in finding ease to carry out further calculations without any prior optimizations on TTR proteins, both computationally and experimentally.

CRediT authorship contribution statement

E. Srinivasan: Methodology, Investigation, Software. **Nandhini Natarajan:** Data curation, Writing - original draft. **R. Rajasekaran:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.compbiolchem.2020.107290.

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