scientific reports

Check for updates

OPEN

Exploring *TTN* variants as genetic insights into cardiomyopathy pathogenesis and potential emerging clues to molecular mechanisms in cardiomyopathies

Amir Ghaffari Jolfayi[1], Erfan Kohansal[1], Serwa Ghasemi[1], Niloofar Naderi[2], Mahshid Hesami[1], MohammadHossein MozafaryBazargany[1], Maryam Hosseini Moghadam[2], Amir Farjam Fazelifar[1], Majid Maleki[2] & Samira Kalayinia[2]✉

The giant protein titin (TTN) is a sarcomeric protein that forms the myofibrillar backbone for the components of the contractile machinery which plays a crucial role in muscle disorders and cardiomyopathies. Diagnosing *TTN* pathogenic variants has important implications for patient management and genetic counseling. Genetic testing for *TTN* variants can help identify individuals at risk for developing cardiomyopathies, allowing for early intervention and personalized treatment strategies. Furthermore, identifying *TTN* variants can inform prognosis and guide therapeutic decisions. Deciphering the intricate genotype–phenotype correlations between *TTN* variants and their pathologic traits in cardiomyopathies is imperative for gene-based diagnosis, risk assessment, and personalized clinical management. With the increasing use of next-generation sequencing (NGS), a high number of variants in the *TTN* gene have been detected in patients with cardiomyopathies. However, not all *TTN* variants detected in cardiomyopathy cohorts can be assumed to be disease-causing. The interpretation of *TTN* variants remains challenging due to high background population variation. This narrative review aimed to comprehensively summarize current evidence on *TTN* variants identified in published cardiomyopathy studies and determine which specific variants are likely pathogenic contributors to cardiomyopathy development.

Keywords *TTN*, Titin, Cardiomyopathy, Variant, Genetic

Cardiomyopathies refer to a diverse range of complex diseases affecting heart muscle, which can lead to abnormalities in the structure and function of the myocardium. These abnormalities occur in the absence of other conditions like coronary artery disease, hypertension, or valvular heart disease[1,2]. The American Heart Association (AHA) has categorized cardiomyopathies into genetic, acquired or mixed forms like virally induced post-myocarditis cardiomyopathy. The European Society of Cardiology Organization (ESCO) proposed an alternative classification system dividing cardiomyopathies into two subgroups—familial/genetic cardiomyopathies and non-familial/non-genetic cardiomyopathies[3,4]. Based on morpho-functional phenotypes[5], cardiomyopathies are classified into hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular (ARVC) which each one has their specific features[6]. The hallmark features of cardiomyopathies are genetic and clinical heterogeneity, variable expressivity, and incomplete penetrance. Numerous genes and mutations have been identified that can cause the various types of cardiomyopathies. The majority of known mutations are linked to DCM and HCM, while fewer are associated with RCM and ARVC. Cardiomyopathies demonstrate considerable genetic heterogeneity—mutations in various different genes can lead to cardiomyopathy. There is also phenotypic heterogeneity, where mutations in the same gene can result in diverse types and degrees of severity of cardiomyopathy[7]. Cardiomyopathy following myocarditis is probably the result of an interaction interplay between the viral infection and a person's inherent

[1]Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran. [2]Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran. ✉email: samira.kalayi@yahoo.com

susceptibility. Certain subgroups induced by viral infection may be influenced, at least partially, by genetic factors, suggesting that the elimination of the virus and the immune response could be genetically predetermined[8].

Among the genes involved in cardiomyopathies, the *TTN* gene plays a central role which is attributable to its key structural properties and mechanical function within the striated muscle sarcomeres[9]. *TTN* is a major human muscle disease-related gene that encodes the largest human protein, Titin, which is a fundamental structural and functional unit of striated muscles[10,11]. Due to the size and complexity of this gene, its sequencing was difficult to study the mutations and variants. The initial family studies were performed with primer pairs searching on the exons contained in a 280 kb genomics 2q31 region. This indeed led to the identification of titin mutations causing DCM by Gramlich et al.[12] Subsequently, the introduction of NGS has allowed for the exploration of larger cohorts and various clinical entities.

Following the development of next-generation sequencing (NGS), as a potent tool for sequencing large and complex genes, *TTN* gene sequencing which was previously impossible to conduct a comprehensive analysis, has been performed. This improvement in study tools has led to identifying more than 60,000 *TTN* missense variants reported in the 1000 Genomes Project[13,14]. Determining which TTN variants actually cause disease versus which are benign is challenging. The goal of this review is to discuss the current state of understanding regarding the challenges in establishing clear associations between particular TTN mutations and specific cardiomyopathy subtypes in a clinical context.

Method and materials

Systematic search, selection criteria and data collection

The study systematically collected *TTN* variants associated with cardiomyopathy from the Human Gene Mutation Database (HGMD) and public archive of interpretations of clinically relevant variants (ClinVar). In prioritizing data reliability, only variants with documented reference articles were included, while those lacking reference articles were excluded. The search strategy, extending until February 2023, employed key parameters such as Position on Chromosome, Human Genome Variation Society (HGVS) DNA, HGVS Protein, exon or intron number, and dbSNP identifiers.

Variant annotation and pathogenicity assessment

The annotation of *TTN* variants involved a comprehensive pathogenicity assessment using multiple tools. This included the application of the American College of Medical Genetics and Genomics (ACMG) guidelines, consultation of ClinVar for variant interpretation, insights from Mutation Taster regarding potential pathogenicity, the use of the Combined Annotation Dependent Depletion (CADD) scoring system for deleteriousness prediction, and evaluation through Genomic Evolutionary Rate Profiling (GERP) to assess evolutionary conservation which are explain more in the following.

We determineded the ACMG score for each variant using franklin, an online database (https://franklin.genoox.com/clinical-db). After adding the name in this website, varints ACMG score anongside with other features are provided.

ACMG score

The American College of Medical Genetics and Genomics (ACMG) previously established guidelines for interpreting sequence variants. With the rapid advancements in sequencing technology over the past decade, this report suggests the adoption of standardized terms such as "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" to characterize variants found in genes associated with Mendelian disorders. Additionally, the recommendation outlines a systematic approach for classifying variants into these categories, relying on various types of evidence, including population data (Population, disease-specific, and sequence databases), computational data (using silico tools for missense prediction, splice site prediction and nucleotide conservation prediction), functional data, and segregation data[15,16].

In this classification a variant is considered pathogenic if it meets the requirement of having a very strong criterion (PVS1) along with at least one strong criterion (PS1-PS4), or alternatively, two or more moderate criteria (PM1-PM6), or a combination of one moderate criterion and one supporting criterion (PP1-PP5). Another condition is that a variant can be classified as pathogenic if it satisfies the condition of having at least two strong criteria (PS1-PS4). Additionally, a variant can be considered pathogenic if it meets the criteria of having one strong criterion (PS1-PS4) and either three moderate criteria (PM1-PM6), two moderate criteria and at least two supporting criteria (PP1-PP5), or one moderate criterion and at least four supporting criteria (PP1-PP5)[16].

A variant is considered likely pathogenic if it satisfies the condition of having one very strong criterion (PVS1) in combination with one moderate criterion (PM1-PM6). Alternatively, a likely pathogenic variant may exhibit one strong criterion (PS1-PS4) along with one to two moderate criteria (PM1-PM6). Another criterion designates a variant as likely pathogenic if it possesses one strong criterion (PS1-PS4) and at least two supporting criteria (PP1-PP5). Furthermore, likely pathogenic variants may be identified if they meet the requirement of having three or more moderate criteria (PM1-PM6). Additionally, a variant is classified as likely pathogenic if it has two moderate criteria (PM1-PM6) and at least two supporting criteria (PP1-PP5), or if it exhibits one moderate criterion (PM1-PM6) along with at least four supporting criteria (PP1-PP5)[16]. More information is provided in *"Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology"*[16].

The ACMG score for each variant is determined using Franklin, an online database available at https://franklin.genoox.com/clinical-db. Upon entering the variant's name on this website, the ACMG score, along with other relevant features, is provided.

CADD score

CADD, or Combined Annotation Dependent Depletion, serves as a tool for evaluating the deleteriousness of various genetic variants, including single nucleotide changes, multi-nucleotide substitutions, and insertion/deletion variants within the human genome. In contrast to many other annotation tools that often rely on a singular type of information or have limited applicability, CADD offers a versatile metric that objectively combines diverse annotations. The framework integrates multiple annotations into a unified metric by comparing variants that have undergone natural selection with simulated mutations. It incorporates information from more than 60 genomic features to assess single nucleotide variants and short insertions and deletions across the reference assembly. The C-scores generated by CADD demonstrate robust correlations with allelic diversity, pathogenicity of coding and non-coding variants, and experimentally measured regulatory effects. Notably, C-scores of variants associated with complex traits in genome-wide association studies (GWAS) are significantly higher than matched controls, showing correlation with study sample size, indicative of improved accuracy in larger GWAS. CADD employs a machine learning model that distinguishes between simulated de novo variants, potentially encompassing neutral or harmful alleles, and variants persisting in human populations since the split from chimpanzees.

CADD's capability to quantitatively prioritize functional, deleterious, and disease-causing variants spans a wide range of functional categories, effect sizes, and genetic architectures. This tool enhances the scoring of coding variants through features derived from the ESM-1v protein language model and improves the scoring of regulatory variants using features from a convolutional neural network trained on open chromatin regions. For more information CADD has been detailed in four publications[17–20].

MutationTaster

MutationTaster is a web-based application designed to assess the disease-causing potential of DNA sequence variants. It employs in silico tests to estimate the impact of a variant on the gene product or protein, conducting assessments at both the protein and DNA levels. Unlike tools limited to single amino acid substitutions, MutationTaster can handle a variety of variants, including synonymous and intronic ones[21]. The software, written in Perl programming language and utilizes integrated databases (Ensembl, UniProt, ClinVar, ExAC, 1000 Genomes Project, phyloP and phastCons) to filter out known harmless polymorphisms. Various tests, such as amino acid substitution, conservation, domain functionality, splicing effects, and regulatory element abrogation, are performed on the remaining single-nucleotide polymorphisms (SNPs). The results are evaluated by a Naive Bayes classifier, and the output indicates whether the alteration is known or predicted to be harmless or disease-causing, providing detailed information about the mutation. While the tool demonstrates a raw accuracy of approximately 90%, considering knowledge about common polymorphisms and known disease mutations significantly improves the rate of correct classifications. However, it is important to note that predictions of clinical effects suffer from a lack of specificity, a common constraint across various prediction methods[22,23].

GERP

Comparative genomic approaches have historically identified mutation sites under purifying selection by examining conserved sequences across distantly related species. Additionally, the performance of such approaches may be limited for short-lived functional elements that don't exhibit sequence conservation across numerous species. Genomic Evolutionary Rate Profiling (GERP) score is associated with the strength of selection (Nes). Results indicate that the GERP score is linked to the intensity of purifying selection. Nevertheless, variations in selection coefficients or turnover of functional elements over time can significantly impact the GERP distribution, leading to unexpected relationships between GERP and Nes[24]. The GERP score is characterized as the decrease in the count of substitutions in the multi-species sequence alignment in comparison to the neutral expectation. $GERP^{++}$ scores span from − 12.3 to 6.17, with elevated scores signifying a greater level of evolutionary constraint.

Data integration

Data integration encompassed the consolidation of relevant information, including Position on Chromosome, HGVS DNA, HGVS Protein, exon or intron number, and dbSNP identifiers. Rigorous quality control measures were then applied to ensure the accuracy and consistency of data extraction and annotation.

Statistical analysis

Descriptive statistics were employed for a comprehensive analysis, summarizing the distribution of TTN variants in terms of positions, types, and associated pathogenicity.

Ethical considerations

Ethical considerations are considered in the study, with a commitment to adhering to Data reliability and responsible data handling. In the present study, it is important to note that no human subjects were involved, as this investigation is a comprehensive review rather than an experimental study. The research focused on analyzing reported variants available on PubMed, and ethical approval or consent from human participants was not applicable.

Results

The molecular structure of titin

The *TTN* gene located on the second human chromosome in the 2q31 area. This gene contains 364 exons, which their translation produces a 4200-kDa protein with ~ 38,000 amino acid residues, the largest polypeptide found in the human body. The Titin giant protein, also known as connectin, is the third most abundant protein found

in striated muscle among the vertebrates, after myosin and actin. The Titin is a flexible filament that is more than 1 μm long and 3–4 nm wide and spans half of the sarcomere as the repeating contractile unit that gives striated muscle characteristic striped appearance[25].

Titin has a complex multidomain structure which is composed of four main structural and functional regions: the N-terminal Z-line acts as an anchor for the sarcomeric Z-disk; the I-band provides elastic properties; the A-band stabilizes the thick filament; and the C-terminal M-line extremity overlaps in an antiparallel orientation with another titin molecule's C-terminus, allowing for modulation of titin expression and turnover via the tyrosine kinase domain[26].

The N-terminus contains immunoglobulin (Ig) domains, fibronectin (FN) domains, and a Z-disk region[27]. The rest of the titin molecule includes an elastic I-band region, a spring-like Pro-Glu-Val-Lys (PEVK) domain, three unique sequences called Novex 1, 2, and 3, cardiac-specific N2B and N2A domains, a thick A-band region, and an M-band region where the C-terminus is embedded.

Extensive alternative splicing in the 364 exons of *TTN* leads to forming various molecular isoforms. Previous studies have shown three main titin isoforms expressed in cardiomyocytes: the adult N2B isoform, the adult N2BA isoform, and the fetal cardiac titin (FCT) isoform. The distinct characteristics of each titin isoform arise from differences in their I-band sequences, while the Z-disk, A-band, and M-line regions are highly conserved across all isoforms[28]. Due to the longer extensible I-band region, the N2BA isoform is more compliant than N2B. The N2BA isoform contains additional spring-like elements in the PEVK and tandem Ig regions, leading to lower passive tension in cardiomyocytes compared to other isoforms[29–31].

Molecular structure of sarcomere and the interaction of Titin with thin and thick filaments is demonstrated in Fig. 1.

Z-disk

The Z-disk region spans 826 amino acids horizontally across the structure and contains seven Ig domains separated by Z-insertion sequences. As the site of numerous structural and functional interactions with myofibrillar and sarcolemmal proteins, the Z-disk is critical for myofibril assembly, stability, and signaling. Z-disks anchor essential proteins like titin-Tcap (telethonin), which enables key Z-disk functions including mechanosensing. Mechanosensing involves recruiting other interacting and signaling partners to the Z-disk in response to mechanical stimuli. Overall, Z-disks play indispensable roles in anchoring titin and enabling vital structural and sensory functions[32–34].

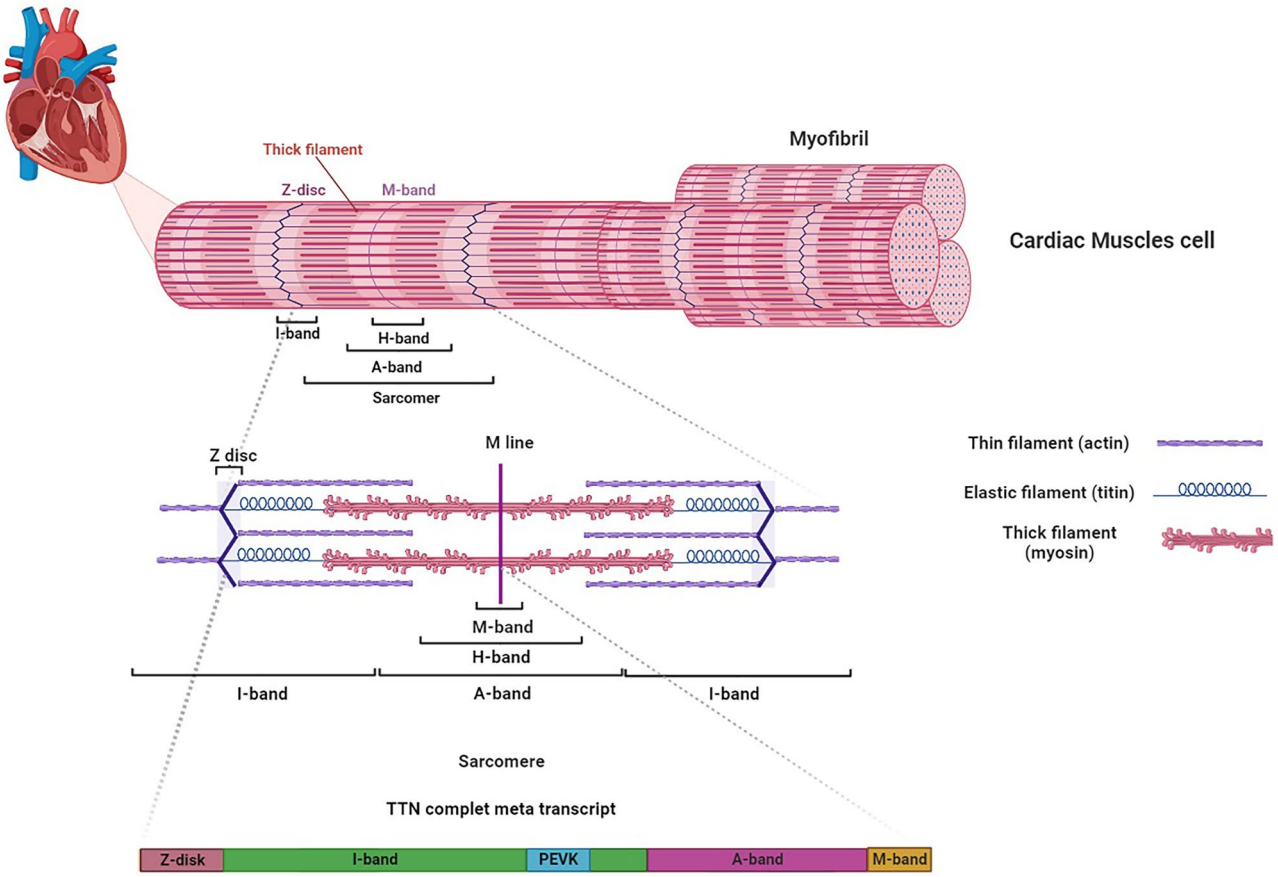

Figure 1. Molecular structure of sarcomere and the interaction of Titin with thin and thick filaments.

The Z-disk interacts with small ankyrin proteins, spectrin, desmin, and obscurin, connecting it to other cytoskeletal structures. Filamin C links the Z-disk to costameres via integrins and sarcoglycans, participating in mechanosensory pathways. Additionally, the Z-disk binds nebulin, which helps stabilize Z-disk anchorage through interactions with actin, desmin, CapZ and myopalladin. α-Actinin binding also enhances Z-disk mechanical stability. Overall, the Z-disk forms critical protein interactions that provide structural support and sensory functions[35–40].

I-band

The I-band region of titin displays extensive alternative splicing, generating diverse isoforms that confer tissue-specific mechanical properties in cardiac and skeletal muscles. Through alternative splicing mechanisms, a spectrum of isoforms emerges, tailoring titin's mechanical functions to meet the needs of different muscle types. The I-band thus acts as a central adapter, converting titin into specialized molecular springs via splicing variability. This interactive segment contains a meta-transcript with principal cardiac and skeletal isoforms. Key components include immunoglobulin folds, the cardiac N2B zone, and the skeletal N2A zone containing nonrepetitive sequences and immunoglobulin domains. The proline-glutamate-valine-lysine (PEVK) domain follows, acting as a spring-like element. Together, the I-band components enable the elasticity of titin[38,41].

The I-band region has distinct proximal and distal segments with specialized roles. The proximal I-band maintains sarcomere integrity, while the medial/distal I-band acts as a bidirectional molecular ruler setting resting length and passive tension[42]. The I-band also functions as a biochemical stress sensor through interactions with αβ-crystallin, a chaperone that stabilizes I-band immunoglobulin domains. Additionally, metabolic enzymes like DRAL, FHL1, and FHL2 associate with I-band sarcomere regions via the Gαq-MAPK pathway[37,43,44]. Indeed, though I-band interactions with the Ca^{+2}-dependent proteases Calpain-1 and Calpain-3, I-band not only contributes to a sarcomeric quality control pathway but also serves as a reservoir for inactive forms of Calpain-3[45,46].

A-band

The A-band spans the sarcomere from M-line to M-line, containing thick filaments of myosin. Within the A-band, titin forms a network that maintains the structural integrity of the thick filaments and regulates their length. The A-band exclusively contains fibronectin type III (FnIII) motifs. Immunoglobulin (Ig) and FnIII motifs are arranged in two super-repeats bisected by Ig folds. Unlike the elastic I-band, the A-band is inextensible, providing myosin binding sites that function as stable anchors. A-band super-repeat domains interact with and position sarcomeric myosin binding protein C (MyBP-C). The A-band also contains binding sites for muscle ring finger proteins MURF1 and MURF2. MURF1 likely facilitates quality control and protein turnover at the sarcomere center, while MURF2 interactions aid formation of mature A-band structures[36,38].

M-band

The M-band integrates structural, signaling, metabolic and protein quality control functions. It contains a putative serine/threonine kinase domain and immunoglobulin cross-hatched rectangle (CII) domains interspersed with M-insertion sequences[47]. While its kinase activity is debated, the M-band kinase domain likely participates in stress sensing through Ca^{2+}-calmodulin-regulated mechanochemical signaling[38,48]. During sarcomerogenesis, myomesin constructs an M-band scaffold linking titin to myosin thick filaments, establishing the myomesin-titin-myosin stability axis[49]. The M-band also senses metabolic stress via ligands DRAL/FHL2 that tether metabolic enzymes, and enables ubiquitin-mediated turnover through interactions with nbr1, p62, MURF1 and MURF2[50]. MURF2 binding facilitates M-band's role in cardiac development[51]. Additionally, the extreme C-terminal TTN/calpain-3/p94 interaction participates in M-band-associated protein turnover[37,52].

The molecular function of titin

Since the discovery of titin, the complexity and diverse functional roles of titin in health and disease continue to emerge. As the third filament system of the sarcomere alongside actin and myosin, titin forms a unique filament network in cardiomyocytes that engages in mechanical and signaling roles[10]. During muscle development, titin likely controls the assembly of actin and myosin contractile proteins, regulating sarcomere size and thick filament structure. In mature muscle, titin contributes to elasticity mechanisms affecting sarcomere resting lengths and tension-related processes[25].

The enormity and intricate three-dimensional structure of titin provides structural support to maintain sarcomere integrity during contraction while generating passive tension during stretching. Additionally, the numerous titin-binding proteins arranged in signaling hotspots allow titin to participate in mechanosensing and signal transduction[26,53]. Thus, titin has multifaceted roles beyond viscoelastic force generation: (a) centering thick filaments for optimal active force; (b) assembling sarcomeres; (c) mechanochemical signaling through binding partners; and (d) potentially enabling length-dependent activation underlying the Frank-Starling law[54].

Comparative analysis of *TTN* variants

In this study we found 611 distant TTN variant which were not benign and they were pathogenic, likely pathogenic or variant of uncertain significance (VUS).

85% of the variants were reported in exon fragments, while 15% were reported in intron fragments. In ACMG classification, 69.6% of the variants were classified as Pathogenic, 21.6% as Likely Pathogenic, and 8.8% as Variants of Uncertain Significance (VUS). Substitution accounted for 57.25% of the variants, deletion for 29.62%, duplication for 7.36%, and insertion for 5.72%.

The majority of variants occurred in the interval from exon 200 to the end of the molecule, with the hotspot regions identified at exon 326 and 358 being the most common points for variations (Fig. 2).

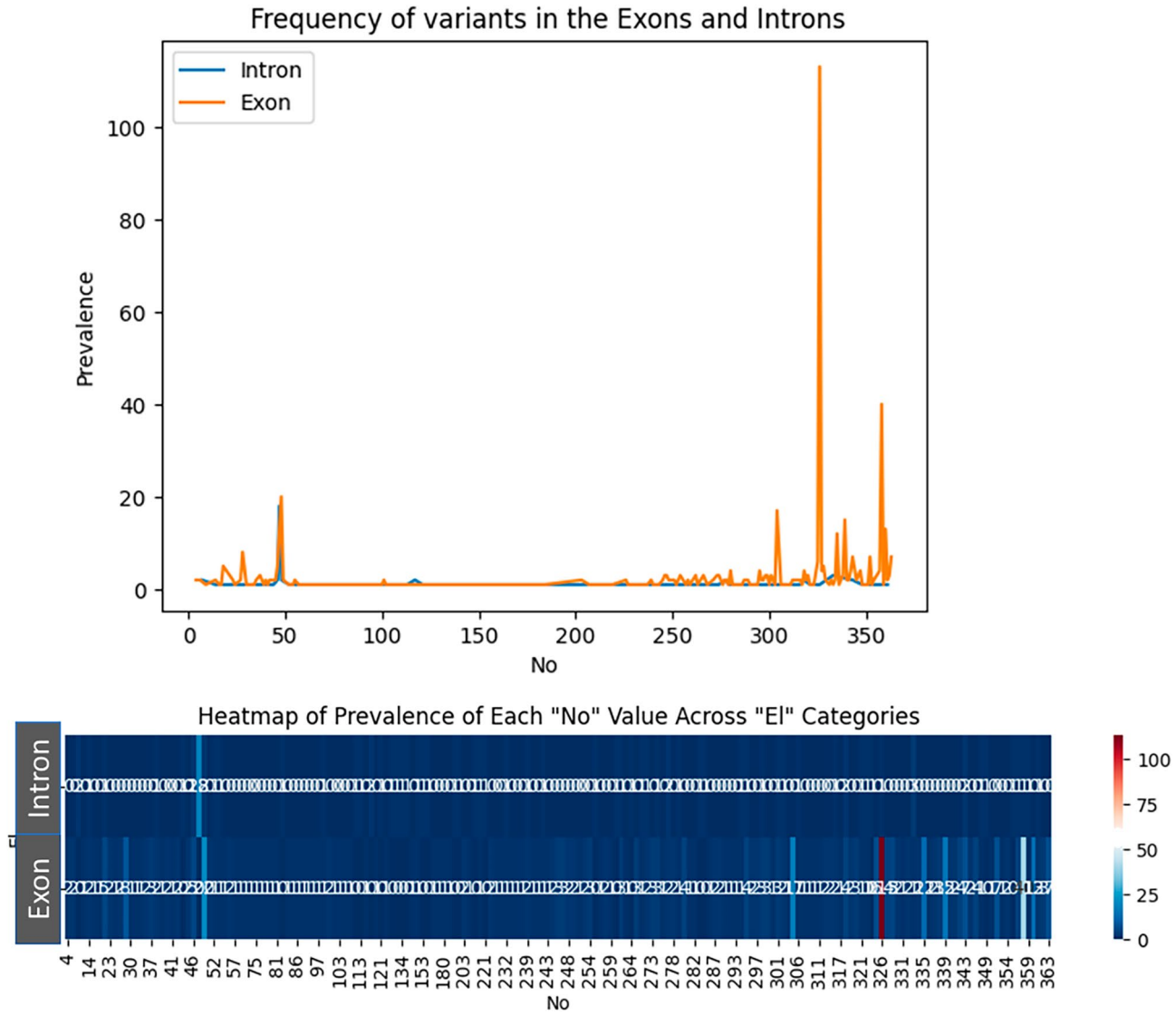

Figure 2. Prevalence of variants in different exons and introns in *TTN*.

Most pathogenic variants are located after the exon 326 to the end of the molecule which has higher CADD number compared to others (Fig. 3A). The Genomic Evolutionary Rate Profiling (GERP) score is used to compare the gene nucleotides among the species in the TTN gene[24]. It is supposable that the nucleotides and exons which are conserved in the evolution, can be considered a vital element for survive and loss of function of these components are associated with death and the prevention of its inheritance. In the comparison of the conservity of the gene nucleotides, it can be concluded that most the variants have a notable GERP score which indicates their conservity (Fig. 3B).

In comparing the average CADD score of various exons, it can be concluded that exons with higher CADD scores are located in the end of the gene and the middle part of the gene, the average CADD score is not notable. The first few exons of the gene have a higher CADD score but in the last exons, the CADD score is increased considerably especially in the last 50 exons. VUS variants have less CADD score and likely pathogenic variants also have lesser scores compared to pathogenic variants (Fig. 3C,D).

In the comparing type of genetic alternation in variants, it can be concluded that the most common alternations are substitution and deletions. Most of the deletions have high score numbers while substitutions have various CADD scores. Most of the insertion and duplications also have notable CADD score because of frameshift events while in the substitutions we can observe some lesser CADD score which is not exists in other types of alternations. As demonstrated, most of the pathogenic variants in the first parts of the gene are deletions but the most pathogenic variants in the last parts of the gene have substitutions (Fig. 3E,F).

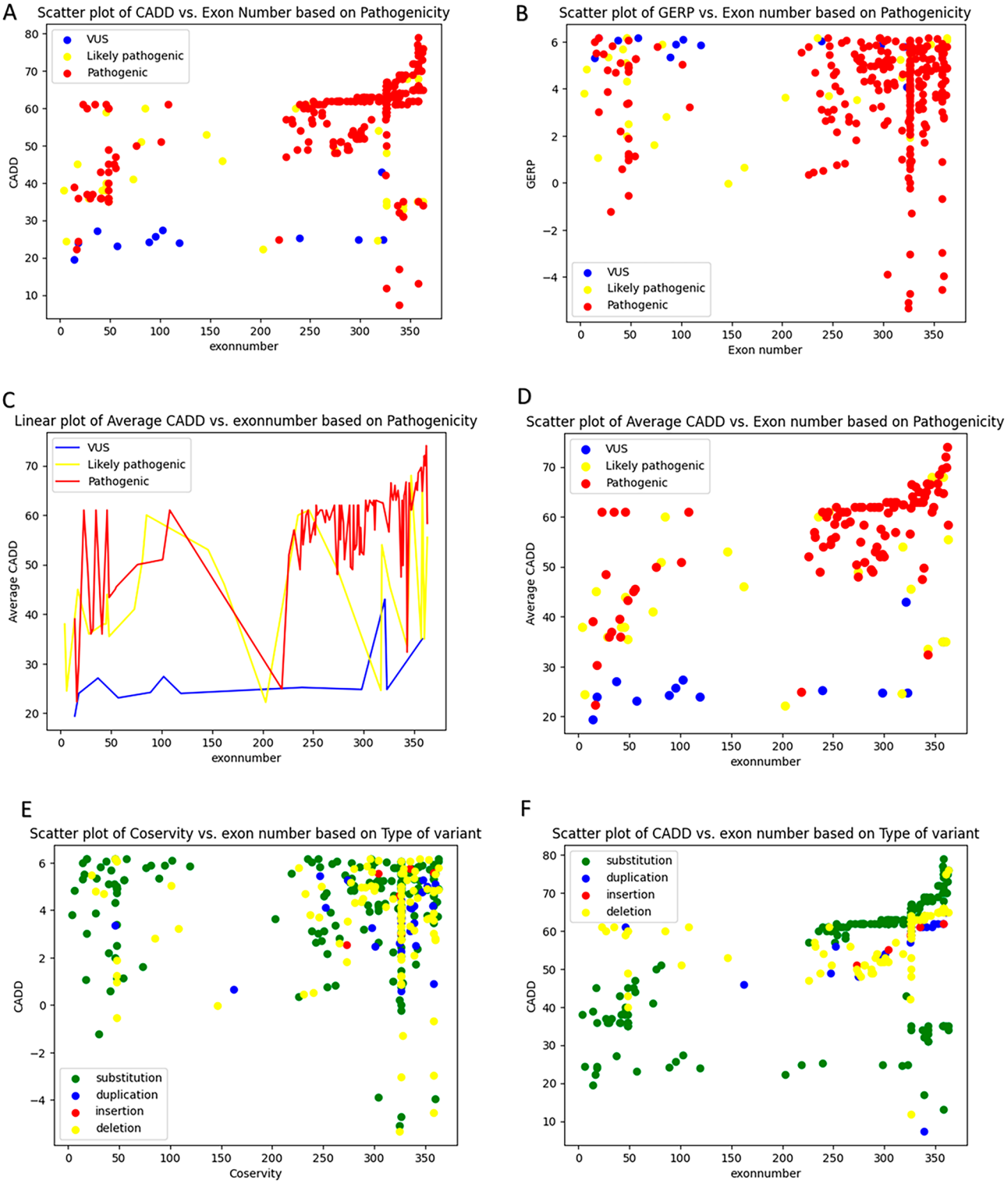

Figure 3. Comparative analysis of *TTN* variants with their pathogenicity, type of alternation, and conservity.

The biogenesis pathways of *TTN*

Role of alternative splicing

TTN gene consists of 364 exons as translatable parts according to NCBI[55] and is estimated to code 34,350 amino acid residues according to UniProt[56]. *TTN* can be spliced in different ways to produce different transcript forms. Since alternative splicing of *TTN*, the protein has various sizes. The I-band, M-line, and Z-disc areas of Titin are the most variable parts, which lead to various isoforms with a wide range of elasticity. Due to variations in the I-band area, different muscle types have varying degrees of elasticity. The Titin gene's I-band encoding region

is the site of many splicing processes resulting in isoforms with various spring compositions. This process even can discriminate cardiac Titin with skeletal muscle Titin.

All cardiac Titin isoforms have exon 49, which contains the N2B sequence; however, skeletal muscle does not[57]. The cardiac isoform known as N2B Titin is a small 2970-kDa weight protein produced by splicing exons 49/50. Deletion of Titin N2B region causes diastolic dysfunction and cardiac atrophy[58]. Another isoform is N2BA which is made up of exons 102 to 109, which code for the N2A element. A specific property of this isoform is that it contains more PEVK segments and is longer with more Ig domains[58].

I-band and its isoforms in cardiac compliance and DCM

Protein composition patterns can change among different populations and even in various stages of human life. The isoform transforming of sarcomeric proteins in the troponin complex, Myosine heavy chain (MHC), Myosine light chain (MLC) and Titin from fetal to adult through transcriptional changes or alternative splicing is the essential element of myofibril maturation[59].

A study by Lahmers et al.[59] revealed that fetal titin isoforms are expressed in neonates, containing additional spring elements in the tandem Ig and PEVK regions. This leads to lower stiffness compared to adults, explained by the unique spring composition of fetal cardiac titin in neonates. Changes in titin expression during development likely impact functional transitions and diastolic filling as the heart matures. The fetal cardiac titin isoform, with its extra Ig and PEVK spring elements, gradually disappears postnatally in a species-dependent manner.

In the human heart, the ratio of titin isoform expression is established based on passive tension. There is a high correlation between titin-based passive tension and I-band region size, with lower tension associated with a larger, more elastic I-band. In healthy adult hearts, the N2BA and N2B titin isoforms express at 30–40% and 60–70% respectively. The relative levels of these two isoforms are a key determinant of cardiomyocyte stiffness[60]. Titin plays a central role in the passive ventricular tension. Animal studies have proved that the N2BA isoform is present in the near-term fetus 6 days before birth but after birth disappears and is replaced by a smaller N2B isoform, which predominates in 1-week-old neonate and adults. Adult cardiomyocytes have 15 times more passive tension compared to fetal cardiomyocytes which is confirmed by immunofluorescence microscopy. This transformation is compatible with the heart's function in each stage of life which after birth needs more passive tension to pump the blood effectively through the vessels[61].

Alternative splicing of the TTN gene plays significant roles in cardiac diseases like dilated cardiomyopathy (DCM). In DCM, the more compliant N2BA isoform is upregulated, decreasing passive stiffness and increasing chamber compliance. Overall, variable expression and splicing of titin isoforms critically influence myocardial passive tension and compliance[30,31,62,63].

Hidalgo et al.[64] conducted sophisticated experiments to identify the mechanisms influencing myocardial passive stiffness by modifying the phosphorylation state of titin. The study revealed that titin serves as a substrate not only for protein kinase A but also for protein kinase G and protein kinase C α (PKCα). The researchers pinpointed the PEVK region of titin as the primary site for PKCα phosphorylation, demonstrating that phosphorylation at this site enhances passive tension in the myocardium.

Novex variants and tiny titin results alternative splicing

The whole sequence of the human *TTN* gene contains three isoform-specific mutually exclusive exons named novel exons (novex), which encode for the I-band sequence. Novex1 is presented in exon 45, novex-2 is located in exon 46, and novex-3 is placed in exon 48. The novex-1 and novex-2 Titin isoforms are encoded by transcripts that either include the novex-1 or novex-2 exons. Early stop-gain codon in the novex-3 transcript produces a remarkably tiny isoform (700 kDa) known as novex-3 Titin. The 'tiny Titin' isoform, expressed in all striated muscles, stretches from the Z-disc to the novex-3 domain (C-terminus). Therefore, stress-induced sarcomeric rearrangement may be mediated by novex-3 Titin because of its regulatory involvement in calcium level and GTPase-associated myofibrillar pathways[65]. Furthermore, novexes 2 and 3 may be linked to DCM or ARVC based on the expression levels of novex variations in human cardiac tissues affected by cardiomyopathies. Previous research suggests that novex variations may be attributable to cardiomyopathy[66].

Splicing regulation of alternative splicing

Encoding Titin by a single gene into various forms is the result of different mRNA splice pathways which leads to Titin isoform classes[57]. The titin gene contains 409 introns, enabling generation of 57 distinct mRNA transcripts through extensive alternative splicing. These include 29 unspliced forms and 28 spliced isoforms. Additional diversity arises from 5 alternative promoters, 9 non-overlapping final exons, and 9 verified polyadenylation sites. The resulting mRNAs vary in: 3' end truncations, 5' end truncations, presence/absence of 173 cassette exons, overlapping exons with different borders, and splicing versus retention of 3 introns[67].

RBM20 regulates a subset of genes involved in developing the heart's muscles by modulating their mRNA alternative splicing. Titin, known to undergo extremely complex alternative splicing, is one of the RBM20's targets. RBM20 specifically manipulates alternative splicing within the I-band of *TTN* pre-mRNA, which possesses the highest frequency of the alternative splicing process. It has been demonstrated that some alterations in the protein can produce pathogenic *TTN* isoforms, which are believed to lead to DCM[68]. Surprisingly, Khan et al.[69], detected 80 distinct circRNAs among nearly a thousand from human hearts, indicating that the I-band of Titin is a hotspot region of circRNAs. Remarkably, the introns on each side of the back-spliced junctions were enriched in RBM20 binding sites, and the introns related to the *TTN* circRNAs had a five-fold higher frequency of RBM20 binding sites compared to a control set of introns. Studies on the RBP20 knock-out animals, and a cardiac sample of heterozygous RBM20 mutation carrier with substantially compromised synthesis of *TTN* circRNAs, both provided evidence that RBP20 is involved in the biogenesis of these *TTN* circRNAs[69]. Furthermore, the

most recent study by Czubak et al.[70], also found that Type 1 diabetes patients' human skeletal muscles included a significant amount of circRNAs primarily derived from the I-band of Titin. Titin has considerable interaction with other functional and structural proteins of sarcomeres. So, it is assumable that it has numerous binding sites for muscle-associated proteins and serves as an adhesion template for contractile machinery assembly in cardiac cells. So, it should be considered a dynamic and transformable molecule.

The role of *TTN* variants in cardiomyopathies

Heterozygous mutations in *TTN* are commonly associated with cardiomyopathies and *TTN* has been reported as the most common gene involved in cardiomyopathies[71]. The mutations can be broadly classified into two categories, which are truncating or missense mutations. Truncating mutations lead to premature termination of Titin protein synthesis, resulting in either an altered protein or the loss of functional domains. In contrast, missense mutations result in the replacement of amino acids, potentially causing interference with the typical operation of the Titin protein[36].

The ongoing inquiry into the exact molecular mechanisms by which *TTN* mutations lead to cardiomyopathies illuminates the intricate relationship between *TTN* mutations and various forms of cardiomyopathies. The haploinsufficiency model is a notable mechanism that proposes the presence of truncating mutations in one allele of the *TTN* gene results in a reduction in Titin expression, consequently inducing a functional deficit of Titin protein. The phenomenon mentioned above possesses the capability to disrupt the sarcomere assembly process, alter the mechanical properties of cardiac muscle cells, and prevent the heart's contractile function, leading to the manifestation of cardiomyopathy. Another proposed mechanism which even can be manifest in dominant pattern is missense mutations. This occurrence takes place when the mutated form of the Titin protein impairs the normal functioning of the unaltered Titin protein, leading to compromised assembly and operation of the sarcomere.

Moreover, it is plausible that *TTN* mutations may trigger aberrant splicing occurrences, leading to the production of deficient or abnormal Titin isoforms, thus playing a role in the pathogenesis of cardiomyopathy c. The bioinformatics analysis of reported variants in *TTN* related to cardiomyopathies has been shown in Table 1.

Dilated cardiomyopathy

Idiopathic factors are just as significant in the pathophysiology of DCM as acquired variables (such as infections, poisons, or autoimmune diseases). Individuals harboring *TTN* mutations exhibit a higher susceptibility to developing DCM compared to other forms of the disease[36,72–74]. Idiopathic DCM, including familial and sporadic instances, has a genetic etiology, according to a vast number of studies[75,76].

A review study by Chauveau et al.[26] reported that Among the *TTN* mutations linked to DCM, 29 are categorized as nonsense mutations, with three of them occurring in the I-band, while the remaining 26 are located in the A-band. Additionally, 17 frameshift mutations are reported, with three in the I-band and 14 in the A-band. Furthermore, 18 mutations are predicted to affect *TTN* splicing *TTN* mutations, particularly truncating variants (*TTN*tv) in the A-band region and in exons that are highly utilized across the range of titin isoforms, have been shown in a number of studies to be strongly associated with the occurrence of DCM and its severity, accounting for the majority of cases[77–80].

Although fewer *TTN*tv have been identified in pediatrics, a study by Fatkin et al.[81] on the young population showed that the prevalence between adolescents and adults is similar, indicating that they need to have multiple clinical and genetic risk factors other than a single *TTN*tv to present with CDM. *TTN*tv accounts for 25% of familial cases and 18% of sporadic cases of idiopathic dilated cardiomyopathy[82]. The aforementioned *TTN*tv have demonstrated a remarkably low prevalence within the broader populace.

According to Fatkin et al. the prevalence of *TTN*tv is 20% among individuals with DCM, whereas only 0.5% of the general population carries this type of mutation[83,84]. The aforementioned data aligns with the results of Fang et al.[85] survey, which indicated an overall prevalence rate of 17%. The survey also revealed that 23% of cases were familial, while 16% were sporadic. For example, mutations in the A-band are implicated as predominant genetic causes of DCM[86–88].

An important question is how minor TTNtv carrier populations can avoid presenting with DCM. A convincing explanation comes from a study by Roberts et al.[77] showing that the two major adult cardiac titin isoforms, N2BA and N2B, are responsible. These abundant full-length isoforms predominantly contain distal A-band exons, where most DCM-causing TTNtvs are located. However, mutations in proximal exons not present in all TTN transcripts do not cause DCM.

Hypertrophic cardiomyopathy

HCM is the most common inherited cardiomyopathy, frequently arising from sarcomere gene defects. Characterized by arrhythmias and heart failure symptoms due to left ventricular outflow obstruction, diastolic dysfunction, ischemia, or mitral regurgitation, HCM displays autosomal dominant inheritance. Mutations, predominantly missense, in one or more sarcomere genes underlie most cases of HCM. To date, over 1400 mutations have been identified in genes encoding primarily sarcomeric proteins[89].

Due to the involvement of a vast range of mutations with distinctive penetrance, a comprehensive understanding of the pathophysiological mechanisms underlying the development of HCM in the presence of sarcomere-related gene mutations is still unfulfilling[90]. In a study conducted by Ingles et al.[91] on 33 genes reported to have an association with HCM, only 8 genes (*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL2*, and *MYL3*) were shown to have a definitive impact on occurring HCM. It is estimated that around 30% of HCM patients have unidentified genes responsible for the condition.

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
1	179391826	c.107889del	p.Lys35963AsnfsTer9	E.363	rs281864930	P	P	DC	76	5.79	[195]
2	179391848	c.107867T > C	p.Leu35956Pro	E.363	rs267607156	LP	LP	DC	35	6.17	[196]
3	179391875	c.107840T > A	p.Ile35947Asn	E.363	rs281864928	P	VUS	DC	34	4.9	[196]
4	179391915	c.107800G > T	p.Gly35934Ter	E.363	rs368277535	LP	VUS	DC	76	6.05	[197]
5	179391925	c.107780-107790delinsTGAAAGAAAAA	p.Glu35927-Trp35930delinsValLysGluLys	E.363	rs281864927	P	P	DC	65	4.87	[198]
6	179391925	c.107780-107781insTGAAAGAAAAA	p.Glu35927AspfsTer6	E.363	NA	LP	NA	PO	65	4.52	[196]
7	179391972	c.107743A > C	p.Thr35915Pro	E.363	NA	LP	NA	DC	32	6.06	[196]
8	179392207	c.107646del	p.Ser35883GlnfsTer10	E.362	NA	LP	NA	DC	75	5.76	[199]
9	179392218	c.107635C > T	p.Gln35879Ter	E.362	rs757082154	P	VUS	DC	75	4.87	[196]
10	179392275	c.107578C > T	p.Gln35860Ter	E.362	rs1009131948	P	LP/P	DC	73	3.75	[200]
11	179393000	c.107377 + 1G > A	–	I.361	rs112188483	P	P/LP	NA	NA	4.96	[201]
12	179393027	c.107351del	p.Ser35784Ter	E.361	rs778765016	P	NA	DC	81	4.97	[202]
13	179393094	c.107284C > T	p.Arg35762Ter	E.361	rs1477669354	P	LP	DC	70	4.36	[203]
14	179393272	c.107208del	p.Phe35736LeufsTer15	E.360	NA	P	NA	DC	75	5.17	[204]
15	179393329	c.107149C > T	p.Gln35717Ter	E.360	rs369157062	P	NA	DC	81	5.56	[202]
16	179393480	c.106998dup	p.Ala35667SerfsTer6	E.360	rs1031891465	LP	NA	NA	65	4.56	[202]
17	179393500	c.106978C > T	p.Gln35660Ter	E.360	rs1687693219	P	NA	DC	81	5.56	[205]
18	179393519	c.106959T > A	p.Tyr35653Ter	E.360	rs369450212	LP	NA	DC	41	– 7.15	[202206]
19	179393524	c.106954C > T	p.Arg35652Ter	E.360	rs565675340	P	P	DC	70	– 3.94	[207]
20	179393564	c.106914G > C	p.Trp35638Cys	E.360	rs758497512	LP	VUS	DC	35	5.55	[205]
21	179393709	c.106768dup	p.His35590ProfsTer2	E.360	NA	P	LP	NA	65	5.10	[202]
22	179393738	c.106740del	p.Ala35581GlnfsTer36	E.360	NA	P	LP	DC	75	5.53	[202]
23	179393845	c.106668del	p.Lys35556AsnfsTer6	E.360	rs587776772	P	P	DC	75	2.76	[208]
24	178529118	c.106632-106633del	p.Leu35545LysfsTer3	E.360	NA	P	NA	DC	9.91	1.97	[204]
25	179393849	c.106629del	p.Ala35544ProfsTer2	E.360	rs869312069	P	LP	DC	75	2.82	[202]
26	179393907	c.106571del	p.Lys35524ArgfsTer22	E.360	rs199469666	P	NA	DC	73	3.39	[208]
27	179394686	c.106531 + 1G > A	–	I.359	rs760915007	P	P	NA	NA	5.61	[209]
28	179394796	c.106422del	p.Phe35475SerfsTer3	E.359	NA	LP	NA	DC	72	– 0.80	[206]
29	179394967	c.106374 + 1del	–	I.358	rs763404256	LP	VUS	NA	NA	5.13	[202]
30	179395292	c.106050del	p.Glu35351AsnfsTer54	E.358	NA	LP	NA	DC	74	– 10.5	[206]
31	179395323	c.106019del	p.Gly35340ValfsTer65	E.358	rs727504482	P	NA	DC	74	5.23	[210]
32	179395428	c.105910-105914del	p.Thr35304CysfsTer3	E.358	NA	P	NA	DC	73	3.24	[206]
33	179395510	c.105832C > T	p.Gln35278Ter	E.358	NA	LP	NA	DC	11.95	2.7	[211]
34	179395528	c.105814del	p.Thr35272HisfsTer21	E.358	rs759645441	LP	NA	DC	66	0.59	[202]
35	179395600	c.105739-105742dup	p.Lys35248SerfsTer2	E.358	rs866421715	LP	NA	NA	62	0.88	[202]
36	179395807	c.105528-105535del	p.Gln35176HisfsTer9	E.358	rs199469665	P	LP	DC	66	3.57	[212]
37	179395811	c.105523-105531del	p.His35175-Val35177del	E.358	NA	VUS	NA	PO	53	3.49	[199]
38	179395856	c.105486del	p.Trp35162CysfsTer8	E.358	rs1553485330	P	P	DC	66	4.78	[213]
39	179395919	c.105423C > A	p.Tyr35141Ter	E.358	NA	LP	NA	DC	64	– 4.25	[214]
40	179396571	c.104771C > A	p.Ser34924Ter	E.358	rs1559003939	P	LP	DC	75	5.56	[215]
41	179396675	c.104666-104667del	p.Pro34889ArgfsTer3	E.358	NA	P	LP	DC	66	– 0.66	[216]

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
42	179396929	c.104413C>T	p.Arg34805Ter	E.358	rs750519430	P	LP/P	DC	71	4.59	217
43	179397250	c.104092C>T	p.Arg34698Ter	E.358	rs727504184	P	LP	DC	79	4.19	202218
44	179397397	c.103945C>T	p.Arg34649Ter	E.358	rs995029896	P	LP	DC	74	3.46	219
45	179397492	c.103850-103851insAAC	p.Lys34618AspfsTer2	E.358	NA	VUS	NA	PO	62	0.00	210
46	179397546	c.103796G>A	p.Arg34599Lys	E.358	rs1362778188	LP	NA	DC	35	5.80	205
47	179397637	c.103705A>T	p.Lys34569Ter	E.358	rs1553490574	P	LP	DC	75	5.94	202
48	179397824	c.103518del	p.Ala34507LeufsTer8	E.358	rs1553491220	P	LP	DC	66	− 4.55	209
49	179397934	c.103408G>T	p.Glu34470Ter	E.358	rs769023413	LP	VUS	DC	68	5.78	202
50	179397982	c.103360del	p.Glu34454AsnfsTer3	E.358	rs760768093	P	P	DC	66	3.87	220
51	179398245	c.103096-103097insSVAelement	–	E.358	rs1575266261	NA	LP	NA	NA	NA	202
52	179398266	c.103073-103076dup	p.Ser34359ArgfsTer2	E.358	NA	P	LP	NA	62	0.89	202
53	179398340	c.103002-103003insA	p.Ala34335SerfsTer7	E.358	NA	P	NA	DC	62	3.24	205
54	179398393	c.102949C>T	p.Gln34317Ter	E.358	rs397517787	P	LP	DC	75	5.5	80
55	179398396	c.102946del	p.Tyr34316ThrfsTer3	E.358	NA	P	NA	DC	66	4.28	205
56	179398410	c.102932C>G	p.Ser34311Ter	E.358	NA	LP	NA	DC	72	5.6	221
57	179398712	c.102630del	p.Val34211Ter	E.358	rs869312101	p	VUS	DC	66	4.82	202
58	179398819	c.102523C>T	p.Arg34175Ter	E.358	rs752697861	P	P	DC	13.12	4.23	221
59	179398833	c.102509G>A	p.Trp34170Ter	E.358	NA	P	NA	DC	73	5.38	205
60	179399071	c.102271C>T	p.Arg34091Trp	E.358	rs140319117	P	VUS	DC	35	4.82	205
61	179399128	c.102214T>A	p.Trp34072Arg	E.358	NA	LP	NA	DC	34	5.88	204
62	179399285	c.102057del	p.Asn34020ThrfsTer9	E.358	NA	P	LP	DC	66	− 2.96	204
63	179400115	c.101227C>T	p.Arg33743Ter	E.358	rs794729305	P	LP	DC	76	4.63	222
64	179400229	c.101113del	p.Ser33705LeufsTer4	E.358	NA	P	NA	DC	65	4.4	213
65	179400244	c.101098-101099insT	p.Asp33700ValfsTer13	E.358	rs869312122	P	LP	DC	62	5.59	202
66	179400320	c.101021-101022del	p.Arg33674IlefsTer4	E.358	rs869312087	P	LP	DC	65	3.01	202
67	179400405	c.100936-100937del	p.Val33646HisfsTer26	E.358	NA	LP	NA	DC	65	4.08	205
68	179400516	c.100826G>A	p.Arg33609Gln	E.358	rs771243505	VUS	VUS	DC	35	5.3	223
69	179400517	c.100825C>T	p.Arg33609Ter	E.358	rs1057518195	P	LP/P	DC	72	5.3	224
70	179400577	c.100766-1G>T	–	I.357	rs185589320	LP	NA	NA	NA	5.3	202
71	179400887	c.100587G>A	p.Trp33529Ter	E.357	rs1064793560	P	LP	DC	70	5.76	225
72	179400913	c.100558-100561dup	p.Gly33521AspfsTer25	E.357	rs1553501572	P	LP	NA	62	4.18	213
73	179401029	c.100445C>A	p.Ser33482Ter	E.357	rs869312086	P	LP	DC	77	5.76	202
74	179401230	c.100244C>T	p.Pro33415Leu	E.357	rs72648282	LP	VUS	DC	35	5.76	226
75	179402067	c.99865+2T>C	–	I.355	rs1453570860	P	NA	NA	NA	5.53	199
76	179403522	c.99034A>T	p.Lys33012Ter	E.354	rs771511344	P	LP	DC	72	5.71	199
77	179403562	c.98994del	p.Lys32998AsnfsTer63	E.354	rs727504535	P	P	DC	65	3.68	222
78	179403888	c.98774del	p.Gly32925ValfsTer56	E.353	NA	P	LP	DC	65	6.15	210
79	179404189	c.98603del	p.Phe32868SerfsTer11	E.352	NA	P	NA	DC	65	3.44	201
80	179404241	c.98551C>T	p.Arg32851Ter	E.352	rs553821887	P	VUS	DC	69	3.78	202
81	179404286	c.98506C>T	p.Arg32836Ter	E.352	rs869312085	P	LP	DC	72	4.88	202
82	179404492	c.98299-98300del	p.Arg32767GlyfsTer2	E.352	rs397517776	P	P	DC	65	4.91	202
Continued											

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
83	179404493	c.98299del	p.Arg32767GlyfsTer26	E.352	rs772061676	P	LP	DC	65	3.65	202
84	179404524	c.98265-98268dup	p.His32757AsnfsTer4	E.352	rs869312067	P	LP	NA	62	5.02	202
85	179404687	c.98105del	p.Pro32702LeufsTer15	E.352	NA	P	NA	DC	65	6.17	213
86	179405030	c.97863G > A	p.Trp32621Ter	E.351	NA	LP	NA	DC	68	5.96	201
87	179406990	c.97492 + 1G > A	–	I.349	rs727505319	P	NA	NA	NA	6.17	227
88	179407385	c.97192 + 4A > G	–	I.348	rs370069759	VUS	VUS	NA	NA	4.4	202
89	179407531	c.97050dup	p.Glu32351ArgfsTer6	E.348	rs794729365	P	P	NA	62	5.27	228
90	179407808	c.96892C > T	p.Gln32298Ter	E.347	rs201108270	LP	VUS	DC	68	5.91	202
91	179408200	c.96500-96501insAGAATTC	p.Gly32168GlufsTer27	E.347	NA	P	NA	DC	61	6.03	205
92	179408240	c.96460dup	p.Thr32154AsnfsTer39	E.347	rs869312084	P	LP	NA	61	4.75	202
93	179408364	c.96336-96337insC	p.Lys32113GlnfsTer3	E.347	NA	P	NA	DC	61	5.32	80
94	179408990	c.95966del	p.Asn31989ThrfsTer2	E.345	rs72648265	P	LP	DC	64	6.17	199
95	179409084	c.95872C > T	p.Arg31958Ter	E.345	NA	P	LP	DC	69	5.23	229
96	179410544	c.95416 + 3–95416 + 4insCCT	–	I.343	NA	LP	NA	NA	NA	3.31	199
97	179410545	c.95415–95416 + 2del	–	I.343	rs769407533	P	LP	NA	NA	5.82	202
98	179410592	c.95371G > C	p.Gly31791Arg	E.343	NA	P	VUS	DC	31	5.82	230
99	179410605	c.95358C > G	p.Asn31786Lys	E.343	rs869320743	P	P	DC	31	4.95	231
100	179410622	c.95341C > T	p.Arg31781Ter	E.343	NA	P	NA	DC	69	2.95	205
101	179410768	c.95195C > T	p.Pro31732Leu	E.343	rs753334568	P	LP/P	DC	35	5.82	231
102	179410778	c.95185T > C	p.Trp31729Arg	E.343	rs869320741	LP	P	DC	34	5.82	231
103	179410799	c.95164C > T	p.Gln31722Ter	E.343	NA	P	NA	DC	66	4.95	199
104	179410829	c.95134T > C	p.Cys31712Arg	E.343	rs869320740	LP	P	DC	33	5.82	231
105	179411050	c.95008C > T	p.Arg31670Ter	E.342	rs1322596650	P	P	DC	68	4.78	232
106	179411199	c.94859T > G	p.Leu31620Ter	E.342	rs561946873	LP	NA	DC	70	6.03	207
107	179411200	c.94852-94858del	p.Ala31618TyrfsTer37	E.342	rs869312066	P	LP	DC	64	4.51	202
108	179411203	c.94855C > T	p.Arg31619Ter	E.342	rs869312121	P	LP	DC	68	2.36	202
109	179411339	c.94816C > T	p.Arg31606Ter	E.341	rs1060500435	P	LP	DC	69	1.72	233
110	179411593	c.94562dup	p.Thr31522AsnfsTer12	E.341	rs869312083	P	LP	NA	61	2.50	202
111	179411905	c.94344-94347del	p.Lys31448AsnfsTer8	E.340	rs727503546	P	P	DC	64	5.67	234
112	179411967	c.94285T > A	p.Trp31429Arg	E.340	NA	LP	NA	DC	35	6.03	196
113	179412186	c.94167del	p.Phe31389LeufsTer7	E.339	rs747837187	LP	NA	DC	64	5.26	202
114	179412199	c.94154C > G	p.Ser31385Ter	E.339	rs548010682	LP	NA	DC	72	6.03	207
115	179412246	c.94103-94107del	p.Ile31368SerfsTer34	E.339	rs769488730	P	P	DC	64	5.33	199
116	179412456	c.93897del	p.Phe31299LeufsTer14	E.339	rs397517758	P	P	DC	64	3.15	80
117	179412902	c.93451G > T	p.Glu31151Ter	E.339	NA	P	NA	DC	67	5.65	199
118	179413151	c.93202G > T	p.Glu31068Ter	E.339	NA	P	NA	DC	68	5.65	205
119	179413187	c.93166C > T	p.Arg31056Ter	E.339	rs72648250	P	LP/P	DC	69	5.65	202
120	179413477	c.92876G > A	p.Trp30959Ter	E.339	rs72648249	P	NA	DC	67	5.22	199
121	179413670	c.92683C > T	p.Asp30885SerfsTer30895Ter	E.339	rs869312065	P	LP	DC	16.84	5.3	202
122	179413694	c.92652-92659del	p.Asp30885SerfsTer3	E.339	rs1559175090	P	LP	DC	63	2.1	224
123	178549148	c.92478dup	p.Val30827SerfsTer22	E.339	NA	P	LP	NA	7.36	3.45	235

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
124	179414036	c.92317C > T	p.Arg30773Ter	E.339	rs794729301	P	LP/P	DC	68	3.79	225
125	179414065	c.92284-92288dup	p.Ser30763ArgfsTer7	E.339	rs756367933	P	VUS	NA	64	4.17	202
126	179414119	c.92234C > A	p.Ser30745Ter	E.339	NA	P	NA	DC	67	5.74	205
127	179414186	c.92167C > T	p.Pro30723Ser	E.339	rs758537709	P	VUS	DC	32	5.73	213
128	179414303	c.92146C > T	p.Gln30716Ter	E.338	NA	P	NA	DC	70	5.73	205
129	179414366	c.92083T > C	p.Ser30695Pro	E.338	rs768267695	LP	NA	DC	31	5.74	236
130	179414574	c.91875del	p.Pro30626GlnfsTer2	E.338	rs757451467	P	P	DC	63	4.82	205
131	179414812	c.91753T > G	p.Phe30585Val	E.337	rs1060500507	P	VUS	DC	34	5.74	237
132	179414850	c.91715dup	p.Asn30572LysfsTer16	E.337	rs779129892	P	VUS	NA	61	4.08	202
133	179415706	c.91551-91552del	p.Asp30519Ter	E.336	NA	P	NA	DC	63	3.18	205
134	179416527	c.91097-91100dup	p.Asn30367LysfsTer3	E.335	NA	P	NA	NA	61	5.07	79
135	179416849	c.90778dup	p.Tyr30260LeufsTer12	E.335	rs397517750	P	LP	NA	61	4.10	199
136	179416870	c.90757G > A	p.Gly30253Arg	E.335	–	P	NA	DC	35	5.9	205
137	179417040	c.90587del	p.Lys30196ArgfsTer94	E.335	rs397517749	P	LP	DC	63	6.06	238
138	179417257	c.90370G > T	p.Glu30124Ter	E.335	rs1553539995	P	LP	DC	67	5.76	239
139	179417305	c.90322-90323insT	p.Glu30108ValfsTer6	E.335	rs869312082	P	LP	DC	61	5.76	202
140	178552691	c.90208-90209insSVAelement	–	E.335	NA	NA	LP	NA	NA	NA	202
141	179417539	c.90087-90088del	p.Glu30029AspfsTer7	E.335	rs869312064	P	LP	DC	63	3.32	202
142	179417542	c.90085del	p.Glu30029LysfsTer11	E.335	NA	P	NA	DC	63	5.76	238
143	179417543	c.90084del	p.Glu30029LysfsTer11	E.335	NA	LP	NA	DC	63	-9.19	199
144	179417724	c.89900-89903del	p.Asn29967MetfsTer27	E.335	rs869312081	P	LP	DC	63	4.36	202
145	179417877	c.89750dup	p.Val29918SerfsTer3	E.335	rs869312063	P	LP	NA	63	3.12	202
146	179418418	c.89314G > T	p.Glu29772Ter	E.334	NA	P	P	DC	64	4.71	240
147	179418468	c.89265G > A	p.Trp29755Ter	E.334	rs1179247052	P	LP	DC	66	5.6	225
148	179418639	c.89197 + 2T > G	–	I.333	rs1575536935	P	LP	DC	NA	5.61	241
149	179418639	c.89197–89197 + 2del	–	I.333	rs397517741	P	LP	NA	NA	4.10	80
150	179418640	c.89197 + 1G > C	–	I.333	rs1131691873	P	LP	DC	NA	5.61	225
151	179418877	c.88961G > A	p.Trp29654Ter	E.333	NA	P	NA	DC	66	5.61	205
152	179419329	c.88745C > T	p.Ser29582Phe	E.332	NA	LP	NA	DC	35	5.66	237
153	179419370	c.88703-88704del	p.His29568LeufsTer7	E.332	rs794729360	P	P	DC	63	5.29	242
154	179419765	c.88421G > A	p.Trp29474Ter	E.331	rs869025546	P	LP	DC	66	5.66	243
155	179422099	c.87887-87890del	p.His29296ProfsTer104	E.329	rs869312120	P	LP	DC	63	5.77	202
156	179422273	c.87716del	p.Gly29239AspfsTer32	E.329	rs869312028	P	VUS	DC	63	5.56	202
157	179422457	c.87624C > A	p.Tyr29208Ter	E.328	rs772121356	P	LP	DC	66	0.93	202
158	179422552	c.87529A > T	p.Lys29177Ter	E.328	NA	LP	NA	DC	33	4.44	201
159	179422565	c.87516del	p.Tyr29173ThrfsTer24	E.328	rs727503552	P	LP	DC	63	-1.28	199
160	179422726	c.87355del	p.Ala29119LeufsTer17	E.328	rs794729356	P	P	DC	63	5.63	244
161	179422902	c.87179C > A	p.Ser29060Ter	E.328	NA	P	NA	DC	67	5.69	205
162	179423093	c.87093del	p.Pro29032LeufsTer8	E.327	NA	P	NA	DC	63	4.57	205
163	179423146	c.87040C > T	p.Arg29014Ter	E.327	rs776065839	P	P	DC	67	4.77	209
164	179423220	c.86967G > A	p.Trp28989Ter	E.327	rs869312062	P	LP	DC	66	5.76	202

Continued

www.nature.com/scientificreports/

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
165	179423314	c.86872dup	p.Ser28958LysfsTer10	E.327	NA	P	NA	NA	61	0.07	[79]
166	179424036	c.86821 + 2T > A	–	I.326	rs397517735	P	P	DC	NA	5.61	[199]
167	179424057	c.86799-86802del	p.Gly28936Ter	E.326	rs727504856	P	P	DC	63	1.24	[228]
168	179424114	c.86742-86745del	p.Tyr28915ThrfsTer22	E.326	rs1415420768	P	LP	DC	63	0.88	[225]
169	179424219	c.86640C > A	p.Tyr28880Ter	E.326	NA	P	LP	DC	65	3.92	[202]
170	179424219	c.86640delC	p.His28881ThrfsX2	E.326	rs794729298	P	LP	DC	63	3.92	[202]
171	179424399	c.86459-86460del	p.Ser28820TrpfsTer50	E.326	rs869312080	P	LP	DC	63	2.79	[202]
172	179424496	c.86363G > A	p.Trp28788Ter	E.326	rs1064793814	P	P	DC	66	5.87	[199]
173	179424743	c.86116C > T	p.Arg28706Ter	E.326	rs794729384	P	P	DC	64	2.05	[232]
174	179424783	c.86076dup	p.Ser28693IlefsTer2	E.326	rs1285329277	P	P	NA	61	0.61	[245]
175	179424844	c.86015G > A	p.Trp28672Ter	E.326	NA	P	NA	DC	66	5.87	[223]
176	179424968	c.85891del	p.Ala28631LeufsTer3	E.326	rs1575610911	P	LP	DC	63	6.08	[246]
177	179425091	c.85768C > T	p.Arg28590Ter	E.326	rs748689777	P	P	DC	65	2.95	[227]
178	179425207	c.85640-85652del	p.Pro28547GlnfsTer12	E.326	rs762286447	P	LP	DC	63	4.12	[205]
179	179425598	c.85261-85262insAlu	–	E.326	NA	P	LP	NA	NA	NA	[247]
180	179425708	c.85151G > A	p.Arg28384Gln	E.326	rs1465916943	LP	NA	DC	34	5.09	[223]
181	179425709	c.85150C > T	p.Arg28384Ter	E.326	NA	P	LP	DC	65	3.09	[205]
182	179425748	c.85109-85111del	p.Lys28370-Ala28371delinsThr	E.326	NA	P	NA	DC	50	4.99	[223]
183	179425769	c.85090C > T	p.Arg28364Ter	E.326	rs770038577	P	LP/P	DC	66	5.09	[202]
184	179425848	c.85008-85011del	p.Glu28338HisfsTer9	E.326	rs869312100	P	VUS	DC	62	0.90	[202]
185	179426041	c.84819G > A	p.Trp28273Ter	E.326	rs72648222	P	P	DC	66	5.78	[199]
186	179426302	c.84557dup	p.Ile28187AsnfsTer6	E.326	rs1553564589	P	LP	NA	61	2.28	[80]
187	179426383	c.84476del	p.Gly28159ValfsTer15	E.326	rs1553564694	P	LP	DC	62	5.56	[80]
188	179426471	c.84388del	p.Cys28130ValfsTer44	E.326	NA	P	NA	DC	62	− 0.12	[205]
189	179426483	c.84376C > T	p.Gln28126Ter	E.326	rs869312119	P	LP	DC	66	5.22	[202]
190	179426940	c.83919del	p.Asn27973LysfsTer2	E.326	NA	LP	NA	DC	62	− 1.64	[223]
191	179427344	c.83515C > T	p.Arg27839Ter	E.326	rs869312118	P	P	DC	67	5.76	[202]
192	179427362	c.83497G > T	p.Gly27833Ter	E.326	NA	P	P	DC	66	4.87	[199]
193	179428087	c.82772G > A	p.Trp27591Ter	E.326	NA	P	NA	DC	66	5.85	[205]
194	179428202	c.82657G > T	p.Gly27553Ter	E.326	rs869178171	P	P	DC	65	4.96	[248]
195	179428256	c.82603A > G	p.Thr27535Ala	E.326	rs775733174	P	NA	DC	24	4.8	[236]
196	179428346	c.82513del	p.Ile27505PhefsTer20	E.326	rs869312060	P	LP	DC	62	0.86	[202]
197	179428522	c.82337C > T	p.Ala27446Val	E.326	rs780558473	LP	NA	DC	34	5.97	[249]
198	179428586	c.82273C > T	p.Gln27425Ter	E.326	rs371332011	P	LP	DC	65	5.97	[202]
199	179428871	c.81988C > T	p.Gln27330Ter	E.326	rs72648222	P	NA	DC	65	6.07	[199]
200	179428916	c.81943G > T	p.Glu27315Ter	E.326	rs373533040	P	LP	DC	66	6.07	[202]
201	179428920	c.81942del	p.Glu27315AsnfsTer35	E.326	NA	LP	NA	DC	62	0.49	[223]
202	179428980	c.81878-81879del	p.Phe27293CysfsTer3	E.326	rs727504660	P	P	DC	62	3.73	[199]
203	179429341	c.81518del	p.Pro27173HisfsTer17	E.326	rs869312079	P	LP	DC	62	4.63	[202]
204	179429515	c.81340-81344del	p.Lys27114GlnfsTer9	E.326	rs886038928	P	LP	DC	62	4.1	[250]
205	179429538	c.81321C > G	p.Tyr27107Ter	E.326	rs557312035	P	P	DC	64	4.22	[202]
Continued											

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
206	179429590	c.81262-81269del	p.Gln27088CysfsTer5	E.326	rs869312059	P	LP	DC	62	4.82	202
207	179429862	c.80997-81012del	p.Tyr26999Ter	E.326	rs727503559	P	LP	DC	64	2.08	251
208	179430143	c.80716C>T	p.Arg26906Ter	E.326	rs727505284	P	P	DC	64	3.71	252
209	179430224	c.80635C>T	p.Gln26879Ter	E.326	rs79926414	LP	VUS	DC	65	5.49	202
210	179430320	c.80539C>T	p.Gln26847Ter	E.326	rs561152891	P	NA	DC	65	4.59	243
211	179430345	c.80514del	p.Val26839LeufsTer5	E.326	NA	P	P	DC	62	1.22	199
212	179430692	c.80167C>T	p.Arg26723Cys	E.326	rs1412497882	LP	VUS	DC	35	4.92	223
213	179430807	c.80052del	p.Gly26685AspfsTer11	E.326	NA	LP	NA	DC	62	3.32	223
214	179431048	c.79809-79811del	p.Val26604del	E.326	rs776591304	VUS	NA	PO	48	0.24	223
215	179431175	c.79684C>T	p.Arg26562Ter	E.326	rs869025545	P	LP	DC	65	4.03	253
216	179431293	c.79566T>A	p.Tyr26522Ter	E.326	NA	LP	NA	DC	62	− 2.28	205
217	179431416	c.79443del	p.Cys26482ValfsTer16	E.326	NA	P	NA	DC	62	2.22	243
218	179431868	c.78991C>T	p.Arg26331Ter	E.326	rs779996703	P	P	DC	65	1.45	254
219	179431880	c.78979C>T	p.Arg26327Ter	E.326	rs1419374180	P	LP	DC	65	0.75	232
220	179432352	c.78507del	p.Gly26170ValfsTer3	E.326	rs869312058	P	LP	DC	62	3.06	202
221	179432357	c.78502G>A	p.Ala26168Thr	E.326	NA	LP	NA	DC	28.7	5.75	199
222	179432675	c.78184G>T	p.Glu26062Ter	E.326	rs869312057	P	LP	DC	64	5.58	202
223	179432681	c.78178G>T	p.Glu26060Ter	E.326	rs794729289	P	P	DC	64	5.58	225
224	179432761	c.78095-78098del	p.Arg26032ThrfsTer41	E.326	rs869312117	P	LP	DC	62	4.37	202
225	179433095	c.77764C>T	p.Gln25922Ter	E.326	rs794729288	P	VUS	DC	65	5	210
226	179433197	c.77646-77662delinsAGA	p.Ile25883AspfsTer3	E.326	rs794729345	P	LP	DC	11.72	3.33	199
227	179433210	c.77647-77649del	p.Ile25883del	E.326	NA	LP	P	DC	48	1.91	199
228	179433274	c.77585del	p.Lys25862ArgfsTer25	E.326	NA	P	NA	DC	62	6.03	205
229	179433407	c.77452G>T	p.Glu25818Ter	E.326	NA	P	P	DC	63	6.03	205
230	179433438	c.77421dup	p.Ser25808GlnfsTer19	E.326	rs730880343	P	LP	NA	61	3.64	80
231	179433632	c.77227G>T	p.Glu25743Ter	E.326	rs765997807	P	LP	DC	64	5.74	223
232	179433630	c.77226-77229del	p.Ser25742ArgfsTer9	E.326	NA	P	NA	DC	61	3.86	196
233	179433665	c.77194C>T	p.Gln25732Ter	E.326	NA	P	NA	DC	64	5.74	243
234	179433714	c.77145dup	p.Ser25716LeufsTer8	E.326	rs1205409465	P	LP	NA	60	3.91	225
235	179433758	c.77101-77102insT	p.Pro25701LeufsTer9	E.326	NA	P	NA	DC	60	5.83	199
236	179433759	c.77100dup	p.Pro25701ThrfsTer9	E.326	rs794729343	P	P	NA	60	3.71	255
237	179433781	c.77077-77078delATinsGA	p.Ile25693Asp	E.326	NA	LP	NA	DC	60	2.62	256
238	179434010	c.76849-76850insGT	p.Ser25617CysfsTer18	E.326	NA	P	NA	DC	60	3.76	243
239	179434060	c.76790-76799del	p.Arg25597ThrfsTer9	E.326	NA	P	NA	DC	61	4.08	79
240	179434161	c.76697-76698del	p.Leu25566ArgfsTer3	E.326	NA	P	NA	DC	61	2.12	199
241	179434463	c.76393-76396del	p.Asn25465Ter	E.326	rs727504483	P	LP	DC	59	2.75	210
242	179434473	c.76383-76386del	p.Asn25462LysfsTer4	E.326	rs869312078	P	LP	DC	61	3.78	202
243	179434486	c.76373del	p.Pro25458GlnfsTer9	E.326	rs869025553	P	P	DC	60	5.02	243
244	179434743	c.76116-76117insA	p.His25373ThrfsTer4	E.326	rs869312077	P	LP	DC	61	3.03	202
245	179435035	c.75824A>G	p.Tyr25275Cys	E.326	NA	LP	NA	DC	34	5.87	249
246	179435223	c.75633-75636dup	p.Val25213CysfsTer25	E.326	rs1553603036	P	LP	NA	60	4.42	224
Continued											

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
247	179435390	c.75469C > T	p.Arg25157Ter	E.326	rs1553603394	P	P	DC	64	0.01	220
248	179435609	c.75250C > T	p.Arg25084Ter	E.326	rs794729286	P	P	DC	64	4.74	257
249	179435628	c.75231T > A	p.Tyr25077Ter	E.326	NA	P	LP	DC	63	− 4.72	227
250	179435718	c.75138-75141del	p.Lys25046AsnfsTer8	E.326	rs794729340	P	P	DC	60	4.16	258
251	179435736	c.75123T > A	p.Tyr25041Ter	E.326	rs753526510	P	VUS	DC	62	− 0.24	202
252	179435976	c.74880-74883dup	p.Pro24962AsnfsTer9	E.326	rs869312116	P	LP	NA	60	3.48	202
253	179436177	c.74682C > A	p.Tyr24894Ter	E.326	NA	P	NA	DC	63	1.11	223
254	179436456	c.74403del	p.Asn24802MetfsTer20	E.326	NA	P	NA	DC	60	3.495	227
255	179436521	c.74338C > T	p.Arg24780Ter	E.326	rs794729285	P	P	DC	64	5.09	202
256	179436553	c.74306dup	p.Asn24769LysfsTer2	E.326	rs869312056	P	LP	NA	59	4.02	202
257	179437013	c.73846C > T	p.Arg24616Ter	E.326	rs794729284	P	P	DC	64	3.98	259
258	179437291	c.73568del	p.Pro24523HisfsTer4	E.326	rs1559415567	P	P	DC	59	3.58	260
259	179437750	c.73109G > A	p.Trp24370Ter	E.326	rs869312115	P	LP	DC	63	5.19	202
260	179438060	c.72799C > T	p.Gln24267Ter	E.326	NA	P	P	DC	63	4.17	205
261	179438190	c.72669del	p.Asp24224IlefsTer8	E.326	rs727504531	P	P	DC	59	− 3.04	260
262	179438873	c.71980-71986delGCATATGinsTA	p.Ala23994Ter	E.326	rs794729338	P	P	DC	58	4.05	199
263	179439257	c.71602C > T	p.Arg23868Ter	E.326	rs397517689	P	P	DC	64	2.44	227
264	179439359	c.71500C > T	p.Gln23834Ter	E.326	rs730880242	P	LP	DC	63	5.7	243
265	179439438	c.71421T > A	p.Tyr23807Ter	E.326	NA	LP	NA	DC	61	− 4.4	205
266	179439506	c.71353A > G	p.Thr23785Ala	E.326	rs765937279	P	NA	DC	26.9	5.6	223
267	179439852	c.71007dup	p.Gly23670ArgfsTer6	E.326	NA	P	NA	NA	59	3.79	243
268	179439881	c.70978C > T	p.Arg23660Ter	E.326	rs1553612386	P	P	DC	63	5.51	243
269	179439924	c.70935del	p.Ala23647LeufsTer19	E.326	NA	P	NA	DC	59	5.06	205
270	179439980	c.70879C > T	p.Gln23627Ter	E.326	rs1575799625	P	LP	DC	64	4.71	203
271	179440068	c.70791del	p.Gly23598GlufsTer8	E.326	rs869312076	P	LP	DC	58	5.02	202
272	179440084	c.70775del	p.Val23592GlyfsTer4	E.326	rs1216966174	LP	NA	DC	59	3.16	202
273	179440168	c.70690-70691dup	p.Thr23565SerfsTer5	E.326	NA	P	NA	NA	59	4.66	199
274	179440565	c.70294G > C	p.Val23432Leu	E.326	NA	VUS	NA	DC	32	5.76	237
275	179440697	c.70162C > T	p.Arg23388Ter	E.326	rs781540455	P	P	DC	63	2.78	261
276	179440982	c.69877G > T	p.Gly23293Ter	E.326	rs869312114	P	LP	DC	62	5.87	202
277	179440999	c.69860G > A	p.Trp23287Ter	E.326	NA	P	LP	DC	63	5.87	248
278	179441016	c.69843del	p.Val23282Ter	E.326	rs869312075	P	LP	DC	53	3.28	202
279	179441101	c.69758C > T	p.Thr23253Ile	E.326	NA	LP	NA	DC	31	5.74	236
280	179441300	c.69671del	p.Pro23224HisfsTer10	E.325	NA	P	NA	DC	54	4.37	205
281	179441341	c.69630C > A	p.Tyr23210Ter	E.325	rs777602537	P	LP	DC	62	− 5.08	205
282	179441449	c.69522T > G	p.Tyr23174Ter	E.325	NA	P	P	DC	63	0.22	199
283	179441479	c.69491-69492del	p.Val23164GlyfsTer2	E.325	rs869312113	P	LP	DC	42	− 5.32	202
284	179441510	c.69458-69461dup	p.Asn23154LysfsTer14	E.325	rs397517679	P	LP	NA	57	2.62	80
285	179441550	c.69421-69422insAAAAG	p.Gly23141GlufsTer38	E.325	rs1247353236	P	LP	PO	59	4.64	225
286	179441649	c.69412 + 1G > A	–	I.324	rs869312074	P	LP	DC	NA	5.72	202
287	179442329	c.68824 + 5G > C	–	I.323	rs749639627	VUS	VUS	DC	NA	5.79	199

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
288	179442329	c.68824G > A	p.Glu22942Lys	E.323	rs199506676	VUS	VUS	DC	24.8	4.08	[202]
289	179443336	c.68329 + 2–68329 + 3insTT	–	I.321	rs536078303	LP	VUS	NA	NA	5.39	[246]
290	179443339	c.68328A > G	p.Thr22776 =	E.321	rs1553619783	VUS	VUS	DC	43	5.78	[199]
291	179443889	c.67868T > C	p.Ile22623Thr	E.320	NA	LP	NA	DC	31	5.98	[262]
292	179444012	c.67745del	p.Val22582AlafsTer10	E.320	NA	P	NA	DC	57	5.68	[199]
293	179444052	c.67705-67706insLINE1	–	E.320-I.319	NA	P	LP	NA	NA	NA	[219]
294	179444405	c.67519C > T	p.Gln22507Ter	E.319	rs1559490694	P	LP	DC	62	5.78	[196]
295	179444429	c.67495C > T	p.Arg22499Ter	E.319	rs574660186	P	P	DC	63	4.63	[202]
296	179444577	c.67349-2A > C	–	I.318	rs753948675	P	P	DC	NA	5.10	[263]
297	179444661	c.67348 + 5G > A	–	I.318	rs765587170	VUS	VUS	PO	NA	3.7	[199]
298	179444666	c.67348C > T	p.Gln22450Ter	E.318	NA	P	P	DC	62	2.24	[264]
299	179444735	c.67279C > T	p.Arg22427Ter	E.318	rs1200988060	P	LP	DC	63	0.99	[265]
300	179444855	c.67159del	p.Ile22387Ter	E.318	rs869312092	LP	VUS	DC	54	4.48	[202]
301	179444925	c.67089del	p.Lys22364ArgfsTer24	E.318	NA	P	NA	DC	56	1.07	[213]
302	179445166	c.66940G > T	p.Asp22314Tyr	E.317	rs768380109	LP	VUS	DC	24.6	5.25	[236]
303	179446219	c.66769 + 3–66769 + 7delAAGTAinsT	–	I.316	NA	LP	NA	NA	NA	4.29	[266]
304	179446300	c.66695T > A	p.Val22232Glu	E.316	NA	LP	NA	DC	31	5.41	[204]
305	179446471	c.66523-66524del	p.Leu22175IlefsTer8	E.316	rs866120156	P	NA	DC	52	2.96	[202]
306	179447667	c.65860-65863dup	p.Asp21955ValfsTer3	E313 –I.313	NA	P	NA	NA	57	3.88	[229]
307	179447693	c.65837C > G	p.Ser21946Ter	E.313	rs775504996	P	NA	DC	63	5.02	[267]
308	179448411	c.65498G > C	p.Arg21833Thr	E.312	NA	VUS	NA	DC	24.7	5.14	[205]
309	179448433	c.65476G > T	p.Glu21826Ter	E.312	rs763824247	P	LP	DC	63	6.02	[202]
310	179449208	c.65070del	p.Ile21691LeufsTer5	E.311	NA	P	NA	DC	57	4.15	[199]
311	179449453	c.64915C > T	p.Arg21639Ter	E.310	rs1432889079	P	LP	DC	63	4.3	[242]
312	179450018	c.64453C > T	p.Arg21485Ter	E.309	rs768345594	P	LP	DC	62	5.25	[202]
313	179451443	c.64185del	p.Ala21396LeufsTer26	E.308	NA	LP	NA	DC	56	− 10	[205]
314	179452145	c.63794-1G > A	–	I.306	rs2049262622	P	LP	DC	NA	5.98	[268]
315	179452435	c.63601C > T	p.Arg21201Ter	E.306	rs764243269	P	P	DC	63	4.92	[202]
316	179453427	c.63025C > T	p.Arg21009Ter	E.304	rs368452607	P	LP	DC	62	5.27	[202]
317	179453720	c.62733G > A	p.Trp20911Ter	E.304	NA	P	NA	DC	63	6.07	[243]
318	179453730	c.62722C > T	p.Arg20908Ter	E.304	rs543860009	P	P	DC	62	− 3.88	[224]
319	179453946	c.62506C > T	p.Arg20836Ter	E.304	rs757231565	P	VUS	DC	63	4.14	[202]
320	179454235	c.62217T > A	p.Tyr20739Ter	E.304	rs727503586	P	P	DC	62	2.63	[199]
321	179454531	c.61921C > T	p.Arg20641Ter	E.304	rs878854324	P	P	DC	63	5.2	[268]
322	179454576	c.61876C > T	p.Arg20626Ter	E.304	rs72646846	P	P	DC	62	5.17	[242]
323	179454770	c.61682C > G	p.Ser20561Ter	E.304	rs1114167324	P	LP	DC	62	4.21	[244]
324	179454784	c.61668del	p.His20557MetfsTer20	E.304	NA	LP	NA	DC	54	− 0.84	[223]
325	179454957	c.61495C > T	p.Arg20499Ter	E.304	rs869312112	P	LP	DC	62	3.97	[224]
326	179455112	c.61339del	p.Ile20447Ter	E.304	rs1576086839	P	LP	DC	52	6.11	[243]
327	179455162	c.61290T > A	p.Cys20430Ter	E.304	NA	P	NA	DC	63	6.11	[199]
328	179455521	c.60931C > T	p.Arg20311Ter	E.304	rs869312055	P	LP	DC	62	5.23	[202]

Continued

www.nature.com/scientificreports/

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
329	179455598	c.60854-60855insG	p.Asn20286LysfsTer13	E.304	NA	P	LP	DC	55	5.535	[205]
330	179455719	c.60733C > T	p.Arg20245Ter	E.304	rs1057522256	P	P	DC	62	4.26	[205]
331	179455726	c.60726T > A	p.Tyr20242Ter	E.304	rs145423907	LP	NA	DC	61	− 1.83	[202]
332	179455780	c.60672del	p.Gly20225GlufsTer7	E.304	NA	P	NA	DC	55	0.045	[205]
333	179456553	c.59993G > A	p.Trp19998Ter	E.303	NA	P	NA	DC	62	6.16	[79]
334	179456704	c.59926 + 1G > A	–	I.302	rs553526525	P	P	DC	NA	6.16	[269]
335	179456766	c.59865-59866insA	p.Gln19956ThrfsTer9	E.302	NA	P	NA	DC	45	4.98	[205]
336	179456783	c.59848C > T	p.Arg19950Ter	E.302	rs1559598775	P	LP	DC	63	5.16	[253]
337	179457005	c.59627-1G > A	–	I.301	rs869312073	P	LP	DC	NA	6.03	[202]
338	179457273	c.59460G > A	p.Trp19820Ter	E.301	rs1250461669	P	LP	DC	62	6.03	[225]
339	179457321	c.59411dup	p.Arg19805LysfsTer3	E.301	rs755261062	P	LP	NA	54	2.46	[202]
340	179457380	c.59352del	p.Glu19785SerfsTer2	E.301	rs869312111	P	LP	DC	53	5.01	[202]
341	179457644	c.59201-59202del	p.Pro19734ArgfsTer5	E.300	rs752948913	P	LP	DC	52	4.85	[257]
342	179457977	c.58958G > C	p.Arg19653Pro	E.299	NA	LP	NA	DC	32	6.16	[205]
343	179458080	c.58855del	p.Glu19619LysfsTer27	E.299	NA	LP	NA	DC	52	6.16	[199]
344	179458083	c.58852dup	p.Arg19618LysfsTer6	E.299	NA	LP	NA	NA	54	1.43	[205]
345	179458293	c.58732 + 2T > C	–	I.298	rs869312054	P	LP	DC	NA	6.02	[202]
346	179458407	c.58620del	p.Val19541PhefsTer22	E.298	rs1576147786	P	LP	DC	52	5.63	[210]
347	179458459	c.58567-58568dup	p.Lys19524ValfsTer8	E.298	rs1553650442	P	P	NA	53	3.26	[234]
348	179458477	c.58550T > C	p.Ile19517Thr	E.298	rs72646838	VUS	VUS	DC	24.8	5.86	[226]
349	179458850	c.58270G > T	p.Glu19424Ter	E.297	rs72646837	P	P	DC	63	6.17	[199]
350	179458948	c.58172del	p.Asp19391AlafsTer45	E.297	rs869312072	P	LP	DC	52	5.03	[202]
351	179459155	c.58066dup	p.Glu19356GlyfsTer27	E.296	NA	LP	NA	NA	54	4.11	[199]
352	179459226	c.57995del	p.His19332ProfsTer18	E.296	rs397517633	P	LP	DC	52	6.17	[80]
353	179460233	c.57847 + 1G > A	–	I.295	rs397517631	LP	VUS	DC	NA	6.07	[80]
354	179460312	c.57769C > T	p.Arg19257Ter	E.295	rs794729275	P	LP	DC	62	5.08	[270]
355	179460320	c.57761A > G	p.Tyr19254Cys	E.295	NA	VUS	NA	DC	33	5.98	[10]
356	179460363	c.57718C > T	p.Arg19240Ter	E.295	rs2051361827	P	LP	DC	62	3.94	[79]
357	179460478	c.57603C > A	p.Cys19201Ter	E.295	rs1418030810	P	LP	DC	62	5.17	[225]
358	179462264	c.57544 + 1G > A	–	I.294	rs2052045274	P	LP	DC	NA	6.06	[202]
359	179462478	c.57331C > T	p.Arg19111Ter	E.294	rs72646831	P	P	DC	62	4.23	[228]
360	179462682	c.57215del	p.Gly19072GlufsTer12	E.293	rs397517628	P	LP	DC	54	5.87	[80]
361	179463603	c.56834del	p.Gly18945ValfsTer6	E.291	rs869312110	P	LP	DC	53	4.97	[202]
362	179463948	c.56572C > T	p.Arg18858Ter	E.290	rs745376275	P	LP	DC	62	3.19	[271]
363	179464342	c.56286T > A	p.Tyr18762Ter	E.289	NA	P	NA	DC	62	− 1.01	[205]
364	179464422	c.56206del	p.Thr18736ProfsTer8	E.289	rs869312109	P	LP	DC	49	4.5	[202]
365	179466193	c.55525-55531del	p.Asp18509SerfsTer29	E.287	rs869312052	P	LP	DC	50	4.37	[202]
366	179466263	c.55460-55461del	p.Lys18487SerfsTer3	E.287	rs1064796230	P	P	DC	49	4.96	[272]
367	179466466	c.55351C > T	p.Arg18451Ter	E.286	rs1440093502	P	P	DC	62	5.83	[205]
368	179466515	c.55303-1G > A	–	I.285	rs748369265	P	VUS	DC	NA	6.07	[202]
369	179466726	c.55269 + 3A > G	–	I. 284	rs72646820	P	NA	NA	NA	4.92	[199]
Continued											

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
370	179468833	c.54581G > T	p.Gly18194Val	E.282	NA	LP	NA	DC	26.8	6.16	[205]
371	179469477	c.54339del	p.Glu18113AspfsTer10	E.281	rs796122911	P	LP	DC	51	4.33	[205]
372	179469738	c.54166C > T	p.Arg18056Ter	E.280	rs768431507	P	LP	DC	62	5.05	[272]
373	179469837	c.54067C > T	p.Arg18023Ter	E.280	rs1553682168	P	P	DC	62	4.83	[273]
374	179469882	c.54022G > A	p.Glu18008Lys	E.280	NA	P	NA	DC	23.9	5.74	[237]
375	179469986	c.53918del	p.Gly17973GlufsTer18	E.280	rs1486129583	P	P	DC	51	5.74	[199]
376	179470140	c.53881 + 1G > T	–	I.279	rs869312051	P	LP	DC	NA	5.63	[202]
377	179470359	c.53656-53663del	p.Pro17886Ter	E.279	NA	P	NA	DC	49	3.10	[205]
378	179471841	c.53488G > T	p.Gly17830Ter	E.278	rs759231562	P	LP	DC	62	5.35	[202]
379	179471975	c.53355G > A	p.Trp17785Ter	E.278	rs794729273	P	P	DC	62	5.99	[274]
380	179472042	c.53288-1G > C	–	I.277	rs1553685927	P	LP	DC	NA	5.99	[199]
381	179472127	c.53287 + 1G > T	–	I.277	rs1064794266	P	VUS	DC	NA	5.99	[199]
382	179472156	c.53259del	p.Lys17753AsnfsTer7	E.277	rs1389777522	P	LP	DC	48	5.19	[205]
383	179472209	c.53206C > T	p.Arg17736Ter	E.277	rs571702144	P	LP	DC	62	4.84	[275]
384	179472611	c.52903C > T	p.Arg17635Ter	E.276	NA	P	LP	DC	62	5.16	[276]
385	179473206	c.52406-2A > C	–	I.274	rs753798236	P	LP	DC	NA	5.72	[199]
386	179473427	c.52311-52312insTTGA	p.Gly17438LeufsTer12	E.274	NA	P	NA	DC	46	4.90	[205]
387	179473511	c.52223-52227dup	p.Asp17410ArgfsTer25	E.274	rs869312050	P	LP	NA	48	5.29	[202]
388	179473610	c.52128del	p.Phe17376LeufsTer27	E.274	rs869312095	LP	VUS	DC	49	3.55	[202]
389	179474002	c.52035-52036insTT	p.Leu17346PhefsTer4	E.273	rs869312049	P	LP	DC	51	2.55	[202]
390	179474121	c.51913-51916del	p.Lys17305ValfsTer13	E.273	rs747513278	P	LP	DC	50	1.81	[79]
391	179474220	c.51817G > T	p.Gly17273Ter	E.273	NA	P	NA	DC	61	5.85	[205]
392	179474816	c.51436 + 1G > A	–	I.271	rs761807131	P	P	DC	NA	5.48	[244]
393	179474817	c.51436C > T	p.Gln17146Ter	E.271	rs906494713	P	P	DC	62	5.48	[224]
394	179474936	c.51317G > A	p.Trp17106Ter	E.271	NA	P	NA	DC	61	5.48	[199]
395	179476484	c.50551 + 1G > A	–	I.268	rs188050862	LP	NA	DC	NA	5.18	[202]
396	179476569	c.50467C > T	p.Gln16823Ter	E.268	NA	P	NA	DC	62	5.08	[205]
397	179477005	c.50247del	p.Phe16749LeufsTer15	E.266	rs869312071	P	LP	DC	56	2.6	[202]
398	179477082	c.50170C > T	p.Arg16724Ter	E.266	rs794729265	P	P	DC	62	2.83	[202]
399	179477226	c.50026G > T	p.Glu16676Ter	E.266	NA	P	NA	DC	62	5.71	[196]
400	179477886	c.49648 + 2del	–	I.264	rs727504851	P	P	NA	NA	5.95	[199]
401	179478553	c.49458G > A	p.Trp16486Ter	E.263	rs869312108	P	LP	DC	61	6.07	[202]
402	179478665	c.49346-1G > A	–	I.262	rs869312070	P	P	DC	NA	6.07	[202]
403	179478861	c.49263C > A	p.Tyr16421Ter	E.262	NA	P	P	DC	58	0.84	[205]
404	179478865	c.49259del	p.Glu16420GlyfsTer23	E.262	NA	LP	NA	DC	55	6.07	[199]
405	179478953	c.49171C > T	p.Arg16391Ter	E.262	rs570046043	P	LP	DC	59	3.38	[277]
406	179479481	c.48761-1G > C	–	I.260	rs876657665	P	LP	DC	NA	5.63	[80]
407	179480145	c.48527G > A	p.Trp16176Ter	E.259	rs869312048	P	LP	DC	61	5.96	[202]
408	179480423	c.48405T > A	p.Cys16135Ter	E.258	rs371722903	LP	NA	DC	61	4.62	[202]
409	179480446	c.48382-48383insT	p.Lys16128IlefsTer6	E.258	rs771146720	LP	NA	DC	49	5.76	[202]
410	179481235	c.48283C > T	p.Arg16095Ter	E.257	rs374140736	P	P	DC	61	3.9	[202]

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
411	179481846	c.47875+1G>A	–	I.255	rs869312047	P	LP	DC	NA	5.76	[202]
412	179482115	c.47697C>A	p.Cys15899Ter	E.254	rs373040154	P	LP	DC	59	2.18	[202]
413	179482120	c.47692C>T	p.Arg15898Ter	E.254	rs775186117	P	LP	DC	61	0.77	[202]
414	179482230	c.47582G>A	p.Ser15861Asn	E.254	NA	VUS	NA	DC	28.2	6.08	[236]
415	179482584	c.47494C>T	p.Arg15832Ter	E.253	rs751746401	P	P	DC	62	4.74	[232]
416	179482662	c.47416del	p.Asp15806IlefsTer4	E.253	NA	P	NA	DC	53	5.63	[205]
417	179483042	c.47142-47143dup	p.Glu15715ValfsTer19	E.252	rs869312107	P	LP	NA	56	4.12	[202]
418	179483495	c.46782C>A	p.Tyr15594Ter	E.251	rs397517587	P	LP	DC	60	4.5999	[202]
419	179483504	c.46773T>A	p.Tyr15591Ter	E.251	rs397517586	P	LP	DC	57	3.1199	[80]
420	179485012	c.46236C>A	p.Cys15412Ter	E.248	rs368200299	P	LP	DC	60	2.73	[202]
421	179485178	c.46069-46070del	p.Met15357ValfsTer4	E.248	rs397517584	P	LP	DC	51	5.0099	[80]
422	179485525	c.45812T>G	p.Leu15271Ter	E.247	rs869312046	P	LP	DC	60	5.83	[202]
423	179485581	c.45756dup	p.Tyr15253IlefsTer15	E.247	rs869312045	P	LP	NA	49	5.44	[202]
424	179485589	c.45732-45748del	p.Glu15245PhefsTer17	E.247	NA	P	NA	DC	60	4.23	[205]
425	179485878	c.45567C>A	p.Tyr15189Ter	E.246	NA	LP	NA	DC	48	-9.56	[278]
426	179485878	c.45566dup	p.Tyr15189Ter	E.246	NA	P	NA	DC	48	0.73	[218]
427	179486054	c.45391delA	p.Ile15131TyrfsTer46	E.246	rs869312091	LP	VUS	DC	61	3.71	[202]
428	179486229	c.45322C>T	p.Arg15108Ter	E.245	rs1060500405	P	VUS	DC	61	6.17	[243]
429	179486244	c.45307C>T	p.Arg15103Ter	E.245	rs397517580	P	VUS	DC	61	3.01	[80]
430	179487411	c.44899C>T	p.Arg14967Ter	E.243	rs727505350	P	VUS	DC	60	2.65	[205]
431	179487495	c.44816-1G>A	–	I.242	rs749705939	P	VUS	DC	NA	5.54	[210]
432	179489209	c.44798G>A	p.Cys14933Tyr	E.242	NA	LP	NA	DC	28	5.72	[279]
433	179490056	c.44492G>C	p.Gly14831Ala	E.241	NA	LP	NA	DC	27.3	5.95	[279]
434	179494088	c.44364del	p.Tyr14789ThrfsTer15	E.240	rs397517576	P	VUS	DC	54	0.52	[205]
435	179494967	c.44281+1G>A	–	I.239	rs771562210	P	VUS	DC	NA	6.04	[202]
436	179494968	c.44281C>T	p.Pro14761Ser	E.239	rs192766485	VUS	VUS	DC	25.2	6.04	[202]
437	179494977	c.44272C>T	p.Arg14758Ter	E.239	rs140743001	P	VUS	DC	61	3.14	[202]
438	179495983	c.43792del	p.Val14598Ter	E.237	rs869312044	P	LP	DC	49	3.81	[202]
439	179497082	c.43539-43540insA	p.Ala14514SerfsTer10	E.236	NA	P	NA	DC	45	5.51	[199]
440	179497414	c.43319G>A	p.Trp14440Ter	E.235	rs372663057	LP	VUS	DC	60	6.16	[202]
441	179498055	c.42947-2A>G	–	I.232	rs1553741357	P	VUS	DC	NA	6.17	[199]
442	179498176	c.42909-42910del	p.Cys14303TrpfsTer12	E.232	rs1114167333	P	LP	DC	56	4.69	[244]
443	179498592	c.42636del	p.Ala14213LeufsTer6	E.231	rs869312106	P	LP	DC	57	0.47	[202]
444	179500295	c.41756A>G	p.Asp13919Gly	E.227	NA	VUS	NA	DC	24.4	6.05	[237]
445	179500825	c.41473C>T	p.Arg13825Ter	E.226	rs869312043	P	VUS	DC	57	0.36	[202]
446	179500851	c.41447del	p.Gly13816AlafsTer18	E.226	rs869312042	P	LP	DC	47	5.8	[202]
447	179505267	c.40723+1G>T	–	I.221	rs371770198	LP	VUS	DC	NA	0.36	[202]
448	179506963	c.40558+1G>A	–	I.219	rs368219776	LP	VUS	DC	NA	5.55	[199]
449	179506964	c.40558G>C	p.Val13520Leu	E.219	rs587780488	P	VUS	DC	24.9	5.55	[199]
450	179514543	c.39895+1G>T	–	I.211	179514543	LP	VUS	DC	NA	5.58	[202]
451	179516234	c.39492dup	p.Glu13165Ter	E.207	NA	P	NA	DC	55	5.22	[213]

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
452	179516991	c.39211G > T	p.Val13071Phe	E.203	rs1334646153	LP	VUS	DC	22.2	3.64	[199]
453	179516996	c.39204-39206dup	p.Thr13069dup	E.203	NA	VUS	NA	NA	60	0.72	[210]
454	179517379	c.39043 + 1G > T	–	I.201	rs373516134	LP	NA	DC	NA	5.64	[202]
455	179517464	c.38960–3-38960-1del	–	I.200	rs773282707	LP	NA	DC	NA	5.64	[202]
456	179523240	c.37579-37582del	p.Lys12527HisfsTer419	E.184	NA	P	NA	DC	43	2.07	[266]
457	179526509	c.37262del	p.Lys12421SerfsTer526	E.180	rs867008501	LP	NA	DC	60	2.65	[202]
458	179532021	c.35739dup	p.Pro11914SerfsTer7	E.162	rs968544783	LP	VUS	NA	46	0.67	[202]
459	179532190	c.35692A > T	p.Arg11898Ter	E.161	rs188568710	LP	NA	DC	52	4.84	[202]
460	179535816	c.35308 + 1G > T	–	I.156	rs1423135750	P	VUS	DC	NA	5.92	[199]
461	179537361	c.34855 + 1G > A	–	I.153	rs377319699	LP	VUS	DC	NA	5.25	[202]
462	179542346	c.34291 + 2T > C	–	I.146	rs186084940	LP	NA	DC	NA	6.17	[202]
463	179542507	c.34132del	p.Leu11378TyrfsTer90	E.146	rs869025551	LP	VUS	DC	53	-0.01	[243]
464	179544666	c.33535del	p.Glu11179SerfsTer3	E.140	rs757135518	LP	NA	DC	47	3.86	[202]
465	179544980	c.33418 + 1G > A	–	I.139	rs746588865	LP	VUS	DC	NA	4.98	[202]
466	179547631	c.32888-1del	–	I.134	rs869312041	P	VUS	DC	NA	5.18	[202]
467	179549632	c.32554 + 1G > C	–	I.130	rs376018437	LP	VUS	DC	NA	5.81	[202]
468	179549717	c.32471-1G > A	–	I.129	rs371725574	P	VUS	DC	NA	5.81	[202]
469	179554062	c.31966A > T	p.Lys10656Ter	E.124	rs368775510	LP	NA	DC	40	2.7	[202]
470	179554624	c.31763-1G > A	–	I.121	rs202234172	P	VUS	DC	NA	5.29	[227]
471	179558336	c.31594G > T	p.Val10532Phe	E.119	rs763955552	VUS	VUS	DC	24	5.85	[202]
472	179558736	c.31427-1G > A	–	I.117	NA	P	NA	DC	NA	6.16	[199]
473	179559325	c.31426 + 1G > C	–	I.117	rs6749719	LP	VUS	DC	NA	6.07	[202]
474	179559557	c.31347del	p.Val10450TyrfsTer25	E.116	NA	P	NA	DC	61	5.26	[205]
475	179560998	c.30803-2A > G	–	I.113	rs869312089	LP	VUS	DC	NA	5.5	[202]
476	179563643	c.30683-2del	–	I.111	rs1553868981	LP	VUS	DC	NA	5.57	[202]
477	179566913	c.30484-30493del	p.Thr10162CysfsTer3	E.108	rs727504452	P	LP	DC	61	3.22	[199]
478	179567322	c.30292G > T	p.Glu10098Ter	E.107	NA	P	NA	DC	58	5.72	[227]
479	179569962	c.29543G > A	p.Arg9848Gln	E.103	rs773444238	VUS	NA	DC	24.4	5.82	[280]
480	179571370	c.29231G > A	p.Arg9744His	E.102	rs760305440	VUS	VUS	DC	27.4	6.1	[280]
481	179571652	c.29071A > T	p.Lys9691Ter	E.101	rs376189903	LP	NA	DC	60	6.14	[202]
482	179571661	c.29062del	p.Ala9688GlnfsTer7	E.101	rs869312040	P	LP	DC	51	5.05	[202]
483	179571683	c.29042-2A > C	–	I.100	rs6716782	P	VUS	DC	NA	6.16	[202]
484	179572327	c.28967dup	p.Asp9656GlufsTer8	E.100	NA	LP	NA	NA	42	3.43	[202]
485	179575947	c.28016dup	p.Pro9340AlafsTer23	E.97	rs954237155	LP	NA	NA	54	2.52	[202]
486	179577042	c.27607G > A	p.Glu9203Lys	E.95	rs769097909	VUS	VUS	DC	25.7	5.88	[202]
488	179580418	c.25723G > A	p.Gly8575Arg	E.89	rs397517517	VUS	VUS	DC	24.2	5.33	[205]
489	179582078	c.25383del	p.Lys8461AsnfsTer5	E.88	rs1452206214	LP	NA	DC	61	-1.14	[202]
500	179582856	c.24863-24877del	p.Asp8288-Ile8293delinsVal	E.86	NA	LP	NA	DC	60	3.55	[210]
490	179583072	c.24749-24761del	p.Gly8250ValfsTer8	E.85	NA	LP	VUS	DC	60	2.80	[199]
491	179583429	c.24498dup	p.Val8167CysfsTer13	E.84	rs1282574211	P	NA	NA	41	3.99	[243]
492	179583702	c.24227-2A > G	–	I.83	rs373060681	P	NA	DC	NA	5.71	[202]

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
493	179584983	c.23386C > T	p.Arg7796Ter	E.81	rs748111134	LP	VUS	DC	51	5.91	202
495	179585718	c.23014-2328del	p.Ser7672-Ser7676del	E.79	NA		NA	DC	61	2.29	210
496	179586600	c.22788-22790delCATinsG	p.Asp7596GlufsTer16	E.78	NA	LP	NA	DC	60	5.08	266
497	179587599	c.22027C > T	p.Gln7343Ter	E.76	rs886043434	P	VUS	DC	50	5.8	196
498	179587773	c.21961G > A	p.Glu7321Lys	E.75	NA	P	NA	DC	23.2	5.95	210
499	179588844	c.21142C > T	p.Arg7048Ter	E73	rs770579313	LP	VUS	DC	41	1.61	202
501	179590572	c.20477C > A	p.Ser6826Ter	E.70	NA	P	NA	DC	42	4.85	214
502	179591958	c.20134del	p.Asp6712IlefsTer5	E.69	NA	P	NA	DC	51	6.17	199
503	179596801	c.16895T > C	p.Ile5632Thr	E.57	rs727504971	VUS	VUS	DC	23.1	6.17	262
504	179597846	c.16057C > T	p.Arg5353Ter	E.55	rs267599069	P	VUS	DC	47	5.29	281
505	179597615	c.16288C > T	p.Arg5430Ter	E.55	rs772235481	P	VUS	DC	44	5.29	282
506	179598224	c.15796C > T	p.Arg5266Ter	E.54	rs372277017	P	VUS	DC	45	1.13	202
507	179598245	c.15776-1G > T	–	I.53	rs869312094	LP	VUS	DC	NA	5.86	202
509	179598437	c.15679del	p.Ile5227SerfsTer29	E.53	NA	LP	NA	DC	59	0.07	202
510	179599054	c.15496 + 1G > A	–	I.52	rs397517481	P	VUS	DC	NA	5.86	210
511	179599091	c.15460G > A	p.Gly5154Ser	E.52	rs772907723	VUS	NA	DC	25.5	5.86	205
512	179602835	c.14344-14345delAGinsGA	p.Ser4782Asp	E.49	NA	VUS	NA	DC	61	5.8	97
513	179602866	c.14314T > C	p.Cys4772Arg	E.49	NA	VUS	NA	DC	26.6	5.8	205
514	179603088	c.14093-1G > A	–	I.48	rs869312099	P	VUS	DC	NA	5.37	202
515	179603867	c.14092 + 1G > T	–	I.48	NA	P	NA	DC	NA	5.62	205
516	179603904	c.14056del	p.Thr4686GlnfsTer9	E.48	rs869312104	P	LP	DC	49	0.97	202
517	179604264	c.13696C > T	p.Gln4566Ter	E.48	rs775072385	LP	VUS	DC	36	4.95	199
518	179604345	c.13615-13616insT	p.Asn4539IlefsTer5	E.48	NA	LP	NA	DC	61	– 5.64	205
519	179604363	c.13597del	p.Glu4533LysfsTer38	E.48	NA	LP	NA	DC	55	3.84	205
520	179604368	c.13592C > G	p.Ser4531Ter	E.48	NA	P	P	DC	36	4.91	199
521	179604528	c.13432-13433insA	p.Cys4478Ter	E.48	NA	LP	NA	DC	56	4.66	230
522	179604852	c.13108C > T	p.Gln4370Ter	E.48	rs267607158	P	P	DC	35	5.02	97
523	179604950	c.13010del	p.Lys4337SerfsTer14	E.48	NA	P	NA	DC	49	3.46	199
524	179598224	c.15796C > T	p.Arg5266Ter	E.48	rs372277017	P	VUS	DC	45	1.13	202
525	179605203	c.12757C > T	p.Gln4253Ter	E.48	rs869312039	P	LP	DC	38	4.91	202
526	179605317	c.12643C > T	p.Gln4215Ter	E.48	rs368329612	LP	VUS	DC	35	2.51	202
527	179605373	c.12587C > A	p.Ser4196Ter	E.48	rs370912401	P	VUS	DC	35	3.39	202
528	179605482	c.12478del	p.Thr4160ProfsTer8	E.48	NA	P	VUS	DC	40	1.23	205
529	179605512	c.12438-12448del	p.Ser4147ThrfsTer20	E.48	rs1553939749	P	LP	DC	43	– 0.52	244
530	179605752	c.12208G > T	p.Glu4070Ter	E.48	rs397517830	P	LP	DC	36	4.73	283
531	179606008	c.11952C > A	p.Tyr3984Ter	E.48	NA	P	NA	DC	38	0.85	205
532	179606286	c.11674T > C	p.Cys3892Arg	E.48	NA	VUS	NA	DC	21.9	6.08	205
533	179606303	c.11657del	p.Asp3886ValfsTer22	E.48	rs397517826	P	VUS	DC	60	6.08	80
534	179606362	c.11598C > A	p.Tyr3866Ter	E.48	NA	P	NA	DC	37	6.08	205
535	179606445	c.11497-11515del	p.Met3833CysfsTer3	E.48	NA	P	VUS	DC	60	1.90	205
536	179612657	c.11311 + 5184_11311 + 5194dup	–	I.47	rs869312088	VUS	VUS	NA	NA	0.83	202

Continued

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
537	179611822	c.11312-5174del	–	I.47	rs869312097	VUS	VUS	DC	NA	3.71	202
538	179611814	c.11312-5166C > T	–	I.47	rs376396183	VUS	NA	DC	NA	1.64	202
539	179610598	c.11312-3950del	–	I.47	rs774991940	VUS	VUS	DC	NA	3.58	202
540	179612712	c.11311 + 5139del	–	I.47	rs750893661	VUS	NA	DC	NA	1.18	202
541	179616552	c.11311 + 1299T > A	–	I.47	rs1561044021	P	NA	DC	NA	3.52	205
542	179616684	c.11311 + 1167del	–	I.47	rs869312096	VUS	VUS	DC	NA	2.36	202
543	179613467	c.11311 + 4384dup	–	I.47	rs771985828	VUS	VUS	DC	NA	1.80	202
544	179613188	c.11311 + 4663del	–	I.47	rs781363456	VUS	VUS	DC	NA	3.13	202
545	179613422	c.11311 + 4429G > T	–	I.47	rs372994805	VUS	VUS	DC	NA	4.68	202
546	179613717	c.11311 + 4134dup	–	I.47	rs768458450	VUS	VUS	NA	NA	3.61	202
547	179610611	c.11312-3963G > T	–	I.47	rs148430495	VUS	VUS	DC	NA	5.94	202
548	179614105	c.11311 + 3746C > G	–	I.47	rs763408700	LP	VUS	DC	NA	5.25	202
549	179610383	c.11312-3735G > T	–	I.47	rs143376837	VUS	VUS	DC	NA	6.17	202
550	179614541	c.11311 + 3310G > T	–	I.47	rs372772094	VUS	NA	DC	NA	4.95	202
551	179615375	c.11311 + 2476G > T	–	I.47	rs373480236	VUS	NA	DC	NA	4.66	202
552	179616345	c.11311 + 1506del	–	I.47	rs777963995	VUS	NA	DC	NA	2.31	202
553	179620948	c.11254 + 1G > C	–	I.46	rs192945689	LP	NA	DC	NA	2.21	202
554	179620947	c.11254 + 2T > C	–	I.46	rs199565715	LP	VUS	DC	NA	0.77	202
555	179621013	c.11190C > G	p.Tyr3730Ter	E.46	rs373667402	LP	VUS	DC	38	1.99	202
556	179621020	c.11183dup	p.Leu3729ThrfsTer9	E.46	rs778172350	P	VUS	NA	61	3.38	202
557	179621090	c.11113del	p.Arg3705AspfsTer2	E.46	rs746386040	LP	VUS	DC	59	6.16	202
558	179621351	c.10852C > T	p.Gln3618Ter	E46	rs779064556	LP	VUS	DC	39	4.31	202
559	179621404	c.10799C > A	p.Ser3600Ter	E.46	rs374300381	LP	VUS	DC	40	6.17	202
560	179622355	c.10592C > G	p.Ser3531Ter	E.45	rs767420661	LP	VUS	DC	38	5.12	202
561	179622472	c.10475-10476insAGAC	p.Lys3493AspfsTer10	E.45	NA	LP	NA	DC	60	5.57	210
562	179623709	c.10303 + 2T > C	–	I.44	rs371596417	P	VUS	DC	NA	6.03	202
563	179629492	c.9749-9750del	p.Val3250AlafsTer40	E.42	rs1445295628	LP	NA	DC	60	1.06	202
564	179629515	c.9727C > T	p.Gln3243Ter	E.42	rs869312093	LP	VUS	DC	38	5.69	202
565	179631234	c.9577C > T	p.Arg3193Ter	E.41	rs746115846	P	VUS	DC	36	0.59	211
566	179632509	c.9448C > T	p.Arg3150Ter	E.40	rs146572907	P	VUS	DC	43	5.11	202
567	179632576	c.9381C > A	p.Tyr3127Ter	E.40	NA	P	LP/P	DC	36	2.19	205
568	179632841	c.9205del	p.Val3069TyrfsTer23	E.39	NA	P	NA	DC	61	2.956	243
569	179632884	c.9164-2A > T	–	I.38	rs777369921	LP	VUS	DC	NA	5.73	202
570	179633403	c.9160G > C	p.Glu3054Gln	E.38	–	VUS	NA	DC	23.2	5.81	249
571	179633431	c.9132del	p.Ala3045GlnfsTer14	E.38	rs36059692	LP	NA	DC	52	3.46	202
572	179634417	c.8891-8892insC	p.Thr2965AspfsTer17	E.37	NA	P	NA	DC	59	4.49	205
573	179634544	c.8764G > T	p.Glu2922Ter	E.37	NA	P	NA	DC	38	5.93	205
574	179634621	c.8687C > T	p.Thr2896Ile	E.37	rs72647884	VUS	VUS	DC	27.1	6.06	226
575	179635166	c.8353G > T	p.Gly2785Ter	E.35	NA	P	NA	DC	36	5.19	243
576	179635211	c.8307-8308del	p.Ala2770HisfsTer4	E.35	rs869312037	P	VUS	DC	61	4.71	202
577	179636183	c.7871dup	p.Pro2625AlafsTer9	E.34	rs1553997502	LP	NA	NA	43	3.86	202
Continued											

No	Position on Chr. 2	HGVS DNA	HGVS Protein	Exon/Intron	dbSNP	ACMG	ClinVar	Mutation Taster	CADD	GERP	Reference
578	179638333	c.7450C > T	p.Gln2484Ter	E.32	NA	P	VUS	DC	37	5.82	[199]
579	179639171	c.6820C > T	p.Gln2274Ter	E.30	rs145649088	P	VUS	DC	36	− 1.22	[202]
580	179640236	c.6355G > T	p.Glu2119Ter	E.28	rs869312098	LP	VUS	DC	36	5.33	[202]
581	179640343	c.6248del	p.Arg2083LysfsTer56	E.28	rs72647879	P	NA	DC	61	3.54	[236]
582	179640343	c.6248G > T	p.Arg2083Ile	E.28	rs781676050	VUS	NA	DC	21.9	3.54	[236]
583	179640344	c.6247del	p.Arg2083GlufsTer56	E.28	NA	P	NA	DC	57	3.68	[199]
584	179640468	c.6123G > A	p.Trp2041Ter	E.28	179640468	LP	NA	DC	36	5.19	[202]
585	179640970	c.5622G > A	p.Trp1874Ter	E.28	rs777078420	P	NA	DC	38	5.09	[197]
586	179641014	c.5577G > C	p.Arg1859Ser	E.28	NA	VUS	NA	B	23.6	1.41	[284]
587	179641524	c.5067G > A	p.Trp1689Ter	E.28	rs375648277	P	NA	DC	37	5.33	[202]
588	179641962	c.4724-4728del	p.Met1575SerfsTer6	E.27	rs756433029	P	VUS	DC	60	4.81	[202]
589	179641976	c.4714C > T	p.Arg1572Ter	E.27	rs1554008881	P	VUS	DC	37	3.89	[203]
590	179643691	c.4118C > A	p.Ala1373Glu	E.24	NA	VUS	NA	DC	25	5.91	[205]
591	179644006	c.3913G > A	p.Gly1305Arg	E.23	NA	VUS	NA	DC	25.9	5.72	[205]
592	179644174	c.3742-3745del	p.Ser1248ProfsTer14	E.23	NA	P	LP	DC	61	5.48	[205]
593	179647331	c.3101-2A > T	–	I.18	rs1060500467	P	VUS	DC	NA	5.54	[199]
594	179647533	c.3100G > A	p.Val1034Met	E18	rs142951505	VUS	VUS	DC	24	6.17	[202]
595	179647588	c.3045C > G	p.Cys1015Trp	E.18	NA	VUS	NA	DC	25	4.09	[223]
596	179647599	c.3034C > T	p.Arg1012Ter	E.18	rs397517547	P	VUS	DC	36	3.03	[80]
597	179647707	c.2926T > C	p.Trp976Arg	E.18	rs267607155	P	LP	DC	24.5	6.17	[285]
598	179647707	c.2926T > A	p.Trp976Arg	E.18	rs267607155	P	NA	DC	24.5	6.17	[10]
599	179648447	c.2841G > T	p.Ser947 =	E.17	rs774074192	LP	VUS	DC	45	1.07	[202]
600	179649078	c.2494G > T	p.Ala832Ser	E.16	rs376133574	P	VUS	DC	22.3	5.52	[202]
601	179650574	c.2370 + 1G > T	–	I.14	rs375796806	LP	NA	DC	NA	4.99	[202]
602	179650717	c.2228C > T	p.Ala743Val	E.14	rs267607157	VUS	P	PO	19.42	5.3	[97]
603	179650808	c.2137C > T	p.Arg713Ter	E.14	rs727505277	P	VUS	DC	39	5.99	[205]
604	179658212	c.1455dup	p.Ala486SerfsTer26	E.9	rs758662735	P	NA	NA	60	3.36	[243]
605	179659281	c.1246-3del	–	I.7	NA	VUS	NA	DC	NA	1.44	[199]
606	179659646	c.1245 + 3A > G	–	I.7	rs757221300	LP	VUS	DC	NA	5.87	[202]
607	179613717	c.11311 + 4134dup	–	I.47	rs768458450	VUS	VUS	NA	NA	3.61	[202]
608	179664231	c.897-898insT	p.Thr300TyrfsTer23	E.6	NA	P	NA	DC	58	− 2.83	[205]
609	179664293	c.835C > T	p.Arg279Trp	E.6	rs138060032	LP	VUS	DC	24.5	4.82	[191]
610	179665172	c.533C > A	p.Ala178Asp	E.4	NA	LP	NA	DC	23.4	5.16	[286]
611	179665380	c.325C > T	p.Arg109Ter	E.4	rs150954246	LP	VUS	DC	38	3.8	[202]

Table 1. Bioinformatics analysis of Pathogenic, Likely pathogenic, Unknown Significance reported variants in *TTN* related to cardiomyopathies.

The gene *MYBPC3*, which codes for cardiac myosin-binding protein C, is the most important gene in this process accounting for up to half of the mutations identified[92–94]. In the second place, *MYH7*, which is responsible for encoding the beta-myosin heavy chain, is present in approximately 15–25% of patients diagnosed with HCM[92,95].

In comparison to other plausible etiologies of HCM, the presence of the *TTN* gene mutations exhibits a relatively low ranking. Several studies reported four *TTN* variants resulting in gain-of-function effects in HCM patients. Satoh et al.[96] found a Z-line mutation (c.2219G > T, p.Arg740Leu) which increases alpha-actinin binding affinity. Two studies, similarly, reported a mutation in cardiac-specific N2B exon 49 [c.12347C > A, p.Ser4116Tyr] resulting in increased TTN binding to DRAL/FHL2[97,98]. The TTN/T-CARP interaction is reinforced by the presence of two mutations located in exons 103 and 104-N2A, c.29231G > A, p.Arg9744 (initially reported as p.Arg8500His) and c.29543G > A, p.Arg9848Gln (initially reported as p.Arg8604Gln), as reported by Arimura et al.[99]. Lopes et al., in a different study, reported 219 *TTN* variants in a population of unrelated HCM patients. Of those 87% coexisted with mutations in HCM-related sarcomere gene defects and only 13% were found isolated[26,100]. However, in a study on 90 HCM patients and their close relatives, the mutation screening revealed no clue of the *TTN* gene being involved in their pathogenesis[101]. Similarly, Martijn Bos et al.[102] detected no *TTN* mutation in a group of 389 HCM patients.

Restrictive cardiomyopathy

Restrictive cardiomyopathy is a diverse collection of disorders that primarily affect the myocardium, with a lesser impact on the endocardium and sub-endocardium. It is characterized by increased stiffness of the ventricular walls leading to restricted ventricular filling, which consequently results in significant diastolic dysfunction, elevated end-diastolic pressure, and reduced ejection fraction in the advanced stages[103,104].

The epidemiology of this disease is not well understood in the literature due to classification and etiology reporting difficulties, but RCM is surely the least common form of cardiomyopathies, representing 2% to 5% of cases[2,105]. There are a variety of diseases that can cause it, including infiltrative disorders like amyloidosis and sarcoidosis, non-infiltrative disorders like diabetes and scleroderma, storage disease, endomyocardial disease, and cardiotoxicity brought on by chemotherapy or radiotherapy[2].

Numerous genes that encode non-sarcomeric, sarcomeric, and sarcomere-associated proteins have been shown to play a role in RCM occurrence and inheritance. Examples include the *TTR* gene variants (V122I; I68L; L111M; T60A; S23N; P24S; W41L; V30M; V20I) and *APOA1* gene in Amyloidosis; *GLA* gene in Fabry disease; GBA gene in Gaucher disease; *HAMP*, *HFE*, *HFE2*, *HJV*, *PNPLA3*, *SLC40A1*, *TfR2* genes in Hereditary hemochromatosis; *NPC1*, *NPC2* and *SMPD1* genes in Niemann-Pick disease; *AG3, CRYAB, DES, DNAJB6, FHL1, FLNC, LDB3,* and *MYOT* genes in Myofibrillar myopathies; *ABCC6* gene in Pseudoxanthoma elasticum; *ACTC, MHC, TNNT2, TNNI3, TNNC1, DES, MYH, MYL3*, and *CRYAB* genes in Sarcomeric protein disorders; *WRN* gene in Werner's syndrome; and *BMP5*, *BMP7* and *TAZ* genes in Endocardial fibroelastosis[1,2,106,107].

The role of *TTN* variants in RCM is relatively unknown and more investigations are needed to illustrate this fact. In 2013, for the first time, Peled et al. discovered a novel missense mutation (c.50057A > G, p.Tyr16686Cys) in the intersection of the A and I regions of Titin (IA junction). This mutation was found to play a role in early-onset familial RCM, which affected six members of a family. They asserted that Titin determines the sarcomere's resting tension, and their study offers genetic proof of its critical significance in diastolic function.[36,108,109]. In another study, Kizawa et al.[110] found another novel *TTN* missense mutation (c.22769C > A, p.P7590Q) in a young boy with neurofibromatosis type 1, which is thought to be responsible for RCM co-occurrence. This de novo mutation is also located at the IA junction.

Arrhythmogenic right ventricular cardiomyopathy (ARVC)

Arrhythmogenic cardiomyopathy (ACM), is a rare and potentially life-threatening heart muscle disease with a prevalence of approximately 1:1000 to 1:5000[111–113]. Although asymptomatic in most instances upon diagnosis, it is characterized by palpitations, atypical chest pain, and syncope caused by cardiac arrhythmia, mostly in the right ventricle, which leads to the term "arrhythmogenic right ventricular cardiomyopathy (ARVC)"[114–116]. This condition is characterized by the progressive replacement of the myocardium with fibrofatty tissue, a process that begins at the epicardium, turns into a regional wall motion abnormality, and eventually spreads throughout the myocardium, resulting in the development of ventricular dilation and multiple aneurysms[117–119].

The primary etiology of ACM is attributed to mutations in genes that encode desmosomal proteins, mainly with an autosomal dominant pattern of inheritance and over 30 percent of cases being familial. *JUP*, *DSP*, *PKP2*, *DSG2*, and *DSC2* genes are the most probable to be involved. *LMNA* and *TMEM43* are two additional genes that have been linked to the nuclear envelope, and there are genes that are shared with other cardiomyopathies (such as *DES*, *PLN*, *TGFB3*, *TTN*, and *SCN5A*)[112,120–123].

Several studies have been conducted on the role of *TTN* variations in the pathogenesis of ARVC. In one study by Taylor et al.[121], eight novel *TTN* variants (c.C29453T, p.Thr2896Ile; c.A97341G, p.Tyr8031Cys; c.C106734T, p.His8848Tyr; c.T215598C, p.Ile16949Thr; c.G221380A, p.Ala18579Thr; c.G226177T, p.Ala19309Ser; c.C272848T, p.Pro30847Leu; c.T281801C, p.Met33291Thr) were identified in seven unrelated families with well-established ARVC. They claimed the most prominent variant was Thr2896Ile, showing strong segregation evidence. In another investigation on the phenotype-genotype relationship of ARVC in 39 families, Brun et al.[123] found 13% of their studied population, had rare *TTN* variants (c.29453C > T, p.Thr2896Ile; 281801T > C, Ala18579Thr; c.221380AG > T, p.Met33291Thr; c.226177G > T, p.Ala19309Ser; c.97341G > A, p.Tyr8031Cys; c.272848C > T, p.Pro30847Leu). In the investigation of the levels of Novex variant expression in human hearts with cardiomyopathies, Chen et al.[124] came to the conclusion that this factor was altered in cardiomyopathies such as DCM and ARVC.

Other muscle disorders

Beyond cardiomyopathies, TTN mutations are implicated in numerous non-cardiac muscle disorders. According to Chauveau et al.[26], 39 TTN mutations have been identified so far in four pure skeletal muscle myopathies: limb girdle muscular dystrophy type 2J (LGMD2J), late-onset autosomal dominant tibial muscular dystrophy (TMD), hereditary myopathy with early respiratory failure (HMERF), and congenital centronuclear myopathy (CNM). Additional conditions associated with TTN variants include early adult onset recessive distal titinopathy, early-onset myopathy with fatal cardiomyopathy, multi-minicore disease with heart disease, childhood-juvenile Emery-Dreifuss-like phenotype without cardiomyopathy, and adult-onset recessive proximal muscular dystrophy[125].

Frequent TTN-related molecules in cardiomyopathies

There are several molecules which play a considerable role in the signaling and function of Titin. In the present study, we evaluated their interaction with Titin and consider their interaction with Titin in the pathogenesis of cardiomyopathies (Fig. 4).

Calpain

Calpain, a family of Ca^{2+}-dependent cytosolic cysteine proteases, plays a role in various cellular processes, including cell death and tissue remodeling[126]. It has been implicated in several cardiac conditions, including dilated cardiomyopathy, alcohol-related cardiomyopathy, chemotherapy-induced cardiomyopathy, arrhythmogenic cardiomyopathy, and diabetic cardiomyopathy[127–131]. Sustained over-expression of calpain-2, specifically in cardiomyocytes, induced age-dependent dilated cardiomyopathy in mice[127].

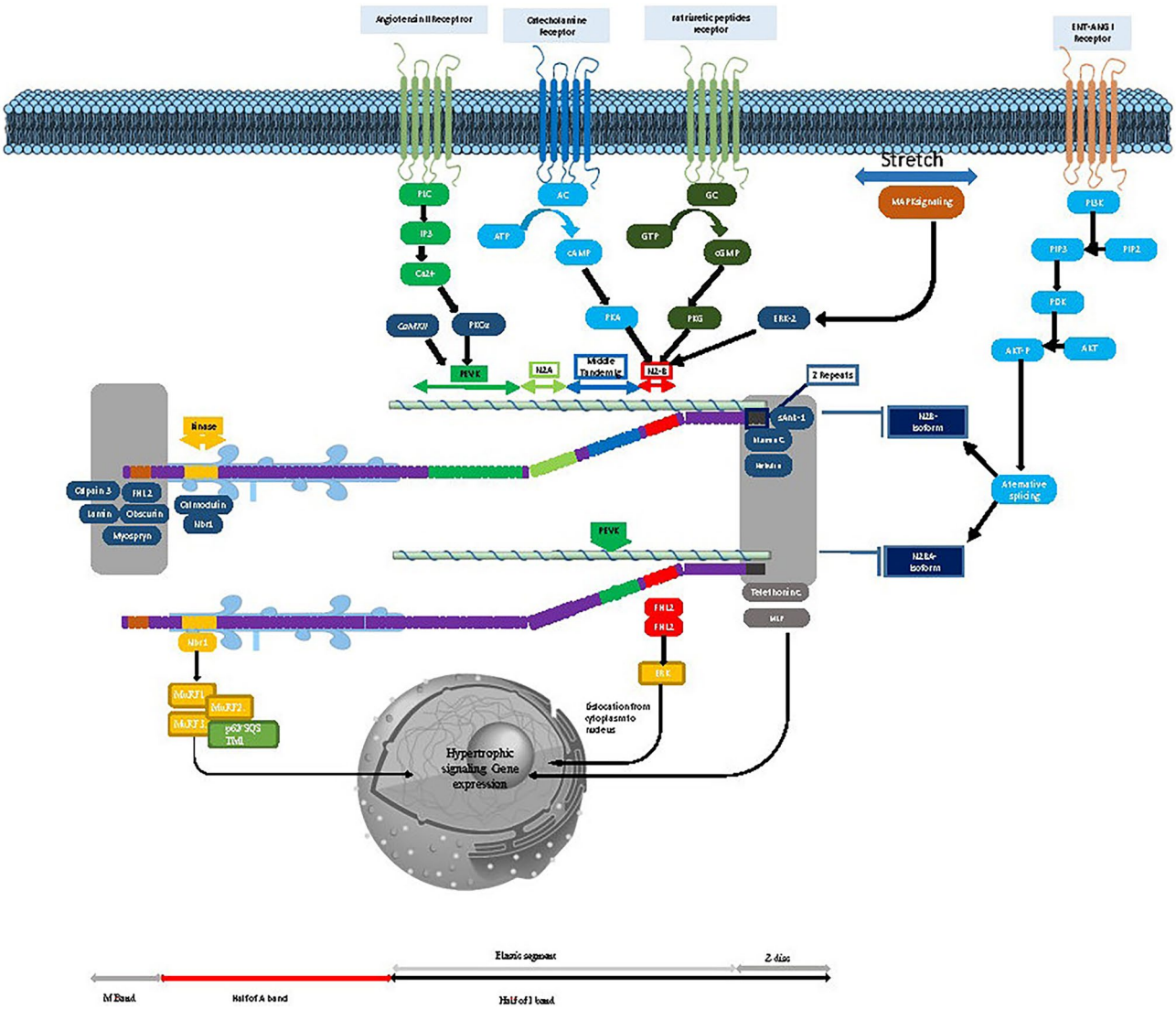

Figure 4. Illustration of the intricate signaling pathway implicated in the development of cardiomyopathy associated with Titin and other related proteins.

MuRF1/2

Muscle ring finger (MuRF) proteins are muscle-specific ubiquitin E3 ligases that regulate the ubiquitin–proteasome system and modulate cardiac mass and function[132]. A study by Su et al.[133] showed a higher prevalence of rare MuRF1 and MuRF2 variants in hypertrophic cardiomyopathy (HCM) patients compared to controls. HCM patients with these rare MuRF1/2 variants were younger and had greater maximum left ventricular wall thickness than those without the variants[133].

ERK

ERK (Extracellular signal-regulated kinase) plays a central role in cardiac physiology and hypertrophy[134–136]. ERK signaling is implicated in various forms of cardiac hypertrophy and progression to heart failure[135]. Altered ERK activity has been linked to HCM[134]. ERKs are considered key regulators of cardiac hypertrophy since they are activated by most, if not all, stress stimuli known to induce hypertrophic growth[137]. Studies show that concurrently eliminating ERK1 and ERK2 in the heart leads to eccentric hypertrophy with chamber dilatation and cardiomyocyte elongation[136].

NFAT

Nuclear factor of activated T-cells (NFAT) transcription factors are implicated in developing cardiac hypertrophy and heart failure[138]. Activation of NFAT signaling induces pathological remodeling of cardiomyocytes[139]. Inhibition of NFAT prevents maladaptive cardiac growth in response to stress stimuli[140]. Targeting NFAT signaling pathways may be therapeutic for specific cardiomyopathies[141,142].

FHL1/2

Mutations in the four-and-a-half LIM domain proteins 1 and 2 (FHL1 and FHL2) are associated with reducing body myopathy and hypertrophic cardiomyopathy[143]. FHL1/2 are involved in sarcomere assembly and signaling and highly expressed in skeletal and cardiac muscle[144,145]. Abnormal FHL proteins cause structural defects in sarcomeres and impaired muscle contraction[146]. FHL1 mutations account for 8–10% of familial reducing body myopathy cases which can include cardiomyopathy[147,148]. Chu et al.[145] reported FHL1 upregulation in Cardiac ventricles of two mouse models with cardiac hypertrophy and dilated cardiomyopathy.

MARP

Muscle ankyrin repeat proteins (MARPs), including CARP, Ankrd1/2, and DARP, are a family of ankyrin repeat proteins expressed in striated muscle that are induced by stress. MARPs play regulatory roles in the muscle stress response and hypertrophy pathogenesis[149]. Overexpression of CARP is linked to dilated cardiomyopathy in animal models[150]. In addition, Patients with hypertrophic, dilated, ischemic, and arrhythmogenic right ventricular cardiomyopathy are more likely to develop CARP upregulation[62,149,151,152]. Missense mutations in the Ankrd1 gene have recently been identified as the cause of dilated and hypertrophic cardiomyopathy in humans[99,149,153,154]. CARP modulation of gene expression may contribute to adverse ventricular remodeling in cardiomyopathies[155].

Nbr1

Neighbor of BRCA1 gene 1 (Nbr1) is a cardiac-expressed protein involved in autophagy, protein degradation and sarcomere organization[156]. Several studies suggested role of Nbr1 overexpression in developing dilated cardiomyopathy[157–159].

SRF

Serum response factor (SRF) is a transcription factor regulating cardiac gene expression important for adaptation to stress[160]. SRF inactivation in animal models causes dilated cardiomyopathy[160]. SRF likely controls genes involved in maintaining normal cardiac structure and function[161]. Alterations in SRF-dependent gene regulation may underlie some cardiomyopathies[162].

MLP

Muscle LIM protein (MLP) is involved in mechanosensing and stretch response in cardiomyocytes[163]. MLP knockout mice develop dilated cardiomyopathy[164]. Loss of MLP leads to impaired myocyte stretch signaling and contraction[165]. MLP deficiency is implicated in some forms of familial dilated cardiomyopathy[166].

MyBP-C

Myosin binding protein C (MyBP-C) is important for maintaining sarcomere structure and regulating muscle contraction[167]. Mutations in cardiac MyBP-C are the most common cause of hypertrophic cardiomyopathy[168]. Abnormal MyBP-C disrupts sarcomere function leading to reduced contractility and development of hypertrophy[169].

Myomesin

Myomesin is a major component of the sarcomeric M-band involved in thick filament organization[170]. Myomesin mutations have been associated with hypertrophic and dilated cardiomyopathy in some patients[171]. Altered myomesin disrupts myofilament integrity and crosstalk resulting in cardiomyocyte damage[172].

Sh2 domain
Src homology 2 (SH2) domains mediate protein–protein interactions in cell signaling cascades[173]. Mutations affecting SH2 domains of ZASP/Cypher proteins are linked to dilated cardiomyopathy[174]. Disruption of ZASP protein interactions likely impairs structural organization and signaling processes in cardiac muscle[175].

Ras
Ras family small GTPases regulate growth and survival signaling[176]. Constitutively active mutant Ras expressed in mouse hearts causes dilated cardiomyopathy phenotype[177]. Hyperactive Ras leads to increased cell growth, altered metabolism and myocardial dysfunction[178].

Raf
Raf kinases act downstream of Ras to activate MEK/ERK signaling involved in cell proliferation and differentiation[179]. Cardiac-specific expression of activated Raf in transgenic mice induces dilated cardiomyopathy[180].

Alpha actinin
Alpha-actinin-2 (ACTN2) is the sole muscle isoform of α-actinin expressed in cardiac muscle[181]. Previous studies have shown that novel ACTN2 variants are associated with familial HCM[182]. Previous studies have shown that novel ACTN2 variants are associated with[181]. Mutations in ACTN2 have been linked to mild to moderate forms of HCM[181]. Disease modeling of an ACTN2 mutation has guided clinical therapy in HCM[183]. Genome-wide analyses have also demonstrated that ACTN2 mutations can cause HCM[184].

Filamin C
In striated muscle, different forms of the Ank3 gene product (ankyrins-G) are produced due to tissue-specific alternative splicing. These ankyrins-G have a shared segment called the Obscurin/Titin-Binding-related Domain (OTBD), which is consistent across ankyrin genes and links obscurin and Titin to Ank1 gene products. Previously, it was suggested that the OTBD segment in ankyrins plays a unique role in muscle protein interactions. In recent studies, muscle proteins that can bind to the ankyrin-G OTBD were identified as plectin and filamin C, both crucial for muscle development and structure. These three proteins (ankyrin-G, plectin, and filamin C) are found together in skeletal muscle and are observed in the same regions (costameres) of adult muscle fibers[185]. Filamin C (FLNC) is an actin-binding cytoskeletal protein encoded by the FLNC gene, instrumental in maintaining sarcomeric integrity. While first identified as causative in myofibrillar myopathy, recent evidence reveals a key role for FLNC in cardiomyopathy pathogenesis. Truncated FLNC variants predominate in DCM and ARVC, while non-truncated forms are more common in hypertrophic cardiomyopathy and restrictive cardiomyopathy. The primary mechanisms underlying FLNC-associated cardiomyopathies are protein aggregation from non-truncating mutations and haploinsufficiency resulting from filamin C truncation[186].

Nebulin
Members of the nebulin protein family, which includes nebulin, nebulette, LASP-1, LASP-2, and N-RAP, are diverse in size, expression pattern, and function, but they all bind to actin. While nebulin's presence in the heart is minimal, nebulette stands out for its heart-specific expression. Crucially, mutations in the nebulette gene have been linked to DCM. Transgenic mice with these mutations display symptoms that mirror this human heart condition[187].

Mechanosensory signaling mechanism of titin

Titin plays a crucial role in mechanosensing, which is the ability of cells to sense mechanical forces. When muscles undergo stretch or contraction, Titin is subjected to mechanical stress and strain. This mechanical deformation of Titin can trigger mechanotransduction pathways, converting mechanical signals into biochemical signals. These pathways involve the activation of various signaling molecules, including kinases, phosphatases, and transcription factors, leading to cellular responses such as gene expression changes, protein synthesis, and remodeling of the contractile apparatus[188] (Fig. 4).

Z disk region
The Z-disc region of Titin consists of Z-repeats and Ig-domains Z1 and Z2, forming the very NH2-terminal end. Telethonin connects two Titin molecules from one sarcomere, which is essential for sarcomere integrity. Cardiac telethonin undergoes phosphorylation by various kinases and mutations in telethonin are linked to various cardiac cardiomyopthies. Some mutations might disrupt its phosphorylation and, thus, its function. Telethonin interacts with the muscle LIM protein (MLP), together with actinin, MLP, Titin, and telethonin might form a complex that senses mechanical stretch[50].

N2-B region
Cardiac-specific N2-B region which made up of Ig-domains can bind to two isoforms of the LIM domain protein, FHL-1 and FHL-2 which respond strongly to biomechanical stress, and can move to the nucleus to work as transcriptional co-activators. FHL-2's activity could suppress calcineurin, inhibiting pathological cardiac growth while FHL1 might connect to the MAPK signaling cascade. Under non-stimulating conditions, MEK1/2 anchors ERK in the cytoplasm, but after activation, it shifts ERK to the nucleus, activating specific transcription factors.

ERK2 has been seen to phosphorylate Titin's N2-Bus sequence, potentially affecting myofilament stiffness. Knocking down FHL1 in mice changed myofibrillar responsiveness and reduced hypertrophic signaling. Hence, the N2-B/FHL-1/MAPK complex might be a key biomechanical stress sensor in cardiomyocytes[44,58,137,189,190].

M-band region

The M-band region of Titin, particularly the Titin kinase (TK) domain, is a significant area for hypertrophic signaling. TK's conformational changes, suggesting its role as a biomechanical stress sensor, might be biomechanically induced. When activated, TK interacts with Nbr1, forming a complex with p62/SQSTM1 and muscle-specific ubiquitin E3 ligases MuRF1, MuRF2, and MuRF3.

The TK signaling complex with the zinc-finger protein nbr1 is involved in mechanically-activated signaling. Nbr1 directs the ubiquitin-binding protein p62/SQSTM1 to sarcomeres where it interacts with the muscle-specific E3 ligase MuRF2, linked to the transactivation domain of serum response factor (SRF). Mechanical inactivity triggers MuRF2 nuclear migration, decreasing nuclear SRF and suppressing transcription. Mutations in the TK domain disrupt this mechanism, resulting in hereditary muscle disorders[50,191].

Of course, it should be considered that subsequent investigations have proposed that TK functions as an inactive pseudokinase, utilizing its kinase scaffold to recruit MuRF1 for biomechanically regulated autophagy pathways[192,193].

The hotspot region for *TTN* variants

In a quantitative analysis of variants, it was revealed that the most common hotspot region for variants is the exon number 326 which is located in the A band as the Fibronectin type III domain[194] and has a more considerable number of variants compared to other parts which are followed by exon 358 (containing Ig-like domain and Fibronectin type III domain)[194] and exon 48. Among the introns, intron 47 can be considered as the hotspot point for variants compared to other introns[194] (Fig. 2).

Discussion

This study identified 611 distant TTN variants, classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS). These variants predominantly occurred in exon fragments (85%), with 69.6% classified as pathogenic, 21.6% as likely pathogenic, and 8.8% as VUS in ACMG classification. Substitutions accounted for 57.25% of the variants, deletions for 29.62%, duplications for 7.36%, and insertions for 5.72%. The majority of pathogenic variants were located after exon 326, exhibiting higher CADD scores. GERP scores indicated conservity among gene nucleotides, with most variants having notable GERP scores. Exons at the end of the gene displayed higher average CADD scores. VUS variants had lower CADD scores.

TTN, a functionally and structurally essential component of striated muscles, is the largest human protein[10,11]. It consists of four functional regions including N-terminal, I-band, A-band, and C-terminal[26]. The N-terminal is an anchor for Z-disk, which not only plays a crucial role in myofibril assembly and stability but also in sensory functions, protein interactions, and signaling pathways[32–40]. Owing to alternative splicing, I-band is the central adopter specializing titin for specific tissues. The elasticity of the titin is mostly attributable to the I-band unit[38,41]. On the contrary to the I-band, the A-band is not extensible and is a stable anchor for myosin fibers. It also interacts with various proteins contributing to protein turnover at the sarcomeric center[38,41]. The M-band constitutes the myomesin-titin-myosin and also senses and responds to the metabolic stress[50].

The passive tension of the human heart is determined by the pattern of expression of titin isoforms. Expression of more elastic and larger I-band isoforms is associated with lower titin passive tension. The ratio of N2BA and N2B isoform expression determines the stiffness of cardiomyocytes[60]. If the balance between N2BA and N2B is disrupted and N2BA isoform upregulates, the decrease in passive stiffness of the heart brings about DCM[30,31,62,63]. Mutations in the TTN gene are speculated to bring about cardiomyopathies through disruption in sarcomere assembly or contractility, or triggering aberrant splicing[30,31,62,63].

In accordance with our study, another study demonstrated that most TTN variants associated with TTN are located in the A-band unit followed by the I-band[26]. Truncating TTN variants located in the A-band region are the predominant TTN mutations associated with the DCM[77–80,86–88]. The N2BA and N2B isoforms contain distal exons of the A-band. Therefore, variants affecting the A-band and its distal regions are more frequently reported to manifest with DCM, while, the N-terminal mutations are less likely to bring about DCM, considering they are not expressed in N2BA and N2B isoforms[77].

TTN mutations are not as prominent in HCM compared to DCM. HCM is speculated to arise from mutations in sarcomere-related genes; nonetheless, the exact pathophysiology of HCM is yet to be found[90]. Mutations in Sarcomeric, non-sarcomeric, and sarcomere-associated proteins are proposed to contribute to the development and inheritance of RCM[1,2,106,107]. Although the role of TTN variants in the pathogenesis and inheritance of RCM is not fully understood, it is known that titin is the key determinant of sarcomere resting tension and diastolic function[36,108,109]. Similarly, the impact of TTN mutations in ARVC is not yet determined. However, rare TTN variants have been reported in probands and family members of ARVC patients[121,123].

The most common hotspot for mutations is exon 326 of the TTN gene which is located in the A-band region. Notably, the exon containing the most TTN variants is 358, also in the A-band. As presented, the TTN variants were primarily located in a small number of exons which are mostly situated at A- and I-bands. This localization of TTN variants might stem from the higher fatality of mutations in other locations, or conversely, these mutations do not exhibit clinical symptoms to prompt genetic evaluation.

The conservatory TTN exons seem to be associated with the pathogenicity of the variants This might be explained, at least in part, by the theory that more conserved nucleotides could be essential, and mutations affecting this nucleotide could be more pathogenic.

Data availability

The datasets generated and/or analyzed during the current study are available in the the public archive of interpretations of clinically relevant variants (ClinVar) repository, (https://www.ncbi.nlm.nih.gov/clinvar/?term=TTN%5Bgene%5D&redir=gene).

Received: 22 November 2023; Accepted: 1 March 2024
Published online: 04 March 2024

References

1. Ormerod, J.O. and A. Yavari, *Cardiomyopathies.* Medicine, 2022.
2. Ciarambino, T. *et al.* Cardiomyopathies: An Overview. *Int. J. Mol. Sci.* **22**(14), 7722 (2021).
3. Maron, B. J. *et al.* Contemporary definitions and classification of the cardiomyopathies: An American heart association scientific statement from the council on clinical cardiology, heart failure and transplantation committee; quality of care and outcomes research and functional genomics and translational biology interdisciplinary working groups; and council on epidemiology and prevention. *Circulation* **113**(14), 1807–1816 (2006).
4. Elliott, P. *et al.* Classification of the cardiomyopathies: A position statement from the European society of cardiology working group on myocardial and pericardial diseases. *Eur. Heart J.* **29**(2), 270–276 (2008).
5. Maurizia Grasso, B., Favalli, V. & Riccardo Bellazzi, M. The MOGE (S) classification of cardiomyopathy for clinicians. *J. Am. College Cardiol.* **64**(3), 304–318 (2014).
6. Brandenburg, R. Report of the WHO/ISFC task force on the definition and classification of cardiomyopathies. *Br. Heart J.* **44**, 672–673 (1980).
7. Czepluch FS, Wollnik B, Hasenfuß G, (2018) *Genetic determinants of heart failure: facts and numbers*, Wiley Online Library. Cham. 211-217
8. Cannata, A. *et al.* Myocarditis evolving in cardiomyopathy: When genetics and offending causes work together. *Eur. Heart J. Suppl.* **21**(Suppl B), B90-b95 (2019).
9. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**(7145), 661–678 (2007).
10. Gerull, B. The rapidly evolving role of titin in cardiac physiology and cardiomyopathy. *Can. J. Cardiol.* **31**(11), 1351–1359 (2015).
11. Tabish, A. M. *et al.* Genetic epidemiology of titin-truncating variants in the etiology of dilated cardiomyopathy. *Biophys. Rev.* **9**(3), 207–223 (2017).
12. Gerull, B. *et al.* Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat. Genet.* **30**(2), 201–204 (2002).
13. Arbustini, E. *et al.* Desmin accumulation restrictive cardiomyopathy and atrioventricular block associated with desmin gene defects. *Eur. J. Heart Fail.* **8**(5), 477–483 (2006).
14. Merlo, M. *et al.* Poor prognosis of rare sarcomeric gene variants in patients with dilated cardiomyopathy. *Clin. Transl. Sci.* **6**(6), 424–428 (2013).
15. Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet. Med.* **17**(5), 405–424 (2015).
16. Richards, S. *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet. Med.* **17**(5), 405–423 (2015).
17. Kircher, M. *et al.* A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* **46**(3), 310–315 (2014).
18. Rentzsch, P. *et al.* CADD: Predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res.* **47**(D1), D886-d894 (2019).
19. Rentzsch, P. *et al.* CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med.* **13**(1), 31 (2021).
20. Schubach, M. *et al.* CADD v1.7: Using protein language models, regulatory CNNs and other nucleotide-level scores to improve genome-wide variant predictions. *Nucleic Acids Res.* **52**(D1), D1143-d1154 (2024).
21. Schwarz, J. M. *et al.* MutationTaster evaluates disease-causing potential of sequence alterations. *Nat. Methods* **7**(8), 575–576 (2010).
22. Steinhaus, R. *et al.* MutationTaster2021. *Nucleic Acids Res.* **49**(W1), W446-w451 (2021).
23. Šimčíková, D. & Heneberg, P. Refinement of evolutionary medicine predictions based on clinical evidence for the manifestations of Mendelian diseases. *Sci. Rep.* **9**(1), 18577 (2019).
24. Huber, C. D., Kim, B. Y. & Lohmueller, K. E. Population genetic models of GERP scores suggest pervasive turnover of constrained sites across mammalian evolution. *PLoS Genet.* **16**(5), e1008827 (2020).
25. Tskhovrebova, L. & Trinick, J. Titin: Properties and family relationships. *Nat. Rev. Mol. Cell Biol.* **4**(9), 679–689 (2003).
26. Chauveau, C., Rowell, J. & Ferreiro, A. A rising titan: TTN review and mutation update. *Hum. Mutat.* **35**(9), 1046–1059 (2014).
27. Tskhovrebova, L. *et al.* Elasticity and unfolding of single molecules of the giant muscle protein titin. *Nature* **387**(6630), 308–312 (1997).
28. LeWinter, M. M. & Granzier, H. Cardiac titin: A multifunctional giant. *Circulation* **121**(19), 2137–2145 (2010).
29. Lahmers, S. *et al.* Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. *Circ. Res.* **94**(4), 505–513 (2004).
30. Cazorla, O. *et al.* Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circ. Res.* **86**(1), 59–67 (2000).
31. Neagoe, C. *et al.* Gigantic variety: expression patterns of titin isoforms in striated muscles and consequences for myofibrillar passive stiffness. *J. Muscle Res. Cell Motil.* **24**(2), 175–189 (2003).
32. Knoll, R., Buyandelger, B. & Lab, M. The sarcomeric Z-disc and Z-discopathies. *J. Biomed. Biotechnol.* **2011**, 569628 (2011).
33. Knoll, R., Hoshijima, M. & Chien, K. R. Muscle LIM protein in heart failure. *Exp. Clin. Cardiol.* **7**(2–3), 104–105 (2002).
34. Knöll, R. *et al.* A common MLP (muscle LIM protein) variant is associated with cardiomyopathy. *Circ. Res.* **106**(4), 695–704 (2010).
35. Clark, K. A. *et al.* Striated muscle cytoarchitecture: An intricate web of form and function. *Ann. Rev. Cell Dev. Biol.* **18**(1), 637–706 (2002).
36. Gigli, M. *et al.* A review of the giant protein titin in clinical molecular diagnostics of cardiomyopathies. *Front Cardiovasc. Med.* **3**, 21 (2016).
37. Granzier, H. L. & Labeit, S. Titin and its associated proteins: The third myofilament system of the sarcomere. *Adv. Protein Che.* **71**, 89–119 (2005).

38. Kontrogianni-Konstantopoulos, A. *et al.* Muscle giants: Molecular scaffolds in sarcomerogenesis. *Physiol. Rev.* **89**(4), 1217–1267 (2009).
39. Linke, W. A. Sense and stretchability: The role of titin and titin-associated proteins in myocardial stress-sensing and mechanical dysfunction. *Cardiovasc. Res.* **77**(4), 637–648 (2008).
40. Miller, M. K. *et al.* The muscle ankyrin repeat proteins: CARP, ankrd2/Arpp and DARP as a family of titin filament-based stress response molecules. *J. Mol. Biol.* **333**(5), 951–964 (2003).
41. Trombitas, K. *et al.* Titin extensibility in situ: Entropic elasticity of permanently folded and permanently unfolded molecular segments. *J. Cell Biol.* **140**(4), 853–859 (1998).
42. Helmes, M., Trombitas, K. & Granzier, H. Titin develops restoring force in rat cardiac myocytes. *Circ. Res.* **79**(3), 619–626 (1996).
43. Hojayev, B. *et al.* FHL2 binds calcineurin and represses pathological cardiac growth. *Mol. Cell. Biol.* **32**(19), 4025–4034 (2012).
44. Hojayev, B. *et al.* FHL2 binds calcineurin and represses pathological cardiac growth. *Mol. Cell Biol.* **32**(19), 4025–4034 (2012).
45. Granzier, H. L. & Labeit, S. The giant protein titin: A major player in myocardial mechanics, signaling, and disease. *Circ. Res.* **94**(3), 284–295 (2004).
46. Witt, C. C. *et al.* Induction and myofibrillar targeting of CARP, and suppression of the Nkx2.5 pathway in the MDM mouse with impaired titin-based signaling. *J. Mol. Biol.* **336**(1), 145–54 (2004).
47. Obermann, W. M. *et al.* Molecular structure of the sarcomeric M band: Mapping of titin and myosin binding domains in myomesin and the identification of a potential regulatory phosphorylation site in myomesin. *EMBO J.* **16**(2), 211–220 (1997).
48. Gautel, M. *et al.* A calmodulin-binding sequence in the C-terminus of human cardiac titin kinase. *Eur. J. Biochem.* **230**(2), 752–759 (1995).
49. Musa, H. *et al.* Targeted homozygous deletion of M-band titin in cardiomyocytes prevents sarcomere formation. *J. Cell Sci.* **119**(20), 4322–4331 (2006).
50. Kotter, S., Andresen, C. & Kruger, M. Titin: central player of hypertrophic signaling and sarcomeric protein quality control. *Biol. Chem.* **395**(11), 1341–1352 (2014).
51. McElhinny, A. S. *et al.* Muscle-specific RING finger-2 (MURF-2) is important for microtubule, intermediate filament and sarcomeric M-line maintenance in striated muscle development. *J. Cell Sci.* **117**(15), 3175–3188 (2004).
52. Beckmann, J. S. & Spencer, M. Calpain 3, the "gatekeeper" of proper sarcomere assembly, turnover and maintenance. *Neuromusc. Disord.* **18**(12), 913–921 (2008).
53. Granzier, H. *et al.* Titin: Physiological function and role in cardiomyopathy and failure. *Heart Fail. Rev.* **10**(3), 211–223 (2005).
54. Tskhovrebova, L. and J. Trinick, *Roles of titin in the structure and elasticity of the sarcomere.* Journal of Biomedicine and Biotechnology, **2010** (2010).
55. Medicine, N.L.o. *TTN titin [Homo sapiens (human)]*. 2024; Available from: https://www.ncbi.nlm.nih.gov/gene/7273.
56. Resource, T.U.P. *Q8WZ42 · TITIN_HUMAN*. Available from: https://www.uniprot.org/uniprotkb/Q8WZ42/entry.
57. Bang, M. L. *et al.* The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circ. Res.* **89**(11), 1065–1072 (2001).
58. Radke, M. H. *et al.* Targeted deletion of titin N2B region leads to diastolic dysfunction and cardiac atrophy. *Proc. Natl. Acad. Sci. USA* **104**(9), 3444–3449 (2007).
59. Lahmers, S., Wu, Y., Call, D. R., Labeit, S. & Granzier, H. Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. *Circ. Res.* **94**, 505–513 (2004).
60. Hidalgo, C. & Granzier, H. Tuning the molecular giant titin through phosphorylation: Role in health and disease. *Trends Cardiovasc. Med.* **23**(5), 165–171 (2013).
61. Opitz, C. A. *et al.* Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ. Res.* **94**(7), 967–975 (2004).
62. Nagueh, S. F. *et al.* Altered titin expression, myocardial stiffness, and left ventricular function in patients with dilated cardiomyopathy. *Circulation* **110**(2), 155–162 (2004).
63. Opitz, C. A. *et al.* Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ. Res.* **94**(7), 967–975 (2004).
64. Ahmed, S. H. & Lindsey, M. L. Titin phosphorylation: Myocardial passive stiffness regulated by the intracellular giant. *Circ. Res.* **105**(7), 611–613 (2009).
65. Kellermayer, D., Smith, J. E. 3rd. & Granzier, H. Novex-3, the tiny titin of muscle. *Biophys. Rev.* **9**(3), 201–206 (2017).
66. Chen, Z. *et al.* Characterization of TTN novex splicing variants across species and the role of RBM20 in novex-specific exon splicing. *Genes (Basel)* **9**(2), 86 (2018).
67. View, A. *Homo sapiens complex locus CCDC141andTTN, encoding titin and coiled-coil domain containing 141.* . 2010; Available from: https://www.ncbi.nlm.nih.gov/ieb/research/acembly/av.cgi?db=human&term=ttn&submit=Go.
68. Legnini, I. *et al.* Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. *Mol. Cell* **66**(1), 22–37 (2017).
69. Khan, M. A. *et al.* RBM20 regulates circular RNA production from the titin gene. *Circ. Res.* **119**(9), 996–1003 (2016).
70. Czubak, K. *et al.* Global increase in circular RNA levels in myotonic dystrophy. *Front. Genet.* **10**, 649 (2019).
71. Ellepola, C. D. *et al.* Genetic testing in pediatric cardiomyopathy. *Pediatr. Cardiol.* **39**(3), 491–500 (2018).
72. Waldmuller, S. *et al.* Targeted 46-gene and clinical exome sequencing for mutations causing cardiomyopathies. *Mol. Cell Probes.* **29**(5), 308–314 (2015).
73. Marian, A. J., Asatryan, B. & Wehrens, X. H. T. Genetic basis and molecular biology of cardiac arrhythmias in cardiomyopathies. *Cardiovasc. Res.* **116**(9), 1600–1619 (2020).
74. Monserrat, L. *et al.* Genetics of cardiomyopathies: Novel perspectives with next generation sequencing. *Curr. Pharm. Des.* **21**(4), 418–430 (2015).
75. Schultheiss, H. P. *et al.* Dilated cardiomyopathy. *Nat. Rev. Dis. Primers* **5**(1), 32 (2019).
76. Weintraub, R. G., Semsarian, C. & Macdonald, P. Dilated cardiomyopathy. *The Lancet* **390**(10092), 400–414 (2017).
77. Roberts, A. M. *et al.* Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci. Transl. Med.* **7**(270), 270ra6 (2015).
78. Akinrinade, O. *et al.* Genetics and genotype-phenotype correlations in Finnish patients with dilated cardiomyopathy. *Eur. Heart J.* **36**(34), 2327–2337 (2015).
79. van Spaendonck-Zwarts, K. Y. *et al.* Titin gene mutations are common in families with both peripartum cardiomyopathy and dilated cardiomyopathy. *Eur. Heart J.* **35**(32), 2165–2173 (2014).
80. Pugh, T. J. *et al.* The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet. Med.* **16**(8), 601–608 (2014).
81. Fatkin, D. *et al.* Titin truncating mutations: A rare cause of dilated cardiomyopathy in the young. *Progress Pediatr. Cardiol.* **40**, 41–45 (2016).
82. Herman, D. S. *et al.* Truncations of titin causing dilated cardiomyopathy. *N. Engl. J. Med.* **366**(7), 619–628 (2012).
83. Fatkin, D. & Huttner, I. G. Titin-truncating mutations in dilated cardiomyopathy: The long and short of it. *Curr. Opin. Cardiol.* **32**(3), 232–238 (2017).
84. Akinrinade, O., Koskenvuo, J. W. & Alastalo, T. P. Prevalence of titin truncating variants in general population. *PLoS One* **10**(12), e0145284 (2015).

85. Fang, H. J. & Liu, B. P. Prevalence of TTN mutations in patients with dilated cardiomyopathy: A meta-analysis. *Herz* **45**(Suppl 1), 29–36 (2020).
86. Akinrinade, O., Alastalo, T. P. & Koskenvuo, J. W. Relevance of truncating titin mutations in dilated cardiomyopathy. *Clin. Genet.* **90**(1), 49–54 (2016).
87. Schafer, S. *et al.* Titin-truncating variants affect heart function in disease cohorts and the general population. *Nat. Genet.* **49**(1), 46–53 (2017).
88. Yoskovitz, G. *et al.* A novel titin mutation in adult-onset familial dilated cardiomyopathy. *Am. J. Cardiol.* **109**(11), 1644–1650 (2012).
89. Herrero-Galán, E., et al., *Conserved cysteines in titin sustain the mechanical function of cardiomyocytes.* bioRxiv, (2020).
90. Marian, A. J. & Braunwald, E. Hypertrophic cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. *Circ. Res.* **121**(7), 749–770 (2017).
91. Ingles, J. *et al.* Evaluating the clinical validity of hypertrophic cardiomyopathy genes. *Circ. Genom. Precis. Med.* **12**(2), e002460 (2019).
92. Van Driest, S.L., et al. *Sarcomeric genotyping in hypertrophic cardiomyopathy*. in *Mayo Clinic Proceedings*. 2005. Elsevier.
93. Van Driest, S. L. *et al.* Myosin binding protein C mutations and compound heterozygosity in hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **44**(9), 1903–1910 (2004).
94. Richard, P. *et al.* Hypertrophic cardiomyopathy: Distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* **107**(17), 2227–2232 (2003).
95. Van Driest, S. L. *et al.* Comprehensive analysis of the beta-myosin heavy chain gene in 389 unrelated patients with hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **44**(3), 602–610 (2004).
96. Satoh, M. *et al.* Structural analysis of the titin gene in hypertrophic cardiomyopathy: Identification of a novel disease gene. *Biochem. Biophys. Res. Commun.* **262**(2), 411–417 (1999).
97. Itoh-Satoh, M. *et al.* Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem. Biophys. Res. Commun.* **291**(2), 385–393 (2002).
98. Matsumoto, Y. *et al.* Functional analysis of titin/connectin N2-B mutations found in cardiomyopathy. *J. Muscle Res. Cell Motil.* **26**(6–8), 367 (2005).
99. Arimura, T. *et al.* Cardiac ankyrin repeat protein gene (ANKRD1) mutations in hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* **54**(4), 334–342 (2009).
100. Lopes, L. R. *et al.* Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. *J. Med. Genet.* **50**(4), 228–239 (2013).
101. Andersen, P. S. *et al.* Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. *Hum. Mutat.* **30**(3), 363–370 (2009).
102. Bos, J. M. *et al.* Genotype-phenotype relationships involving hypertrophic cardiomyopathy-associated mutations in titin, muscle LIM protein, and telethonin. *Mol. Genet. Metab.* **88**(1), 78–85 (2006).
103. Lewis, A. B. Clinical profile and outcome of restrictive cardiomyopathy in children. *Am. Heart J.* **123**(6), 1589–1593 (1992).
104. Muchtar, E., Blauwet, L. A. & Gertz, M. A. Restrictive cardiomyopathy: Genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. *Circ. Res.* **121**(7), 819–837 (2017).
105. Sayegh, A. L. C. *et al.* Cardiac and peripheral autonomic control in restrictive cardiomyopathy. *ESC Heart Fail.* **4**(3), 341–350 (2017).
106. Yang, Z. *et al.* Genotype-phenotype associations with restrictive cardiomyopathy induced by pathogenic genetic mutations. *Rev. Cardiovasc. Med.* **23**(6), 185 (2022).
107. Pereira, N. L., Grogan, M. & Dec, G. W. Spectrum of restrictive and infiltrative cardiomyopathies: Part 1 of a 2-part series. *J. Am. College Cardiol.* **71**(10), 1130–1148 (2018).
108. Peled, Y. *et al.* Titin mutation in familial restrictive cardiomyopathy. *Int. J. Cardiol.* **171**(1), 24–30 (2014).
109. Neiva-Sousa, M. *et al.* Titin mutations: The fall of Goliath. *Heart Fail. Rev.* **20**(5), 579–588 (2015).
110. Kizawa, M. *et al.* Identification of a novel titin variant underlying myocardial involvement in neurofibromatosis type 1. *Can J Cardiol* **34**(10), 1369 e5-1369 e7 (2018).
111. Groeneweg, J. A. *et al.* Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. *Circ. Cardiovasc. Genet.* **8**(3), 437–446 (2015).
112. Corrado, D. & Thiene, G. Arrhythmogenic right ventricular cardiomyopathy/dysplasia: Clinical impact of molecular genetic studies. *Circulation* **113**(13), 1634–1637 (2006).
113. McKenna, W. J. & Judge, D. P. Epidemiology of the inherited cardiomyopathies. *Nat. Rev. Cardiol.* **18**(1), 22–36 (2021).
114. Marrone, D., et al., *History of the discovery of Arrhythmogenic Cardiomyopathy: The history of arrhythmogenic cardiomyopathy (AC) is a paradigm in the progress of Cardiovascular Medicine knowledge, from nosology to diagnosis, treatment, and prevention. In this review, we focus on the discovery of this heart muscle disease at the beginning of Modern Medicine, something you cannot find on the Internet or PubMed*. 2019, Oxford University Press.
115. Basso, C. *et al.* Arrhythmogenic right ventricular cardiomyopathy. *Lancet* **373**(9671), 1289–1300 (2009).
116. Sen-Chowdhry, S. *et al.* Arrhythmogenic right ventricular cardiomyopathy: Clinical presentation, diagnosis, and management. *Am. J. Med.* **117**(9), 685–695 (2004).
117. Corrado, D. *et al.* Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. *J. Am. Coll. Cardiol.* **30**(6), 1512–1520 (1997).
118. Towbin, J. A. *et al.* 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm* **16**(11), e301–e372 (2019).
119. List, P.G. and D. CTNNA, *Arrhythmogenic Right Ventricular Cardiomyopathy Panel.* 2021.
120. Calabrese, F. *et al.* Arrhythmogenic right ventricular cardiomyopathy/dysplasia: Is there a role for viruses?. *Cardiovasc. Pathol.* **15**(1), 11–17 (2006).
121. Taylor, M. *et al.* Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. *Circulation* **124**(8), 876–885 (2011).
122. Sen-Chowdhry, S., Syrris, P. & McKenna, W. J. Role of genetic analysis in the management of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J. Am. Coll. Cardiol.* **50**(19), 1813–1821 (2007).
123. Brun, F. *et al.* Titin and desmosomal genes in the natural history of arrhythmogenic right ventricular cardiomyopathy. *J. Med. Genet.* **51**(10), 669–676 (2014).
124. Chen, Z. *et al.* Characterization of TTN novex splicing variants across species and the role of RBM20 in novex-specific exon splicing. *Genes* **9**(2), 86 (2018).
125. Savarese, M. *et al.* Increasing role of titin mutations in neuromuscular disorders. *J. Neuromuscul. Dis.* **3**(3), 293–308 (2016).
126. Martinez, J. A. *et al.* Calpain and caspase processing of caspase-12 contribute to the ER stress-induced cell death pathway in differentiated PC12 cells. *Apoptosis* **15**(12), 1480–1493 (2010).
127. Ji, X. Y. *et al.* Sustained over-expression of calpain-2 induces age-dependent dilated cardiomyopathy in mice through aberrant autophagy. *Acta Pharmacol. Sin.* **43**(11), 2873–2884 (2022).
128. Kartkaya, K. *et al.* Protective effect of calpain inhibitor N-acetyl-L-leucyl-L-leucyl-L-norleucinal on acute alcohol consumption related cardiomyopathy. *Mol. Biol. Rep.* **41**(10), 6743–6753 (2014).

129. Ng, R., et al., *Patient mutations linked to arrhythmogenic cardiomyopathy enhance calpain-mediated desmoplakin degradation*. JCI Insight, 2019. **5**(14).
130. Ni, R. *et al.* Mitochondrial calpain-1 disrupts ATP synthase and induces superoxide generation in type 1 diabetic hearts: A novel mechanism contributing to diabetic cardiomyopathy. *Diabetes* **65**(1), 255–268 (2016).
131. Zuo, S. *et al.* CRTH2 promotes endoplasmic reticulum stress-induced cardiomyocyte apoptosis through m-calpain. *EMBO Mol. Med.* **10**(3), e8237 (2018).
132. Willis, M. S. *et al.* Muscle ring finger 1 and muscle ring finger 2 are necessary but functionally redundant during developmental cardiac growth and regulate E2F1-mediated gene expression in vivo. *Cell Biochem. Funct.* **32**(1), 39–50 (2014).
133. Su, M. *et al.* Rare variants in genes encoding MuRF1 and MuRF2 are modifiers of hypertrophic cardiomyopathy. *Int. J. Mol. Sci.* **15**(6), 9302–9313 (2014).
134. Gilbert, C. J., Longenecker, J. Z. & Accornero, F. ERK1/2: An integrator of signals that alters cardiac homeostasis and growth. *Biology (Basel)* **10**(4), 346 (2021).
135. Gallo, S. *et al.* ERK: A key player in the pathophysiology of cardiac hypertrophy. *Int. J. Mol. Sci.* **20**(9), 2164 (2019).
136. Mutlak, M. & Kehat, I. Extracellular signal-regulated kinases 1/2 as regulators of cardiac hypertrophy. *Front. Pharmacol.* **6**, 149 (2015).
137. Bueno, O. F. *et al.* The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J.* **19**(23), 6341–6350 (2000).
138. Heineke, J. & Molkentin, J. D. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat. Rev. Mol. Cell Biol.* **7**(8), 589–600 (2006).
139. Wilkins, B. J. & Molkentin, J. D. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem. Biophys. Res. Commun.* **322**(4), 1178–1191 (2004).
140. Bourajjaj, M. *et al.* NFATc2 is a necessary mediator of calcineurin-dependent cardiac hypertrophy and heart failure. *J. Biol. Chem.* **283**(32), 22295–22303 (2008).
141. He, X. *et al.* Cardiac CIP protein regulates dystrophic cardiomyopathy. *Mol. Ther.* **30**(2), 898–914 (2022).
142. Kura, B. *et al.* Oxidative Stress-Responsive MicroRNAs in Heart Injury. *Int J Mol Sci* **21**(1), 358 (2020).
143. Windpassinger, C. *et al.* An X-linked myopathy with postural muscle atrophy and generalized hypertrophy, termed XMPMA, is caused by mutations in FHL1. *Am. J. Hum. Genet.* **82**(1), 88–99 (2008).
144. Liang, Y. *et al.* Four and a half LIM domain protein signaling and cardiomyopathy. *Biophys. Rev.* **10**(4), 1073–1085 (2018).
145. Chu, P. H. *et al.* Expression patterns of FHL/SLIM family members suggest important functional roles in skeletal muscle and cardiovascular system. *Mech. Dev.* **95**(1–2), 259–265 (2000).
146. Schessl, J. *et al.* Clinical, histological and genetic characterization of reducing body myopathy caused by mutations in FHL1. *Brain* **132**(Pt 2), 452–464 (2009).
147. Selcen, D. *et al.* Reducing bodies and myofibrillar myopathy features in FHL1 muscular dystrophy. *Neurology* **77**(22), 1951–1959 (2011).
148. San Roman, I. *et al.* Unclassifiable arrhythmic cardiomyopathy associated with Emery-Dreifuss caused by a mutation in FHL1. *Clin. Genet.* **90**(2), 171–176 (2016).
149. Bang, M. L. *et al.* The muscle ankyrin repeat proteins CARP, Ankrd2, and DARP are not essential for normal cardiac development and function at basal conditions and in response to pressure overload. *PLoS One* **9**(4), e93638 (2014).
150. Aihara, Y. *et al.* Doxorubicin represses CARP gene transcription through the generation of oxidative stress in neonatal rat cardiac myocytes: Possible role of serine/threonine kinase-dependent pathways. *J. Mol. Cell Cardiol.* **32**(8), 1401–1414 (2000).
151. Wei, Y. J. *et al.* Upregulated expression of cardiac ankyrin repeat protein in human failing hearts due to arrhythmogenic right ventricular cardiomyopathy. *Eur. J. Heart Fail.* **11**(6), 559–566 (2009).
152. Zolk, O. *et al.* Cardiac ankyrin repeat protein, a negative regulator of cardiac gene expression, is augmented in human heart failure. *Biochem. Biophys. Res. Commun.* **293**(5), 1377–1382 (2002).
153. Duboscq-Bidot, L. *et al.* Mutations in the ANKRD1 gene encoding CARP are responsible for human dilated cardiomyopathy. *Eur. Heart J.* **30**(17), 2128–2136 (2009).
154. Moulik, M. *et al.* ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene. *J. Am. Coll. Cardiol.* **54**(4), 325–333 (2009).
155. Kuo, H. *et al.* Control of segmental expression of the cardiac-restricted ankyrin repeat protein gene by distinct regulatory pathways in murine cardiogenesis. *Development* **126**(19), 4223–4234 (1999).
156. Marsh, T. & Debnath, J. Autophagy suppresses breast cancer metastasis by degrading NBR1. *Autophagy* **16**(6), 1164–1165 (2020).
157. Bogomolovas, J. *et al.* Induction of Ankrd1 in dilated cardiomyopathy correlates with the heart failure progression. *Biomed. Res. Int.* **2015**, 273936 (2015).
158. Harris, M. P. *et al.* Perinatal versus adult loss of ULK1 and ULK2 distinctly influences cardiac autophagy and function. *Autophagy* **18**(9), 2161–2177 (2022).
159. Radke, M. H. *et al.* Deleting full length titin versus the Titin M-band region leads to differential mechanosignaling and cardiac phenotypes. *Circulation* **139**(15), 1813–1827 (2019).
160. Parlakian, A. *et al.* Temporally controlled onset of dilated cardiomyopathy through disruption of the SRF gene in adult heart. *Circulation* **112**(19), 2930–2939 (2005).
161. Miano, J. M. Serum response factor: Toggling between disparate programs of gene expression. *J. Mol. Cell. Cardiol.* **35**(6), 577–593 (2003).
162. Kuwahara, K. *et al.* Myocardin-related transcription factor A is a common mediator of mechanical stress-and neurohumoral stimulation-induced cardiac hypertrophic signaling leading to activation of brain natriuretic peptide gene expression. *Mol. Cell. Biol.* **30**(17), 4134–4148 (2010).
163. Knoll, R., Hoshijima, M. & Chien, K. Cardiac mechanotransduction and implications for heart disease. *J. Mol. Med. (Berl)* **81**(12), 750–756 (2003).
164. Arber, S. *et al.* MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. *Cell* **88**(3), 393–403 (1997).
165. Ehler, E. & Perriard, J. C. Cardiomyocyte cytoskeleton and myofibrillogenesis in healthy and diseased heart. *Heart Fail. Rev.* **5**(3), 259–269 (2000).
166. Geier, C. *et al.* Beyond the sarcomere: CSRP3 mutations cause hypertrophic cardiomyopathy. *Hum. Mol. Genet.* **17**(18), 2753–2765 (2008).
167. Karsai, A., Kellermayer, M. S. & Harris, S. P. Mechanical unfolding of cardiac myosin binding protein-C by atomic force microscopy. *Biophys. J.* **101**(8), 1968–1977 (2011).
168. Maron, B. J., Maron, M. S. & Semsarian, C. Genetics of hypertrophic cardiomyopathy after 20 years: Clinical perspectives. *J. Am. Coll. Cardiol.* **60**(8), 705–715 (2012).
169. Harris, S. P. *et al.* Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circ. Res.* **90**(5), 594–601 (2002).
170. Agarkova, I. & Perriard, J.-C. The M-band: An elastic web that crosslinks thick filaments in the center of the sarcomere. *Trends Cell Biol.* **15**(9), 477–485 (2005).

171. Forleo, C. *et al.* Targeted next-generation sequencing detects novel gene-phenotype associations and expands the mutational spectrum in cardiomyopathies. *PLoS One* **12**(7), e0181842 (2017).
172. Lange, S. *et al.* Obscurin and KCTD6 regulate cullin-dependent small ankyrin-1 (sAnk1.5) protein turnover. *Mol. Biol. Cell* **23**(13), 2490–504 (2012).
173. Krishnamoorthy, S. *et al.* A novel phosphopeptide microarray based interactome map in breast cancer cells reveals phospho-protein-GRB2 cell signaling networks. *PLoS One* **8**(6), e67634 (2013).
174. Vatta, M. *et al.* Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. *J. Am. Coll. Cardiol.* **42**(11), 2014–2027 (2003).
175. Hoshijima, M. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. *Am. J. Physiol. Heart Circ. Physiol.* **290**(4), H1313–H1325 (2006).
176. Pylayeva-Gupta, Y., Grabocka, E. & Bar-Sagi, D. RAS oncogenes: Weaving a tumorigenic web. *Nat. Rev. Cancer* **11**(11), 761–774 (2011).
177. Sussman, M. A. *et al.* Altered focal adhesion regulation correlates with cardiomyopathy in mice expressing constitutively active rac1. *J. Clin. Invest.* **105**(7), 875–886 (2000).
178. Yamaguchi, K. *et al.* A spin correction procedure for unrestricted Hartree-Fock and Møller-Plesset wavefunctions for singlet diradicals and polyradicals. *Chem. Phys. Lett.* **149**(5–6), 537–542 (1988).
179. Wellbrock, C. & Hurlstone, A. BRAF as therapeutic target in melanoma. *Biochem. Pharmacol.* **80**(5), 561–567 (2010).
180. Harris, I. S. *et al.* Raf-1 kinase is required for cardiac hypertrophy and cardiomyocyte survival in response to pressure overload. *Circulation* **110**(6), 718–723 (2004).
181. Haywood, N. J. *et al.* Hypertrophic cardiomyopathy mutations in the calponin-homology domain of ACTN2 affect actin binding and cardiomyocyte Z-disc incorporation. *Biochem. J.* **473**(16), 2485–2493 (2016).
182. Girolami, F. *et al.* Novel alpha-actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: A massively parallel sequencing study. *Circ. Cardiovasc. Genet.* **7**(6), 741–750 (2014).
183. Prondzynski, M. *et al.* Disease modeling of a mutation in alpha-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy. *EMBO Mol. Med.* **11**(12), e11115 (2019).
184. Chiu, C. *et al.* Mutations in alpha-actinin-2 cause hypertrophic cardiomyopathy: A genome-wide analysis. *J. Am. Coll. Cardiol.* **55**(11), 1127–1135 (2010).
185. Maiweilidan, Y., Klauza, I. & Kordeli, E. Novel interactions of ankyrins-G at the costameres: The muscle-specific Obscurin/Titin-Binding-related Domain (OTBD) binds plectin and filamin C. *Exp. Cell Res.* **317**(6), 724–736 (2011).
186. Song, S. *et al.* Filamin C in cardiomyopathy: From physiological roles to DNA variants. *Heart Fail. Rev.* **27**(4), 1373–1385 (2022).
187. Bang, M. L. & Chen, J. Roles of nebulin family members in the heart. *Circ. J.* **79**(10), 2081–2087 (2015).
188. Voelkel, T. & Linke, W. A. Conformation-regulated mechanosensory control via titin domains in cardiac muscle. *Pflugers Arch.* **462**(1), 143–154 (2011).
189. Sheikh, F. *et al.* An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. *J. Clin. Invest.* **118**(12), 3870–3880 (2008).
190. El-Bizri, N. *et al.* Abstract 13402: FHL-1 contributes to and colocalizes with titin in cardiac hypertrophy. *Circulation* **142**(Suppl_3), A13402–A13402 (2020).
191. Lange, S. *et al.* The kinase domain of titin controls muscle gene expression and protein turnover. *Science* **308**(5728), 1599–1603 (2005).
192. Bogomolovas, J. *et al.* Titin kinase ubiquitination aligns autophagy receptors with mechanical signals in the sarcomere. *EMBO Rep.* **22**(10), e48018 (2021).
193. Bogomolovas, J. *et al.* Titin kinase is an inactive pseudokinase scaffold that supports MuRF1 recruitment to the sarcomeric M-line. *Open Biol.* **4**(5), 140041 (2014).
194. Yang, K., et al., *Clinical efficacy and safety of atorvastatin for chronic subdural hematoma: A randomized controlled trial.*
195. Campuzano, O. *et al.* Rare titin (TTN) variants in diseases associated with sudden cardiac death. *Int. J. Mol. Sci.* **16**(10), 25773–25787 (2015).
196. Evila, A. *et al.* Targeted next-generation sequencing reveals novel TTN mutations causing recessive distal titinopathy. *Mol. Neurobiol.* **54**(9), 7212–7223 (2017).
197. Lahrouchi, N. *et al.* Utility of post-mortem genetic testing in cases of sudden arrhythmic death syndrome. *J. Am. Coll. Cardiol.* **69**(17), 2134–2145 (2017).
198. Hackman, P. *et al.* Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *Am. J. Hum. Genet.* **71**(3), 492–500 (2002).
199. Herman, D. S. *et al.* Truncations of titin causing dilated cardiomyopathy. *New Engl. J. Med.* **366**(7), 619–628 (2012).
200. Krenn, M. *et al.* Genotype-guided diagnostic reassessment after exome sequencing in neuromuscular disorders: Experiences with a two-step approach. *Eur. J. Neurol.* **27**(1), 51–61 (2020).
201. Harris, E. *et al.* A 'second truncation'in TTN causes early onset recessive muscular dystrophy. *Neuromusc. Disord.* **27**(11), 1009–1017 (2017).
202. Roberts, A. M. *et al.* Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci. Transl. Med.* **7**(270), 270ra6 (2015).
203. Schultze-Berndt, A. *et al.* Reduced systolic function and not genetic variants determine outcome in pediatric and adult left ventricular noncompaction cardiomyopathy. *Front. Pediatr.* **9**, 722926 (2021).
204. Chauveau, C. *et al.* Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Hum. Mol. Genet.* **23**(4), 980–991 (2014).
205. Haas, J. *et al.* Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur. Heart J.* **36**(18), 1123–1135 (2015).
206. De Cid, R. *et al.* A new titinopathy: Childhood-juvenile onset Emery-Dreifuss–like phenotype without cardiomyopathy. *Neurology* **85**(24), 2126–2135 (2015).
207. Deo, R. C. Alternative splicing, internal promoter, nonsense-mediated decay, or all three: explaining the distribution of truncation variants in titin. *Circ. Cardiovasc. Genet.* **9**(5), 419–425 (2016).
208. Mukhtar, M.M. and M.A. Salih, *C-Terminal Titin Deletions Cause a Novel Early-Onset Myopathy with Fatal Cardiomyopathy*. 2007, University of Khartoum.
209. Savarese, M. *et al.* Genotype-phenotype correlations in recessive titinopathies. *Genet. Med.* **22**(12), 2029–2040 (2020).
210. Ceyhan-Birsoy, O. *et al.* Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology* **81**(14), 1205–1214 (2013).
211. Fattori, F. *et al.* Centronuclear myopathies: genotype-phenotype correlation and frequency of defined genetic forms in an Italian cohort. *J. Neurol.* **262**(7), 1728–1740 (2015).
212. Hackman, P., et al., *Salih myopathy.* 2019.
213. Evila, A. *et al.* Atypical phenotypes in titinopathies explained by second titin mutations. *Ann. Neurol.* **75**(2), 230–240 (2014).
214. Witting, N., et al., *Phenotypes, genotypes, and prevalence of congenital myopathies older than 5 years in Denmark.* Neurology Genetics, 2017. **3**(2).
215. Rich, K. A. *et al.* Novel heterozygous truncating titin variants affecting the A-band are associated with cardiomyopathy and myopathy/muscular dystrophy. *Mol. Genet. Genomic. Med.* **8**(10), e1460 (2020).

216. Hackman, P. *et al.* Truncating mutations in C-terminal titin may cause more severe tibial muscular dystrophy (TMD). *Neuromuscul. Disord.* **18**(12), 922–928 (2008).
217. Marschall, C., Moscu-Gregor, A. & Klein, H. G. Variant panorama in 1,385 index patients and sensitivity of expanded next-generation sequencing panels in arrhythmogenic disorders. *Cardiovasc. Diagn. Ther.* **9**(Suppl 2), S292–S298 (2019).
218. Laquerriere, A. *et al.* Mutations in CNTNAP1 and ADCY6 are responsible for severe arthrogryposis multiplex congenita with axoglial defects. *Hum. Mol. Genet.* **23**(9), 2279–2289 (2014).
219. Enriquez, A. *et al.* Substrate characterization and outcomes of ventricular tachycardia ablation in TTN (Titin) cardiomyopathy: A multicenter study. *Circ. Arrhythm. Electrophysiol.* **14**(9), e010006 (2021).
220. Perić, S. *et al.* A novel recessive TTN founder variant is a common cause of distal myopathy in the Serbian population. *Eur. J. Hum. Genet.* **25**(5), 572–581 (2017).
221. Haskell, G. T. *et al.* Whole exome sequencing identifies truncating variants in nuclear envelope genes in patients with cardiovascular disease. *Circ. Cardiovasc. Genet.* **10**(3), e001443 (2017).
222. Nykamp, K. *et al.* Sherloc: A comprehensive refinement of the ACMG-AMP variant classification criteria. *Genet. Med.* **19**(10), 1105–1117 (2017).
223. Dalin, M. G. *et al.* Massive parallel sequencing questions the pathogenic role of missense variants in dilated cardiomyopathy. *Int. J. Cardiol.* **228**, 742–748 (2017).
224. Walsh, R. *et al.* Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. *Genet. Med.* **19**(2), 192–203 (2017).
225. Choi, S. H. *et al.* Association between titin loss-of-function variants and early-onset atrial fibrillation. *Jama* **320**(22), 2354–2364 (2018).
226. Taylor, M. *et al.* Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy–overlap syndromes. *Circulation* **124**(8), 876–885 (2011).
227. Ware, J. S. *et al.* Shared genetic predisposition in peripartum and dilated cardiomyopathies. *N. Engl. J. Med.* **374**(3), 233–241 (2016).
228. Gigli, M. *et al.* Genetic risk of arrhythmic phenotypes in patients with dilated cardiomyopathy. *J. Am. Coll. Cardiol.* **74**(11), 1480–1490 (2019).
229. Goli, R. *et al.* Genetic and phenotypic landscape of peripartum cardiomyopathy. *Circulation* **143**(19), 1852–1862 (2021).
230. Sevy, A. *et al.* Improving molecular diagnosis of distal myopathies by targeted next-generation sequencing. *J. Neurol. Neurosurg. Psychiatry* **87**(3), 340–342 (2016).
231. Pfeffer, G. and P.F. Chinnery, *Hereditary myopathy with early respiratory failure.* GeneReviews®[Internet], 2020.
232. Augusto, J. B. *et al.* Dilated cardiomyopathy and arrhythmogenic left ventricular cardiomyopathy: A comprehensive genotype-imaging phenotype study. *Eur. Heart J. Cardiovasc. Imaging* **21**(3), 326–336 (2020).
233. van Waning, J. I. *et al.* Genetics, clinical features, and long-term outcome of noncompaction cardiomyopathy. *J. Am. Coll. Cardiol.* **71**(7), 711–722 (2018).
234. Norton, N. *et al.* Exome sequencing and genome-wide linkage analysis in 17 families illustrate the complex contribution of TTN truncating variants to dilated cardiomyopathy. *Circ. Cardiovasc. Genet.* **6**(2), 144–153 (2013).
235. Cowan, J. R. *et al.* SOS1 gain-of-function variants in dilated cardiomyopathy. *Circ. Genom. Precis. Med.* **13**(4), e002892 (2020).
236. Kostareva, A. *et al.* Genetic spectrum of idiopathic restrictive cardiomyopathy uncovered by next-generation sequencing. *PLoS One* **11**(9), e0163362 (2016).
237. Song, J. S. *et al.* Identification of pathogenic variants in genes related to channelopathy and cardiomyopathy in Korean sudden cardiac arrest survivors. *J. Hum. Genet.* **62**(6), 615–620 (2017).
238. Gerull, B. *et al.* Identification of a novel frameshift mutation in the giant muscle filament titin in a large Australian family with dilated cardiomyopathy. *J. Mol. Med. (Berl)* **84**(6), 478–483 (2006).
239. Verdonschot, J. A. J. *et al.* Implications of genetic testing in dilated cardiomyopathy. *Circ. Genom. Precis. Med.* **13**(5), 476–487 (2020).
240. Herkert, J.C., *Paediatric cardiomyopathies: an evolving landscape of genetic aetiology and diagnostic applications.* 2019.
241. Vissing, C. R. *et al.* Dilated cardiomyopathy caused by truncating titin variants: Long-term outcomes, arrhythmias, response to treatment and sex differences. *J. Med. Genet.* **58**(12), 832–841 (2021).
242. Morales, A. *et al.* Variant interpretation for dilated cardiomyopathy: Refinement of the american college of medical genetics and genomics/clingen guidelines for the DCM precision medicine study. *Circ. Genom. Precis. Med.* **13**(2), e002480 (2020).
243. Akinrinade, O. *et al.* Genetics and genotype–phenotype correlations in Finnish patients with dilated cardiomyopathy. *Eur. Heart J.* **36**(34), 2327–2337 (2015).
244. Klauke, B. *et al.* High proportion of genetic cases in patients with advanced cardiomyopathy including a novel homozygous Plakophilin 2-gene mutation. *PLoS One* **12**(12), e0189489 (2017).
245. Franaszczyk, M. *et al.* Titin truncating variants in dilated cardiomyopathy–prevalence and genotype-phenotype correlations. *PLoS One* **12**(1), e0169007 (2017).
246. Kuhnisch, J. *et al.* Targeted panel sequencing in pediatric primary cardiomyopathy supports a critical role of TNNI3. *Clin. Genet.* **96**(6), 549–559 (2019).
247. Hancks, D. C. & Kazazian, H. H. Jr. Active human retrotransposons: Variation and disease. *Curr. Opin. Genet. Dev.* **22**(3), 191–203 (2012).
248. Chami, N. *et al.* Nonsense mutations in BAG3 are associated with early-onset dilated cardiomyopathy in French Canadians. *Can. J. Cardiol.* **30**(12), 1655–1661 (2014).
249. Al-Shamsi, A. *et al.* Whole exome sequencing diagnosis of inborn errors of metabolism and other disorders in United Arab Emirates. *Orphanet. J. Rare Dis.* **11**(1), 94 (2016).
250. LaDuca, H. *et al.* Exome sequencing covers >98% of mutations identified on targeted next generation sequencing panels. *PLoS One* **12**(2), e0170843 (2017).
251. Anderson, J. L. *et al.* Discovery of TITIN Gene truncating variant mutations and 5-Year outcomes in patients with nonischemic dilated cardiomyopathy. *Am. J. Cardiol.* **137**, 97–102 (2020).
252. Cuenca, S. *et al.* Genetic basis of familial dilated cardiomyopathy patients undergoing heart transplantation. *J. Heart Lung Transplant* **35**(5), 625–635 (2016).
253. Kolokotronis, K. *et al.* New insights on genetic diagnostics in cardiomyopathy and arrhythmia patients gained by stepwise exome data analysis. *J. Clin. Med.* **9**(7), 2168 (2020).
254. Minoche, A. E. *et al.* Genome sequencing as a first-line genetic test in familial dilated cardiomyopathy. *Genet. Med.* **21**(3), 650–662 (2019).
255. Fokstuen, S. *et al.* Experience of a multidisciplinary task force with exome sequencing for Mendelian disorders. *Hum. Genomics* **10**(1), 24 (2016).
256. Campuzano Larrea, O. *et al.* Post-mortem genetic analysis in juvenile cases of sudden cardiac death. *Forensic Sci. Int.* **245**, 30–37 (2014).
257. van Lint, F. H. M. *et al.* Large next-generation sequencing gene panels in genetic heart disease: Yield of pathogenic variants and variants of unknown significance. *Neth. Heart J.* **27**(6), 304–309 (2019).
258. Yang, Y. *et al.* Molecular findings among patients referred for clinical whole-exome sequencing. *Jama* **312**(18), 1870–1879 (2014).

259. Mazzarotto, F. *et al.* Reevaluating the genetic contribution of monogenic dilated cardiomyopathy. *Circulation* **141**(5), 387–398 (2020).
260. Cowan, J. R. *et al.* Multigenic disease and bilineal inheritance in dilated cardiomyopathy is illustrated in nonsegregating LMNA pedigrees. *Circ. Genomic Precis. Med.* **11**(7), e002038 (2018).
261. Brown, E. E. *et al.* Genetic dilated cardiomyopathy due to TTN variants without known familial disease. *Circ. Genomic Precis. Med.* **13**(6), e003082 (2020).
262. Ceyhan-Birsoy, O. *et al.* Next generation sequencing-based copy number analysis reveals low prevalence of deletions and duplications in 46 genes associated with genetic cardiomyopathies. *Mol. Genet. Genomic Med.* **4**(2), 143–151 (2016).
263. Peat, R. A. *et al.* Diagnosis and etiology of congenital muscular dystrophy. *Neurology* **71**(5), 312–321 (2008).
264. Lu, C. *et al.* Molecular analysis of inherited cardiomyopathy using next generation semiconductor sequencing technologies. *J. Transl. Med.* **16**(1), 241 (2018).
265. Bryen, S. J. *et al.* Recurrent TTN metatranscript-only c.39974–11T>G splice variant associated with autosomal recessive arthrogryposis multiplex congenita and myopathy. *Hum. Mutat.* **41**(2), 403–411 (2020).
266. Cummings, B. B. *et al.* Improving genetic diagnosis in Mendelian disease with transcriptome sequencing. *Sci. Transl. Med.* **9**(386), eaal5209 (2017).
267. Hershberger, R.E., *Exome Sequencing and Genome-Wide Linkage Analysis in 17 Families Illustrates the Complex Contribution of TTN Truncating Variants to Dilated Cardiomyopathy.*
268. Hazebroek, M. R. *et al.* Prevalence of pathogenic gene mutations and prognosis do not differ in isolated left ventricular dysfunction compared with dilated cardiomyopathy. *Circ. Heart Fail.* **11**(3), e004682 (2018).
269. Hoorntje, E. T. *et al.* The first titin (c.59926 + 1G > A) founder mutation associated with dilated cardiomyopathy. *Eur. J. Heart Fail.* **20**(4), 803–806 (2018).
270. Miszalski-Jamka, K. *et al.* Novel genetic triggers and genotype-phenotype correlations in patients with left ventricular noncompaction. *Circ. Cardiovasc. Genet.* **10**(4), e001763 (2017).
271. Fan, L. L. *et al.* Whole-exome sequencing reveals doubly novel heterozygous Myosin Binding Protein C and Titin mutations in a Chinese patient with severe dilated cardiomyopathy. *Cardiol. Young* **28**(12), 1410–1414 (2018).
272. Jansweijer, J. A. *et al.* Truncating titin mutations are associated with a mild and treatable form of dilated cardiomyopathy. *Eur. J. Heart Fail.* **19**(4), 512–521 (2017).
273. Wu, L. *et al.* Next-generation sequencing to diagnose muscular dystrophy, rhabdomyolysis, and HyperCKemia. *Can. J. Neurol. Sci.* **45**(3), 262–268 (2018).
274. Yavarna, T. *et al.* High diagnostic yield of clinical exome sequencing in Middle Eastern patients with Mendelian disorders. *Hum. Genet.* **134**(9), 967–980 (2015).
275. Tester, D. J. *et al.* Cardiac genetic predisposition in sudden infant death syndrome. *J. Am. Coll. Cardiol.* **71**(11), 1217–1227 (2018).
276. Carnevale, A. *et al.* Genomic study of dilated cardiomyopathy in a group of Mexican patients using site-directed next generation sequencing. *Mol. Genet. Genomic. Med.* **8**(11), e1504 (2020).
277. Cambon-Viala, M. *et al.* Phenotype/genotype relationship in left ventricular noncompaction: Ion channel gene mutations are associated with preserved left ventricular systolic function and biventricular noncompaction: Phenotype/genotype of noncompaction. *J. Card. Fail.* **27**(6), 677–681 (2021).
278. Bell, C. J. *et al.* Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci. Transl. Med.* **3**(65), 65ra4 (2011).
279. Willsey, A. J. *et al.* De Novo coding variants are strongly associated with tourette disorder. *Neuron* **94**(3), 486-499 e9 (2017).
280. Arimura, T. *et al.* Cardiac ankyrin repeat protein gene (ANKRD1) mutations in hypertrophic cardiomyopathy. *J. Am. College Cardiol.* **54**(4), 334–342 (2009).
281. Liu, J. S. *et al.* Whole-exome sequencing identifies two novel TTN mutations in Chinese families with dilated cardiomyopathy. *Cardiology* **136**(1), 10–14 (2017).
282. Posey, J. E. *et al.* Resolution of disease phenotypes resulting from multilocus genomic variation. *N. Engl. J. Med.* **376**(1), 21–31 (2017).
283. Golbus, J. R. *et al.* Targeted analysis of whole genome sequence data to diagnose genetic cardiomyopathy. *Circ. Cardiovasc. Genet.* **7**(6), 751–759 (2014).
284. Campuzano, O. *et al.* Post-mortem genetic analysis in juvenile cases of sudden cardiac death. *Forensic Sci. Int.* **245**, 30–37 (2014).
285. Gerull, B. *et al.* Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat. Genet.* **30**(2), 201–204 (2002).
286. Hastings, R. *et al.* Combination of whole genome sequencing, linkage, and functional studies implicates a missense mutation in titin as a cause of autosomal dominant cardiomyopathy with features of left ventricular noncompaction. *Circ. Cardiovasc. Genet.* **9**(5), 426–435 (2016).

Acknowledgements

We appreciate the support from Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.

Author contributions

A.G., E.K. and S.K. wrote the initial manuscript. S.K., M.M., A.F., and M.H. contributed to the research design. S.K. made a comprehensive revise. S.G.H., M.H., M.H.M., and N.N. contributed to the collection of data. All the authors read and approved the final manuscript.

Funding

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to S.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2024