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GENOMIC DATA REANALYSIS AND VARIANT REINTERPRETATION IN GENETIC DISEASES: A BIOINFORMATIC AND CLINICAL REEVALUATION IN INHERITED CARDIAC CONDITIONS AND SUSPECTED FANCONI ANAEMIA PATIENT COHORTS

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DOCTORAL THESIS

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Genomic data reanalysis and variant reinterpretation in genetic diseases: A bioinformatic and clinical reevaluation in inherited cardiac conditions and suspected Fanconi anaemia patient cohorts

Thesis presented by

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Abstract

Next generation sequencing (NGS) has revolutionised genetic diagnosis, offering high-throughput, sensitive, and cost-effective genomic analysis compared to traditional methods. Despite significant advancements, there is still room to improve diagnostic rates after a period of time when initial genetic testing is inconclusive. Reanalysis of existing NGS data can help in increasing diagnostic rates by leveraging updated scientific literature, novel gene-disease associations, and enhanced bioinformatics tools, thereby influencing patient management and genetic counseling. Here, a bioinformatics and clinical reevaluation was conducted to genomic data from two cohorts: the first one included patients with clinical diagnoses of different inherited cardiac conditions (ICCs) (cohort 1, n = 742), while the second one consisted in patients exhibiting a suspected Fanconi anaemia (SFA) clinical phenotype (cohort 2, n = 6). The methodology involved rigorous reanalysis of existing NGS data, including advanced variant filtering and prioritisation, deep intronic variant analysis, and comprehensive copy number variant (CNV) analysis. Phenotype and variant classification were meticulously performed, incorporating updated criteria. Final candidate variants identified after prioritisation were subsequently discussed with cardiologists and Fanconi anaemia (FA) specialists to assess their clinical relevance in the context of each patient's current phenotype. For cohort 1, reanalysis of clinical exome sequencing (CES) data led to a 0.8% (6/742) increase in definitive molecular diagnoses. This improvement was primarily driven by the successful reclassification of five variants of uncertain significance (VUS) to pathogenic/likely pathogenic (P/LP) status, underpinned by evolving scientific evidence and a rigorous clinical expert review process. Furthermore, the identification of a diagnostic variant in the *TTN* gene (not analysed in the patient in the standard genetic testing) underscored the value of expanding gene analysis beyond initially considered phenotype-specific panels. In cohort 2, reanalysis of whole exome sequencing (WES) data yielded a 33.4% (2/6) diagnostic rate by identifying P/LP variants in genes not analysed previously. The systematic reanalysis of inconclusive NGS data increased the molecular diagnostic yield across both cohorts. The primary drivers for these new diagnoses were the reclassification of previously ambiguous VUS and the successful identification of P variants through the inclusion of additional gene-disease associations not considered in initial analyses. Despite these advancements, challenges persist, including limited knowledge of many non-OMIM genes and the complexity of polygenic inheritance as well as technical limitations inherent to short-read sequencing, such as suboptimal detection of structural variants (SVs) and non-coding alterations. This work highlights the critical importance of

continuous expert review by multidisciplinary teams and emphasises that future efforts should focus on integrating longer reanalysis intervals, exploring complementary sequencing technologies like long-read sequencing and whole genome sequencing (WGS), and incorporating advanced computational tools, including artificial intelligence, to further maximise the diagnostic yield and enhance patient care.

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Acronyms

AB allele balance

ACMG/AMP American College of Medical Genetics and Genomics and Association for Molecular Pathology

AD autosomal dominant

ANNOVAR ANNOtate VARiation

AR autosomal recessive

ARVC arrhythmogenic right ventricular cardiomyopathy

BAV bicuspid aortic valve disease

BF Bayes factor

bp base pairs

BrS Brugada syndrome

BWA Burrows-Wheeler Aligner

CAAs coronary artery aneurysms

CCDs cardiac conduction diseases

CES clinical exome sequencing

CHAN channelopathies

CHD congenital heart disease

CMR cardiac magnetic resonance

CNV copy number variant

CPVT catecholaminergic polymorphic ventricular tachycardia

DBA Diamond-Blackfan anaemia

DC dyskeratosis congenita

DCM dilated cardiomyopathy

DEB diepoxybutane

ECG electrocardiogram

EDSs Ehlers-Danlos syndromes

EPS electrophysiological studies

ESC European Society of Cardiology

FA Fanconi anaemia

FH familial hypercholesterolaemia

FSA familial syndromic aortopathies

GATK Genome Analysis Toolkit

Gb Gigabytes

HCM hypertrophic cardiomyopathy

HGMD Human Gene Mutation Database

HSCSP Hospital de la Santa Creu i Sant Pau

IBMFSs inherited bone marrow failure syndromes

ICCs inherited cardiac conditions

IGV Integrative Genomics Viewer

indels insertion-deletions

ITP immune thrombocytopenic purpura

LDL-C low-density lipoprotein cholesterol

LDS Loeys-Dietz syndrome

LOF loss-of-function

LP likely pathogenic

LQTS long QT syndrome

LRT Likelihood Ratio Test

LVEF left ventricular ejection fraction

LVH left ventricular hypertrophy

LVOTO left ventricular outflow tract obstruction

MAF minor allele frequency

MCV mean corpuscular volume

MFS Marfan syndrome

MRI magnetic resonance imaging

NDLVC non-dilated left ventricular cardiomyopathy

NGS next generation sequencing

NMDs neuromuscular disorders

OMIM Online Mendelian Inheritance in Man

P pathogenic

PacBio Pacific Biosciences

PAH pulmonary arterial hypertension

PCR polymerase chain reaction

QC quality control

RCM restrictive cardiomyopathy

REVEL Rare Exome Variant Ensemble Learner

SADS sudden arrhythmic death syndrome

SAM systolic anterior motion

SDS Shwachman-Diamond syndrome

SFA suspected Fanconi anaemia

SMRT single-molecule real-time

SNP single nucleotide polymorphism

SNV single nucleotide variant

SOLiD Sequencing by Oligonucleotide Ligation and Detection

STAR Spliced Transcripts Alignment to a Reference

SV structural variant

TAAs thoracic aortic aneurysms

VCF variant call format

vEDS vascular Ehlers-Danlos syndrome

VEP Variant Effect Predictor

VH ventricular hypertrabeculation

VUS variant of uncertain significance

WES whole exome sequencing

WGS whole genome sequencing

XLR X-linked recessive

1. Introduction

1.1. Reanalysis of clinical next generation sequencing data

The reanalysis of clinical genomic data generated by next generation sequencing (NGS) has emerged in recent years as a powerful strategy in diagnostic laboratories to improve diagnostic outcomes without requiring additional sequencing in cases where initial genetic testing has been inconclusive (Hiatt et al., 2018; Van Der Geest et al., 2024). This is sometimes achieved through collaborative efforts involving multiple specialised centers (Best et al., 2024; Laurie et al., 2025). This reevaluation of previously unresolved cases is essential to capitalise on advancements in genomic interpretation which represents a dynamic process that evolves with the continuous updating of databases, enhancements in analytical pipelines, and emerging clinical insights such as the discovery of new disease-causing genes (Dai et al., 2022; Hiatt et al., 2018; Rosier et al., 2022; Sarmady & Abou Tayoun, 2018). Studies have demonstrated that reanalysis of existing data in individuals with suspected Mendelian disorders can increase the diagnostic rate by up to 10% (Dai et al., 2022; Wenger et al., 2017) with a mean diagnostic yield around 10-15% (Dai et al., 2022; Sarmady & Abou Tayoun, 2018; Van Der Geest et al., 2024), profoundly influencing patient management, encompassing treatment strategies and genetic counselling (Fung et al., 2020; Schobers et al., 2022; Van Der Geest et al., 2024). The range of diagnostic rates in these works fluctuates from 6.5% to 41%, influenced by various factors (**Figure 1.1**) such as the type of NGS strategy (genome-based or exome-based), timespan for reanalysis initiation (ranging from 6 months to 3 years), study cohort size and composition, the degree of automation in the reanalysis process, and the reporting methodology employed (Dai et al., 2022; Van Der Geest et al., 2024).

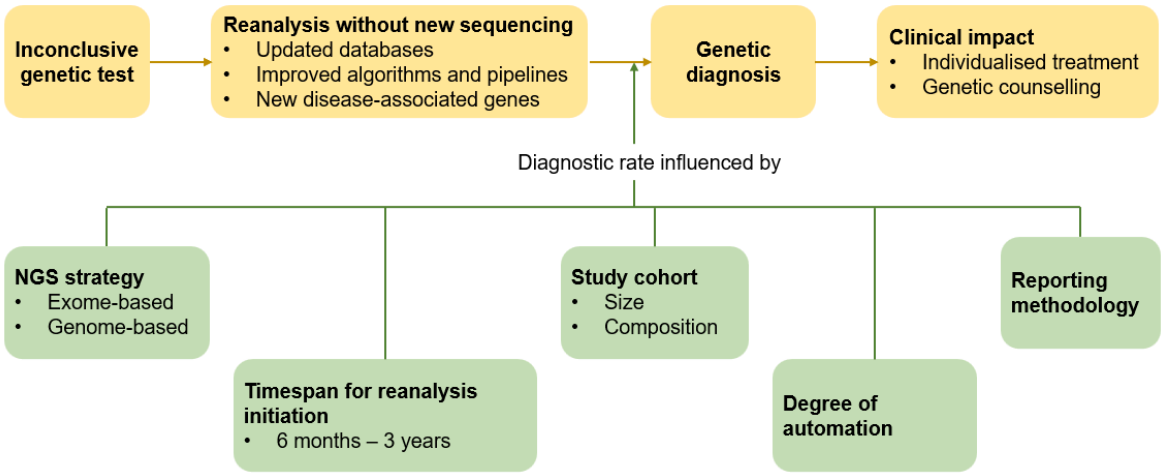

Figure 1.1. Reanalysis of next generation sequencing data in genetic disorders from an inconclusive test to a genetic diagnosis translated to clinical impact, including factors influencing the variability in diagnostic rates.

The increasing importance of reanalysis is intrinsically linked to the introduction of NGS in the early 2000s. NGS, also known as massively parallel or high-throughput sequencing, represented a paradigm shift from traditional Sanger sequencing by enabling the simultaneous sequencing of millions of DNA or RNA fragments, offering several key advantages. These include: 1) high-throughput capabilities with sample multiplexing, allowing for the simultaneous analysis of multiple samples; 2) enhanced sensitivity in detecting low-frequency genetic variants; 3) accelerated processing of large sample volumes, enabling rapid data generation; and 4) a substantial reduction in sequencing costs per patient (Yadav et al., 2023; Zhong et al., 2021). This confluence of factors has transformed NGS into a cost-effective and highly efficient tool for genomic analysis (Satam et al., 2023; Vinkšel et al., 2021), dramatically reducing the price and turnaround time for sequencing a complete human genome from billions of dollars and multiple years in the 2000s to less than one thousand dollars and days currently (Zhong et al., 2021). Consequently, the clinical utility of NGS has been increasingly recognised, leading to its widespread adoption in genetic diagnostics within healthcare institutions since the early 2010s (Gullapalli et al., 2012; Singh et al., 2016), with whole exome sequencing (WES) being the most used approach due to its cost-effectiveness compared to whole genome sequencing (WGS) (Alfares et al., 2018; Dai et al., 2022; Ewans et al., 2022; Fung et al., 2020; Hiatt et al., 2018).

However, while significant progress has been achieved in the interpretation of NGS data, a considerable challenge persists in achieving consistent diagnostic outcomes. Reported diagnostic rates range from 25% to 70%, a variability attributed to a multitude of factors

including phenotypic heterogeneity, technical constraints, bioinformatic pipelines, sequencing approaches, database comprehensiveness, variant classification standards and the quality of clinical information, among others (Fung et al., 2020; Laurie et al., 2025; Rosier et al., 2022; Seaby & Ennis, 2020; Seo & Lee, 2023). For instance, in Mendelian disorders approximately >50% of clinical cases remain inconclusive (Dai et al., 2022; Wenger et al., 2017) while in paediatric neurology the diagnostic yield is around 30% (Schobers et al., 2022). The reanalysis of existing NGS data addresses this challenge by leveraging the evolving knowledge and tools in genomic interpretation.

The enhanced diagnostic success of reanalysis is driven by several interconnected factors. Firstly, the continuous updating of scientific literature and the identification of novel gene-disease associations represent a primary source of new diagnoses. The rapid pace of genetic discovery means that re-examining existing data can reveal genes newly linked to specific phenotypes since the initial analysis, providing reinterpretations that were not possible with the knowledge available at the time of primary testing (Schobers et al., 2022; Van Der Geest et al., 2024; Won et al., 2020). Secondly, significant advancements in bioinformatics tools and analytical methods play a crucial role in enhancing variant detection and interpretation (Schobers et al., 2022; Won et al., 2020). The implementation of updated annotation databases and more sophisticated variant calling algorithms allows for the identification of previously missed or misclassified variants, as demonstrated by cases where pathogenic/likely pathogenic (P/LP) variants were initially overlooked due to annotation to less relevant transcript isoforms (Schobers et al., 2022). In addition, the reinterpretation of variants of uncertain significance (VUS) over time also can significantly impact diagnosis (Thummala et al., 2024; Vears et al., 2018; Walsh et al., 2024).

The increasing knowledge regarding genotype-phenotype correlations and the potential for phenotype expansion over time can significantly aid in the interpretation of molecular findings during reanalysis (Karaca et al., 2018). Detailed reevaluation of clinical information is crucial in this context, as evolving understanding of genetic disorders may reveal that a patient's symptoms align with recently described manifestations of a particular genetic condition. Furthermore, collaborative research efforts and data sharing platforms ease the validation of candidate variants and the identification of novel gene-disease associations. International efforts, exemplified by the European Solve-RD study, demonstrate the added diagnostic value of combined expertise compared to isolated analyses (Laurie et al., 2025).

In light of these significant advantages, recent literature strongly recommends systematic reanalysis of unresolved cases after a period of time, with evidence suggesting that a timespan ranging from 6 months to 3 years after the initial analysis may improve effectiveness by allowing sufficient time for new discoveries and advancements (Van Der Geest et al., 2024). For instance, a minimum of 12 to 18 months is suggested in the case of WES reanalysis in patients with Mendelian diseases or paediatric patients (Ewans et al., 2018; Stark et al., 2019). While the implementation of reanalysis programs faces practical obstacles, including resource allocation, data storage infrastructure, and ethical considerations regarding consent and the communication of new findings (Van Der Geest et al., 2024), the potential for significant diagnostic gains, particularly for patients with rare or complex phenotypes, is substantial.

Finally, automation also plays a key role in reanalysis. While manual curation is often associated with better outcomes (Van Der Geest et al., 2024), automated approaches facilitate the incorporation of up-to-date literature and databases, enabling the identification of novel gene-disease associations and variants, ultimately improving the diagnostic yield (Baker et al., 2019; Dai et al., 2022; James et al., 2020; Sarmady & Abou Tayoun, 2018). Artificial intelligence-based tools have shown promising results and seem to be valuable for clinical laboratories as they reduce the time and resources invested on reanalysis benefiting a larger number of patients (O'Brien et al., 2022; Van Der Geest et al., 2024).

1.1.1. Definition and reanalysis-related terms

As stated by Van Der Geest et al. (2024), various strategies regarding the reevaluation of NGS data have been employed in the literature. Based on the definitions of their work and previous publications (Carrieri et al., 2019; El Mecky et al., 2019), four main terms can be differentiated (summarised at **Table 1.1**). These include: **1) Reanalysis**, the process of reviewing existing raw NGS data for a patient, encompassing all variants and genes, including those not initially associated with the patient's clinical phenotype**; 2) Reinterpretation**, the reassessment of previously analysed and interpreted variants associated with the patient's disease/phenotype to evaluate the ongoing validity of their interpretation or the need for reclassification in the light of new knowledge; **3) Reclassification**, the assignation of a new pathogenicity status to a previously classified variant based on updated information resulting from reinterpretation, such as upgrading a VUS to P/LP; and **4) Retesting**, also known as resequencing or the act of conducting a

new genetic test (e.g. performing WES again using updated approaches, or using other strategy, such as WGS), which generates new data.

Table 1.1. Specification of definitions for reanalysis and related terms.

Term	Definition	Example/s
Retesting	The process in which the patient's sample is tested again using the same or a different technique, resulting in a new set of raw data.	Fung et al. (2020)
Reanalysis	Using the patient's existing raw data to analyse all genes currently associated with the patient's condition.	Costain et al. (2018); Ewans et al. (2018); Jalkh et al. (2019); James et al. (2020); Rosier et al. (2022); Schot et al. (2024); Wenger et al. (2017)
Reinterpretation	Reinterpretation of genetic variants that have been detected, analysed and interpreted previously to assess whether the initial classification should be changed.	Hiatt et al. (2018); P. Liu et al. (2019); Won et al. (2020)
Reclassification	The action in which a variant receives a new classification, as a result of reinterpretation, in regard to the ACMG/AMP classification system.	Hiatt et al. (2018); P. Liu et al. (2019); Won et al. (2020)

Adapted from Van Der Geest et al. (2024). Abbreviations: ACMG/AMP = American College of Medical Genetics and Genomics and Association for Molecular Pathology.

1.1.2. Reevaluation approaches in the literature

The literature provides numerous examples of reanalysis as a prevalent strategy, with a significant proportion of studies focused on the reevaluation of WES or clinical exome sequencing (CES) data in patients with suspected Mendelian disorders (Ewans et al.,

2018; Jalkh et al., 2019; Rosier et al., 2022; Wenger et al., 2017). However, the reanalysis of WGS data is also a widespread option (Costain et al., 2018; James et al., 2020; Schot et al., 2024). Notably, reevaluation approaches are often applied concurrently, such as the combination of reanalysis with retesting for clinical cases with no available raw data (Fung et al., 2020), the integration of reanalysis with reinterpretation and reclassification (Hiatt et al., 2018; Liu et al., 2019; Won et al., 2020), and comprehensive studies encompassing all of them (Schobers et al., 2022; Tan et al., 2020).

While WGS offers advantages over WES and an improved diagnostic yield (Bowman et al., 2025; Brlek et al., 2024; Nomakuchi et al., 2024), studies have demonstrated that reanalysis of WES data can identify a considerable fraction of disease-causing variants detected by WGS at a reduced economic cost (Alfares et al., 2018; Ewans et al., 2022). This suggests that while reanalysis of WES data may exhibit limited diagnostic rates, it presents a cost-effective clinical utility. Consequently, in settings with resource constraints, the reanalysis of existing exome sequencing data represents a valuable strategy for unsolved cases prior to considering genome sequencing (Alfares et al., 2018).

1.2. Next generation sequencing in genetic diagnosis

The advent of NGS has marked a major transition in genetic diagnosis: moving from a gene-by-gene approach (the disease-causing gene had to be identified for genetic diagnosis) to a syndrome-based one (thousands of genes sequenced at once to find the variant explaining the patient's clinical phenotype) (Ki, 2021). This was clearly exemplified by the first use of WES in a genetic diagnosis in the work of M. Choi et al. (2009), where a 5-month-old infant was diagnosed with congenital chloride diarrhea after the identification of a disease-causing variant in the *SLC26A3* gene. Since then, NGS has played a crucial role in the discovery of genes associated with Mendelian disorders, with 87% of gene discoveries by 2017 (Vinkšel et al., 2021).

1.2.1. Next generation sequencing technologies

Prior to the development of NGS technologies, **first generation sequencing** was fundamentally shaped by the introduction of Sanger sequencing in 1977 (Sanger et al., 1977), a technique that established itself as the primary approach up to 2005. Sanger sequencing is widely regarded as the gold standard for the accurate detection of single nucleotide variants (SNVs) and small insertion-deletions (indels). However, it is inherently limited by its capacity to process only a single DNA fragment at a time, with a maximum

read length of approximately 1,000 nucleotides. This constraint renders it inefficient and cost-prohibitive for applications requiring the parallel sequencing of numerous genes or the analysis of the same genomic region across multiple samples. Despite the widespread adoption of NGS in diagnostic settings, Sanger sequencing continues to find utility in specific clinical scenarios, including the diagnosis of some single-gene disorders, targeted screening of at-risk family members for known P variants, and the validation of variants initially identified through massively parallel sequencing. Nevertheless, recent literature has called into question the real clinical utility of Sanger sequencing for validation in certain contexts (Arteche-López et al., 2021; De Cario et al., 2020), arguing that Sanger confirmation should be limited to variants with borderline quality parameters or used only for internal quality control (QC).

Second generation sequencing

The advent of the second generation in the early 2000s inaugurated the era of NGS, characterised by a significantly enhanced throughput capacity, accelerated sequencing speeds, and the fundamental ability to perform parallel sequencing. This technological leap facilitated the development of four principal platforms (Clark et al., 2019; Hu et al., 2021; Satam et al., 2023), each based on distinct methodologies: **1) the Roche 454 system**, which employs pyrosequencing; 2) **Ion Torrent sequencing**, which detects the release of hydrogen ions during DNA synthesis; **3) Illumina sequencing**, which utilises a sequencing-by-synthesis approach based on reversible dye terminators; and **4) SOLiD** (Sequencing by Oligonucleotide Ligation and Detection), which employs a ligation-based strategy with reversible terminators. Notably, the first three platforms are founded on the principle of sequencing by DNA synthesis, while SOLiD utilises sequencing by ligation. The main characteristics of these platforms are described in **Table 1.2**.

A common characteristic of these technologies is the generation of short reads, with a mean length of 150 base pairs (bp) (Oehler et al., 2023). This inherent limitation has strong implications in the difficulties encountered when sequencing repetitive regions and unravelling complex structural variants (SVs), or variants located on high homology and GC-rich regions (Oehler et al., 2023; Sinha et al., 2025; Yadav et al., 2023). Moreover, the library preparation process is characterised by its potential to introduce biases into the sequencing data (Yadav et al., 2023).

Third generation sequencing

To overcome the inherent limitations of preceding technologies, third generation sequencing methodologies were developed, characterised by their capacity to generate significantly longer reads, exceeding 10,000 bp (Olivucci et al., 2024), and eliminating the necessity for DNA fragmentation or polymerase chain reaction(PCR)-based amplification (Oehler et al., 2023; Satam et al., 2023; Yadav et al., 2023). Two primary long-read sequencing platforms are prominent (summarised at **Table 1.2**): **1) Pacific Biosciences** (PacBio), which employs a single-molecule real-time (SMRT) approach utilising fluorescently labeled nucleotides; and **2) Oxford Nanopore Technologies**, wherein a single-stranded DNA molecule is translocated through a nanopore, and the resulting perturbations in electrical current are measured to determine the sequence (Clark et al., 2019; Hu et al., 2021; Mahmoud et al., 2024; Satam et al., 2023). These technologies possess the capability to effectively resolve complex and repetitive genomic regions, leading to enhanced detection of SVs (Iyer et al., 2024; Logsdon et al., 2020; Mantere et al., 2019; Olivucci et al., 2024; Warburton & Sebra, 2023; Wohlers et al., 2023). Moreover, by preserving the native state of the DNA molecule, long-read sequencing enables the direct detection of base modifications, such as DNA methylation (Geysens et al., 2025; Mantere et al., 2019; Oehler et al., 2023; Satam et al., 2023).

While the economic cost associated with achieving comparable sequencing coverage using long-read technologies remains higher than that of short-read sequencing (Espinosa et al., 2024) in addition to higher computational and data storage costs, recent literature has demonstrated their clinical application (Geysens et al., 2025; Mahmoud et al., 2024; Oehler et al., 2023; Olivucci et al., 2024; Sinha et al., 2025).

It is pertinent to note that the scope of this work will be delimited to short-read sequencing technologies, as these methodologies were employed to address the specific research objectives of this thesis dissertation.

Table 1.2. Key features of main next generation sequencing platforms.

Platform	Generation	Use	Sequencing technology	Amplification type	Read length	Advantages	Limitations
Roche 454 pyrosequencing	Second	Short-read sequencing	Sequencing by synthesis	Emulsion PCR	400–1000	Long reads compared to other 2nd generation technologies.	Indel errors at homopolymer regions.
Ion Torrent	Second	Short-read sequencing	Sequencing by synthesis	Emulsion PCR	200–400	Fast sequencing run times.	Indel errors at homopolymer regions.
Illumina	Second	Short-read sequencing	Sequencing by synthesis	Bridge PCR	36–300	Error rate 0.1%.	Error rate >1% in case of sample overloading.
SOLiD	Second	Short-read sequencing	Sequencing by ligation	Emulsion PCR	75	High sequencing accuracy.	Substitution errors and underrepresented GC-rich regions.
PacBio SMRT technology	Third	Long-read sequencing	Sequencing by synthesis	Without PCR	average 10,000–25,000	Long reads, fast turnaround times, real-time data and proper characterisation of SVs.	High economic cost.
Nanopore DNA sequencing	Third	Long-read sequencing	Sequence detection through electrical impedance	Without PCR	average 10,000–30,000	Long reads, fast turnaround times, real-time data and proper characterisation of SVs.	Error rate 2-15%.

Adapted from Satam et al. (2023). Abbreviations: PCR = polymerase chain reaction; indel = insertion–deletion; SOLiD = Sequencing by Oligonucleotide Ligation and Detection; PacBio = Pacific Biosciences; SMRT = single-molecule real-time; SVs = structural variants.

1.2.2. Clinical diagnosis approaches

Clinical diagnosis using short-read NGS has been mostly conducted using one of these three approaches: 1) targeted sequencing; 2) exome sequencing; and 3) genome sequencing.

Targeted sequencing

Targeted sequencing entails the selective analysis of specific coding regions within the genome, concentrating on a defined set of genes associated with a particular disease or a spectrum of related disorders, including syndromic conditions. This methodology enables the study of gene panels typically of a smaller scale than those employed in CES and WES. Target enrichment is achieved through selection and amplification of coding exons and adjacent intronic regions using pools of region-specific oligonucleotide primers (Satam et al., 2023). This strategy possesses the capacity to detect SNVs and small-scale genomic alterations, including deletions, duplications, insertions, and gene rearrangements.

Targeted sequencing has served as the first-line diagnostic test in numerous laboratories for years (Ki, 2021). This preference was attributed to its capacity for achieving higher depth of coverage compared to WES and WGS, alongside improved cost-effectiveness and faster turnaround times (Pei et al., 2023; Satam et al., 2023; Vinkšel et al., 2021; Zhong et al., 2021). As variant detection is restricted to a specific group of genes, the resulting data volume is lower, facilitating more manageable and less time-consuming interpretation while also reducing the likelihood of identifying incidental findings in genes unrelated to the primary clinical indication as well as VUS.

The principal limitation of short-read targeted sequencing lies in its narrow focus, completely missing novel or unexpected variants and single nucleotide polymorphisms (SNPs) outside the targeted regions (Yadav et al., 2023), including limited detection capabilities for SVs and repetitive elements (Vinkšel et al., 2021). Additionally, the discovery of novel gene-disease associations necessitates either the design and implementation of a new gene panel or the consideration of conducting WES, a requirement similarly applicable to CES. Finally, in instances characterised by significant diagnostic uncertainty, the diagnostic rate can be limited (Dillon et al., 2018).

Exome sequencing

Exome sequencing is typically focused on the capture of coding exons and adjacent intronic regions across a broader spectrum of genes than conventional more targeted gene panels. Depending on the targeted genes, two primary strategies exist: 1) **Whole exome sequencing**, which aims to interrogate the entirety of protein-coding genes within the human genome; and **2) Clinical exome sequencing**, which selectively analyses a curated set of genes with established or strong clinical relevance. WES targets the complete exome, which encompasses the protein-coding regions of the genome, representing approximately 1–2% of the complete human genome (Satam et al., 2023; Vinkšel et al., 2021). This technique facilitates the analysis of ~22,000 genes, which harbor about 95% of disease-causing mutations (Posey, 2019; Zhong et al., 2021). In contrast, CES, also known as Mendeliome, involves the analysis of a defined and curated subset of genes, typically ranging between 3,000 and 6,000 (Fazeli et al., 2016; Holla et al., 2022; Lee et al., 2015; Saudi Mendeliome Group, 2015) contingent upon the clinical focus (e.g., all Mendelian diseases, a specific genetic disorder or a set of related conditions). This gene subset is composed of genes with well-established associations to human diseases (Alix et al., 2023; Sainio et al., 2022), as documented in clinical databases such as Online Mendelian Inheritance in Man (OMIM).

Both approaches involve the enrichment of exonic regions through two methodologies: **1) Hybrid capture-based**, utilising biotinylated probes for selective capture and sequencing of exonic DNA; or **2) PCR-based**, wherein PCR is used to amplify and sequence exonic DNA fragments (Satam et al., 2023; Yadav et al., 2023). Exome sequencing permits the identification of SNVs, indels, but presents limited capacity for copy number variants (CNVs) detection (Yao et al., 2017).

Both WES and CES can be used as primary diagnostic options in patients presenting with suspected Mendelian disorders, encompassing those with complex clinical phenotypes or belonging to specific disease groups. In instances where initial analysis studying a virtual panel of genes fails to identify a P variant elucidating the etiology of the disease, leading to unresolved cases, the comprehensive WES or CES dataset can be reanalysed.

As all known disease-causing genes are included, as well as those not yet associated with genetic diseases, WES offers a broader scope compared to CES, allowing for the potential identification of novel disease genes through subsequent reanalysis as variant classification and gene discovery progress (Ewans et al., 2018).

Limitations of both WES and CES include suboptimal coverage of non-coding regions, such as deep intronic and regulatory sequences (Burdick et al., 2020), inconsistent coverage in GC-rich regions, and challenges in the detection of SVs such as large deletions/duplications, inversions, and translocations, as well as variants within repetitive elements and causal variants in cases of somatic mosaicism (Meienberg et al., 2016; Vinkšel et al., 2021). Nevertheless, technological advancements, particularly in long-read sequencing, have led to improved exonic coverage (Vinkšel et al., 2021).

Whole genome sequencing

WGS is the most comprehensive approach of genomic analysis, involving the interrogation of a patient's entire genome, encompassing all protein-coding and non-coding regions, including regulatory elements and intergenic sequences. This approach enables the detection of a wide spectrum of genetic variations, including splicing and regulatory variants. While the initial cost of WGS has historically been higher compared to more targeted sequencing strategies, its increasing cost-effectiveness over recent years has rendered it a progressively viable option for clinical applications (Brlek et al., 2024).

WGS demonstrates significant advantages over targeted gene panels, CES and WES. This methodology exhibits superior diagnostic and analytical sensitivity for the detection of SNVs, indels, and CNVs, including large deletions and duplications, as well as complex genomic rearrangements such as translocations and variants within non-coding regions like deep intronic ones (Ki, 2021; Vinkšel et al., 2021). Furthermore, the enhanced uniformity of sequencing data generated by genome sequencing facilitates the analysis of most GC-rich regions, which are often challenging for other sequencing methods. Similar to exome sequencing, WGS permits the reanalysis of generated data after a period of time. However, it also presents a higher likelihood for the identification of incidental findings and VUS.

One of the drawbacks of WGS lies in the inherent difficulties associated with the clinical interpretation of non-coding variants, largely due to the current limited knowledge and understanding of their phenotypic effect (Brlek et al., 2024). Another significant limitation pertains to the potential for false positives arising from variants with low penetrance. Finally, the substantial volume of data generated by genome sequencing necessitates a considerably longer time for comprehensive interpretation and analysis.

Bioinformatics constraints

From a bioinformatics perspective, the comprehensive nature of WGS necessitates not only a greater economic investment compared to more targeted strategies but also significantly higher computational and data storage demands due to the substantial volume of data generated. Illustratively, data provided by NGS service provider 3billion (https://3billion.io/), indicates that WES produces approximately 10 Gigabytes (Gb) of data per patient at a sequencing depth of 150X, whereas WGS generates approximately 120 Gb of data per patient at a lower sequencing depth of 35X, representing a twelve-fold increase that can pose considerable challenges for data accessibility and management. The broader genomic coverage of WGS typically translates to a reduced average depth of 30-60X, in contrast to WES and targeted sequencing, which commonly achieve depths of 100-200X and 200-500X, respectively (Ki, 2021).

Despite these technical considerations, the mean diagnostic yield of genome sequencing is approximately 40% (Pagnamenta et al., 2023; Wigby et al., 2024), slightly higher than the reported 36% of WES (Fung et al., 2020). However, these values fluctuate depending on factors such as the patient selection criteria and the disease type. These aspects and others mentioned before are summarised in **Table 1.3**.

Table 1.3. Comparison of clinical next generation sequencing approaches

Characteristics	Targeted sequencing	CES	WES	WGS
% of genome studied	0.01-0.1%	Varies by panel	1-2%	90-95%
Sequencing depth	200-500X	100-200X	50-200X	30-60X
Number of genes	100s-1,000s	3,000-6,000	~20,000	~70,000[1]
Capture bias	Yes	Yes	Yes	No
Mean diagnostic yield	Highly variable	High for specific conditions	36%[2]	~40%[3]
Detection capacity of SNVs and indels	High	High to intermediate	High to intermediate	High
Intron variants	Low	Low	Low	High
CNVs	Intermediate	Intermediate	Intermediate	High
SVs	Low	Low	Low	High
Incidental findings rate	Low	High to intermediate	High to intermediate	High
Analysis complexity	Low	Intermediate	High to intermediate	High
Reanalysis potential	Limited to the targeted regions	Limited to selected gene list	Intermediate to high	High
Economic cost	Low	Intermediate	Intermediate	High
Computational cost	Low	Intermediate	Intermediate	High
Data volume	Small	Moderate	~10 Gb[4]	~120 Gb[4]
Turnaround time	Short	Moderate to short	Long to moderate	Long
Data storage cost	Low	Intermediate to low	Intermediate	High
Data access	Simple	Moderate	Moderate to complex	Complex
Utility	Known gene mutations,small genelists	Suspected specific genetic disorders, cost-effective when suspicion is strong	First-tier for many genetic disorders, cost-effective for broad analysis	Complex cases, research, undiagnosed cases after other tests

Edited from Ki (2021). References: 1. https://www.gencodegenes. 2. Fung et al., 2020. 3. Pagnamenta et al., 2023; Wigby et al., 2024. 4. https://3billion.io/. Abbreviations: CES = clinical exome sequencing; WES = whole exome sequencing; WGS = whole genome sequencing; SNVs = single nucleotide variants; indels = insertion–deletions; CNVs = copy number variants; SVs = structural variants; Gb = Gigabytes.

1.2.3. Standard bioinformatics pipeline for next generation sequencing

The generation of raw sequencing NGS data is the initial part of the genetic tests based on that technology; the subsequent bioinformatics analysis pipeline is critical for transforming the data into clinically and biologically meaningful insights. A standard NGS pipeline follows a well-established sequence of computational steps, designed to ensure accuracy, reproducibility, and interpretability of genetic variant information (Metzger et al., 2023; Pereira et al., 2020; Roy et al., 2018; SoRelle et al., 2020).

The core objective of an NGS bioinformatics pipeline is to process raw sequencing reads through a series of validated analytical steps, ranging from initial QC to final variant annotation. These pipelines must be robust and adaptable, particularly in clinical settings where reliability and reproducibility are crucial (Roy et al., 2018; SoRelle et al., 2020). While general structure and tools may be shared across pipelines, customisation is often required to accommodate specific sequencing platforms, sample types (e.g., tumor, germline), and research or clinical goals (Hwang et al., 2018; Khamina et al., 2022).

The key steps in a standard NGS pipeline (**Table 1.4**, **Figure 1.2**) can involve:

1. **Raw data acquisition**. Sequencing platforms generate raw data in the form of FASTQ files containing base calls and quality scores. The integrity and completeness of this data depend on platform-specific parameters and run conditions (Metzger et al., 2023; Pereira et al., 2020).
2. **Quality control** (QC). Before analysis, raw reads undergo quality assessment and filtering to remove low-quality bases, adapters, and potential contaminants. Tools like FastQC (Andrews, S., 2010) and Trimmomatic (Bolger et al., 2014) are widely used to ensure that only high-quality reads progress to downstream analyses (Metzger et al., 2023; Wolfien et al., 2016).
3. **Alignment/mapping**. High-quality reads are aligned to a reference genome (e.g., GRCh38 or GRCh37/hg19) using short-read aligners such as Burrows-Wheeler Aligner (BWA) (Li & Durbin, 2009) or Spliced Transcripts Alignment to a Reference (STAR) (Dobin et al., 2013) to produce alignment files in sam, bam or cram format. Accurate mapping is crucial for reliable variant detection, particularly in regions of high complexity or polymorphism (Metzger et al., 2023; Rosewick et al., 2020).
4. **Post-alignment processing**. Following alignment, several processing steps are performed to refine alignment data quality. These steps can include marking PCR

duplicates, base quality score recalibration, and local realignment around indels. Some of these steps, as recommended by frameworks like the Genome Analysis Toolkit (GATK) (McKenna et al., 2010), improve the specificity and sensitivity of variant calling (Roy et al., 2018; SoRelle et al., 2020).

5. **Variant calling**. This stage identifies deviations from the reference genome, including SNVs, indels, CNVs, and SVs. Tools such as GATK HaplotypeCaller, FreeBayes (Garrison & Marth, 2012), or VarScan (Koboldt et al., 2012) are commonly used to obtain variant call format (VCF) files (Metzger et al., 2023; SoRelle et al., 2020).
6. **Variant annotation**. Detected variants are functionally annotated using tools like ANNOtate VARiation (ANNOVAR) (Wang et al., 2010) or Variant Effect Predictor (VEP) (McLaren et al., 2016). These tools integrate information from population databases, gene models, conservation scores, and clinical databases to provide biological and clinical context (Metzger et al., 2023; SoRelle et al., 2020). The results files of this stage can have different formats (gff, vcf, excel, txt, among others).
7. **Visualisation of results.** Alignment sam, bam or cram files, as well as bed, vcf or gff files, can be visualised using tools such as the Integrative Genomics Viewer (IGV) software (Robinson et al., 2011, 2017, 2023; Thorvaldsdóttir et al., 2013).

Table 1.4. Key steps in a standard bioinformatics pipeline for next generation sequencing.

Step	Purpose	Example tools
Raw Data Acquisition	Obtain sequencing reads	Illumina, PacBio
Quality Control	Filter/trim low-quality reads	FastQC, Trimmomatic
Alignment	Map reads to reference genome	BWA, STAR
Variant Calling	Detect sequence variants	GATK, FreeBayes
Variant Annotation	Add biological/clinical context	ANNOVAR, VEP

Abbreviations: PacBio = Pacific Biosciences; FastQC = Fast Quality Control; BWA = Burrows-Wheeler Aligner; STAR = Spliced Transcripts Alignment to a Reference; GATK = Genome Analysis Toolkit; ANNOVAR = ANNOtate VARiation; VEP = Variant Effect Predictor.

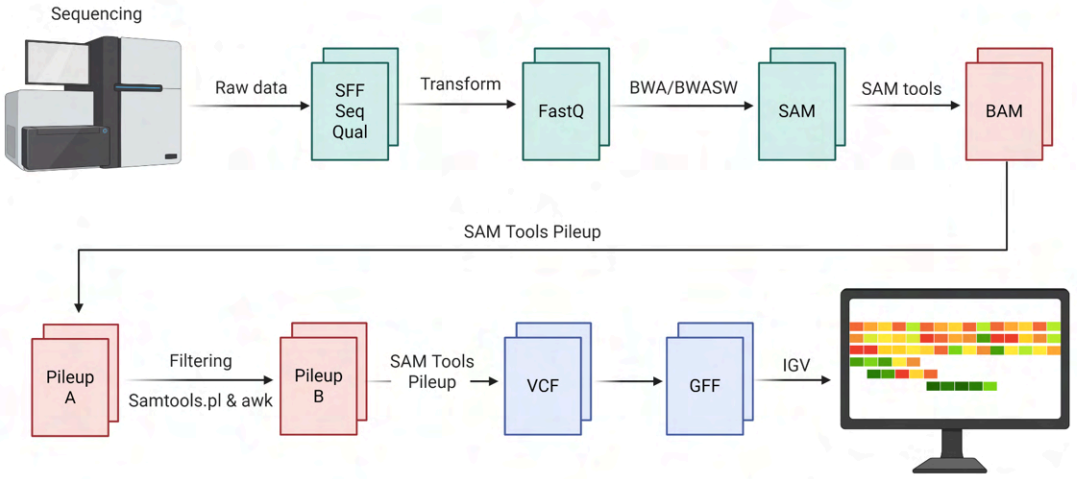

Figure 1.2. Key steps and files in a standard bioinformatics pipeline for next generation sequencing data. Created with BioRender.

1.3. Inherited cardiac conditions

Inherited cardiac conditions (ICCs) constitute an heterogeneous group of diseases, all characterised by heritable aetiologies that primarily affect the heart muscle, the aorta, or cardiac ion channels. These conditions confer a significant predisposition to devastating cardiac events, including heart failure and sudden cardiac death, frequently manifesting at a young age (Alway et al., 2024; Josephs et al., 2023). Consequently, ICCs represent a significant public health concern, with a reported prevalence of up to 1 in 200 (Ormondroyd et al., 2020). Cardiovascular diseases are broadly recognised as the leading cause of global morbidity and mortality, accounting for approximately 32% of all deaths worldwide (Gray et al., 2024), and those with a genetic origin represent more than half of all sudden deaths in individuals under 35 years old (Austin et al., 2024) with ICCs representing a significant proportion of them (Loong et al., 2023).

The inheritance pattern of genetic cardiac diseases is primarily autosomal dominant (AD), although incomplete penetrance, variable expressivity, genetic heterogeneity, oligogenic and modifying variants, overlapping phenotypes, and different disease mechanisms frequently complicate clinical diagnosis and variant interpretation (Josephs et al., 2023; Mio et al., 2024; Serpa et al., 2024). Nonetheless, certain forms of these conditions have also been reported to follow mitochondrial, autosomal recessive (AR), and X-linked recessive (XLR) inheritance patterns (D'Agostino et al., 2022).

1.3.1. Classification of inherited cardiac conditions

ICCs are broadly categorised into three principal classifications: **1) Inherited cardiomyopathies**, characterised by structural and functional abnormalities of the heart muscle, leading to phenotypes such as myocardial hypertrophy, dilation, fibrosis and impaired contractility; **2) Inherited channelopathies**, which predispose to arrhythmias without overt structural abnormalities; and **3) Inherited aortopathies**, predisposing to the development of aortic aneurysms or dissections.

This section primarily focuses on the ICCs found in the studied patient cohort, categorised according to the phenotypic classification proposed in the 2023 European Society of Cardiology (ESC) Guidelines by Arbelo et al. (2023) (see **2. Materials and methods**). **Table 1.5** summarises the estimated population prevalence and the main causative genes associated with each cardiac phenotype, which will be discussed in detail in the following subsections.

Inherited cardiomyopathies

According to the 2023 ESC Guidelines (Arbelo et al., 2023), a cardiomyopathy is precisely defined as 'a myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease (CAD), hypertension, valvular disease, and congenital heart disease (CHD) sufficient to cause the observed myocardial abnormality'. Within this framework, inherited cardiomyopathies constitute a diverse spectrum of cardiac disorders: **hypertrophic cardiomyopathy**, **dilated cardiomyopathy**, **arrhythmogenic right ventricular cardiomyopathy**, **non-dilated left ventricular cardiomyopathy**, **restrictive cardiomyopathy**, and **ventricular hypertrabeculation**. **Figure 1.3** shows cardiac magnetic resonance (CMR) images from cardiomyopathy patients.

Hypertrophic cardiomyopathy (HCM) is diagnostically characterised by a left ventricular wall thickness of ≥15 mm, observed in the absence of other underlying causes such as arterial hypertension, aortic stenosis, or physiological cardiac enlargement (Loong et al., 2023). This condition typically presents with a preserved or even increased ejection fraction. As one of the most prevalent forms of inherited cardiomyopathy, HCM affects an estimated 1 in 500 individuals within the general population (0.2%) (Arbelo et al., 2023; Loong et al., 2023; Serpa et al., 2024). P variants in genes encoding sarcomeric proteins, including *MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL3*, and *MYL2*, collectively

account for approximately 30-50% of cases in affected individuals (Josephs et al., 2023; Loong et al., 2023; Serpa et al., 2024). Among these, variants in *MYH7* (encoding β-myosin heavy chain) and *MYBPC3* (encoding myosin binding protein C) are the most frequently encountered, together representing nearly 70% of identified genetic aetiologies (Gray et al., 2024).

Dilated cardiomyopathy (DCM) is characterised by left ventricular or biventricular dilation coupled with impaired systolic function, in the absence of abnormal loading conditions (e.g., arterial hypertension, valve disease, or CHD) or ischemic heart disease (Arbelo et al., 2023; Loong et al., 2023). This condition exhibits an estimated population prevalence of approximately 1 in 250 individuals (0.4%) including both genetic and acquired forms (Arbelo et al., 2023; Gray et al., 2024). When studying inherited forms, familial DCM is genetically heterogeneous, with causal variants identified in over 50 genes. P variants in the *TTN* gene account for up to 25% of familial DCM cases, while *LMNA* and *MYH7* contribute 6% and 4%, respectively (Gray et al., 2024; Loong et al., 2023). Other relevant genes implicated include *BAG3, DES, FLNC, RBM20, SCN5A, TNNC1, TNNT2* and *PLN* (Josephs et al., 2023).

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterised by right ventricular dilatation and/or dysfunction in the presence of histological involvement and/or electrocardiographic abnormalities (Arbelo et al., 2023). This condition confers a considerable risk of adverse cardiac events, including sustained ventricular arrhythmias, progressive ventricular dysfunction, and sudden cardiac death, with this risk being particularly pronounced in younger patients (Corrado et al., 2017). ARVC shows an incidence of 1 in 1,000-1,500 (approximately 0.078%) (Arbelo et al., 2023). This disease usually arises from defects in cardiac cell-to-cell junctions, primarily involving the desmosome. These abnormalities result in myocyte detachment and altered intracellular signaling, manifesting pathologically as myocardial cell loss and fibrofatty infiltration of the heart muscle (Loong et al., 2023). P variants in *PKP2* are the most frequently observed. Other relevant genes include *DSP, DSG2, DSC2, TMEM43, PLN, DES* and *JUP* (Gray et al., 2024; Josephs et al., 2023; Loong et al., 2023; Serpa et al., 2024).

Non-dilated left ventricular cardiomyopathy (NDLVC) is characterised by regional wall motion abnormalities or myocardial scarring, without left ventricular dilation, and unexplained by abnormal loading conditions or CAD (Eda et al., 2024; Scolari et al., 2024). This diagnostic entity was formally introduced as a new clinical definition of

cardiomyopathy in the 2023 ESC Guidelines (Arbelo et al., 2023). Consequently, its genetic landscape is still being fully elucidated but it shares significant genetic overlap with both DCM and ARVC, given the phenotypic spectrum contained within NDLVC (Scolari et al., 2024). Prominent genes associated with this condition include *DSP, FLNC, DSM, LMNA* and *PLN*.

Restrictive cardiomyopathy (RCM) is characterised by a non-dilated ventricular morphology and the absence of increased wall thickness, coupled with diastolic dysfunction. This includes impaired ventricular relaxation, elevated filling pressures, and consequent atrial enlargement, ultimately leading to heart failure (Scolari et al., 2024). While most RCM-related genes encode sarcomeric proteins (e.g., *TTN* and *MYH7*), the genetic landscape also includes cytoskeletal proteins like *LMNA* and *FLNC* (Scolari et al., 2024). Cardiac amyloidosis, often presented as a RCM, is most commonly associated with variants in the *TTR* gene.

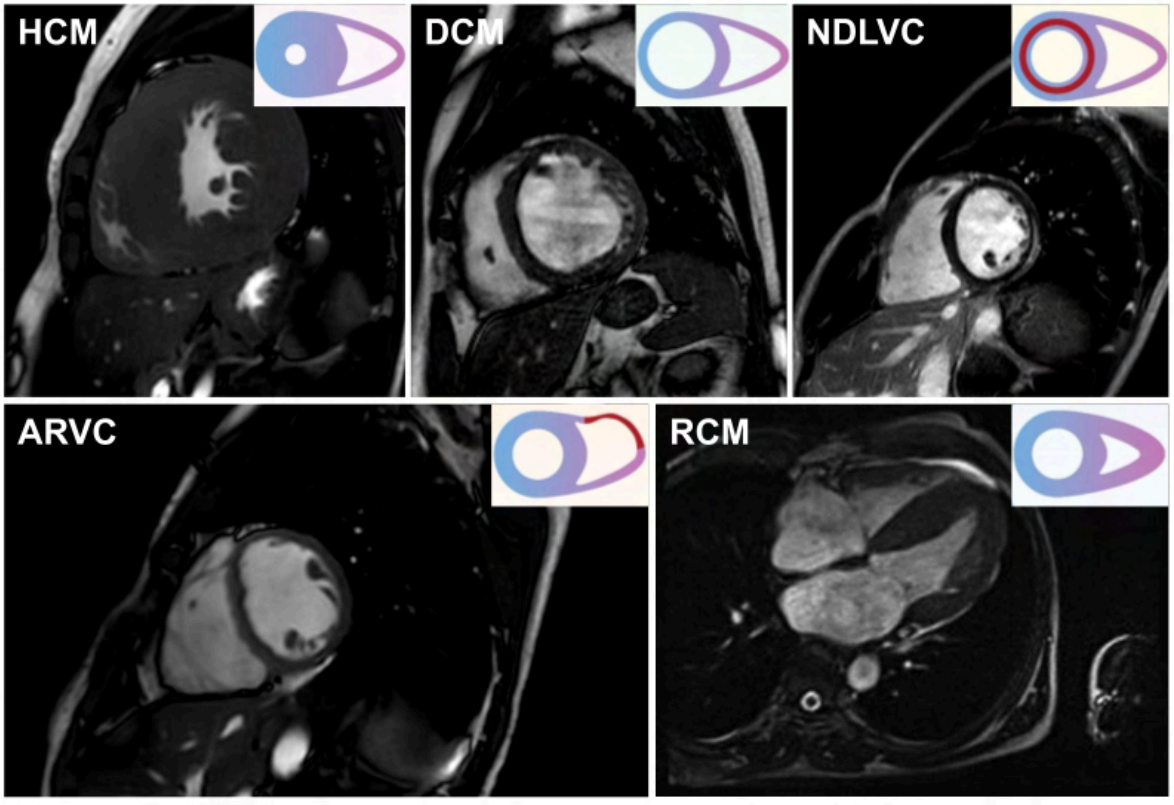

Figure 1.3. Cardiac magnetic resonance images of different cardiomyopathies. The first four images correspond to patients of the cohort analysed in this thesis while the restrictive cardiomyopathy one was extracted from Muchtar et al. (2017). The graphical representations were extracted from Arbelo et al. (2023)**.** Abbreviations: HCM = hypertrophic cardiomyopathy; DCM = dilated cardiomyopathy; NDLVC = non-dilated left ventricular cardiomyopathy; ARVC = arrhythmogenic right ventricular cardiomyopathy; RCM = restrictive cardiomyopathy.

Ventricular hypertrabeculation (VH), historically referred to as left ventricular non-compaction cardiomyopathy, describes a ventricular phenotype characterised by prominent left ventricular trabeculae and deep intertrabecular recesses (Arbelo et al., 2023). According to the 2023 ESC Guidelines, VH is no longer classified as an independent cardiomyopathy but rather as a morphological trait. This phenotypic trait can manifest in isolation or in association with other developmental abnormalities, ventricular hypertrophy, dilatation, or systolic dysfunction (Arbelo et al., 2023). It is important to note that the patients in our cohort exhibiting these characteristics were classified based on the understanding and diagnostic criteria prevalent prior to this reclassification.

Inherited channelopathies

Inherited cardiac channelopathies (CHAN), also known as inherited arrhythmia syndromes or primary electrical heart diseases, encompass a diverse group of genetic conditions. These disorders specifically affect the ion channels within cardiomyocytes, leading to life-threatening arrhythmias. Notably, CHAN are characterised by the absence of structural defects in the heart. The most prevalent ones include **long QT syndrome**, **Brugada syndrome** and **catecholaminergic polymorphic ventricular tachycardia**. Our phenotypic classification also included **cardiac conduction diseases** and **sudden arrhythmic death syndrome,** when a CHAN is suspected but none has specifically been identified. Other remarkable CHAN are short QT syndrome, idiopathic ventricular fibrillation and early repolarization syndrome.

Long QT syndrome (LQTS) patients present a prolonged QT interval on the electrocardiogram (ECG). This prolongation significantly increases the risk of syncope or cardiac arrest, which can occasionally be triggered by emotional or physical stress, and ultimately, sudden cardiac death (Dib Nehme et al., 2025; Loong et al., 2023). This condition has an estimated population prevalence of 1 in 2,500 individuals (Dib Nehme et al., 2025). LQTS is genetically heterogeneous, presenting with up to 17 recognised subtypes, each dependent on the specific disease-causing gene (Dib Nehme et al., 2025). The three major forms, LQT1, LQT2, and LQT3, are associated with P variants in *KCNQ1, KCNH2,* and *SCN5A*, respectively. Other genes significantly implicated in LQTS include *ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5, CALM1, CALM2, CALM3,* and *TRDN*.

Brugada syndrome (BrS) is diagnostically characterised by a distinctive coved-type ST-segment elevation in the absence of structural cardiac abnormalities (Loong et al., 2023). Individuals affected by this disorder may experience life-threatening polymorphic ventricular tachycardia in the right precordial leads of ECG, alongside an elevated risk of sudden cardiac death or ventricular fibrillation. This condition shows an estimated population prevalence of 3-5 per 10,000 people (Dib Nehme et al., 2025). Loss-of-function (LOF) variants in the *SCN5A* gene are strongly associated with BrS, present in 15-30% of clinical cases. Although the disease appears to follow a monogenic AD inheritance pattern, recent advancements in understanding suggest that it is a complex polygenic disorder, involving not only multiple coding genes such as *SCN4A, SCN10A, KCNH2, CACNA1C*, and *CACNA2D1*, but also non-coding ones (Dib Nehme et al., 2025; Loong et al., 2023; Makarawate et al., 2020; Theisen et al., 2023).

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare, inherited arrhythmogenic disorder affecting approximately 1 in 10,000 individuals (Dib Nehme et al., 2025). This condition is characterised by polymorphic ventricular tachycardia, often manifesting as syncopal episodes or sudden cardiac death during periods of physical exercise or emotional stress (Dib Nehme et al., 2025). The primary subtype, CPVT1, is linked to the *RYR2* gene and typically follows an AD inheritance pattern but variants in other genes including *CASQ2, TRDN, TECRL, KCNJ2, CALM1, CALM2, CALM3, SCN5A, PKP2,* and *ANK2*, have also been associated with this form of the condition (Dib Nehme et al., 2025). Homozygous mutations in the *CASQ2* gene have been identified in patients diagnosed with CPVT2 (Dib Nehme et al., 2025; Loong et al., 2023).

Cardiac conduction diseases (CCDs) represent a broad spectrum of clinical conditions defined by abnormalities in cardiac electrical conduction, encompassing both acquired and hereditary etiologies. The familial forms are primarily associated with P variants in *SCN5A* and *TRPM4* (Balla et al., 2025). While often isolated, CCDs can also be observed in conjunction with various cardiomyopathies, including DCM, HCM or arrhythmogenic cardiomyopathy (Balla et al., 2025).

Sudden arrhythmic death syndrome (SADS) denotes sudden cardiac death in young individuals (generally under 35 years of age) where the cause of death remains unexplained after a comprehensive post-mortem examination, including negative toxicology and the finding of a structurally normal heart (Mellor & Behr, 2014; Scrocco et al., 2021). These deaths are frequently linked to undiagnosed inherited CHAN, most

commonly LQTS, BrS, and CPVT (Mellor & Behr, 2014). Indeed, up to 50% of families of individuals with a SADS can be diagnosed with one of these CHAN or, less frequently, an underlying cardiomyopathy (Scrocco et al., 2021).

Inherited aortopathies

Inherited aortopathies represent a heterogeneous group of genetic disorders defined by predisposition to life-threatening aortic aneurysms and dissections (Elendu et al., 2025). These conditions are broadly classified into two main categories: **1) Syndromic aortopathies** (also known as heritable thoracic aortic diseases with systemic features), where aortic disease manifests as part of a broader multisystem disorder exhibiting distinct clinical features across various vessels and systems (e.g., skeletal, ocular, dermal, neurological systems); and **2) Non-syndromic aortopathies** or familial thoracic aortic aneurysms (TAAs) and dissections, which involve aortic disease as the primary or isolated finding, without the pronounced multisystemic characteristics typical of syndromic forms (R. Bhandari et al., 2020; Mills et al., 2024).

This subsection will focus on familial syndromic aortopathies (FSA), as all patients with aortopathies included in the analysed cohort belonged to this category.

The major FSA affecting the thoracic aorta primarily impact connective tissue integrity. The most prominent ones include **Marfan**, **vascular Ehlers-Danlos** and **Loeys-Dietz** syndromes (Asta et al., 2023; R. Bhandari et al., 2020). Other remarkable FSA include Shprintzen-Goldberg syndrome and arterial tortuosity syndrome.

Marfan syndrome (MFS) is an AD multisystem connective tissue disorder, characterised by aortic root aneurysm and aortic dissection, alongside ocular and skeletal abnormalities (R. Bhandari et al., 2020; Loong et al., 2023). This syndrome affects an estimated 1 in 5,000 individuals globally (Dipchand et al., 2024; Elendu et al., 2025). The primary gene implicated, accounting for up to 90% of cases, is *FBN1* (Loong et al., 2023). This gene encodes fibrillin-1, a large extracellular matrix protein critical for maintaining the structural integrity of connective tissue (Asta et al., 2023; R. Bhandari et al., 2020).

Ehlers-Danlos syndromes (EDSs) constitute a heterogeneous group of hereditary connective tissue disorders, marked by extensive clinical and genetic variability across their 13 subtypes (Asta et al., 2023). The specific subtype primarily associated with aortic abnormalities is vascular Ehlers-Danlos syndrome (vEDS), historically known as

Ehlers–Danlos type IV (Mills et al., 2024). This AD connective tissue disorder has an estimated population prevalence of approximately 1 in 100,000 (Dipchand et al., 2024; Elendu et al., 2025). vEDS is caused by P variants in the *COL3A1* gene (Asta et al., 2023). This gene encodes the pro-alpha1 chain of type III procollagen, a protein essential for the structural integrity of arterial walls. Defects in the protein consequently lead to an elevated risk of arterial dissections and aneurysm formation (Mills et al., 2024).

Loeys-Dietz syndrome (LDS) is an AD aortic aneurysm syndrome and connective tissue disorder, clinically characterised by a distinct group of features, including hypertelorism, bifid uvula or cleft palate, and aortic or arterial aneurysms (Asta et al., 2023; Mills et al., 2024). This disorder affects an estimated 1 in 20,000 individuals (Elendu et al., 2025). LDS is caused by P variants in different members of the transforming growth factor beta (TGF-β) signaling pathway. Specifically, mutations in the *TGFBR1* and *TGFBR2* genes are associated with types 1 and 2, respectively (Asta et al., 2023; Dipchand et al., 2024). Other genes implicated in different types of LDS include *SMAD3*, *TGFB2* and *TGFB3* (Asta et al., 2023; Mills et al., 2024). These mutations lead to dysregulated increased TGF-β signaling, which consequently predisposes to aneurysmal formation (Mills et al., 2024).

Table 1.5. Prevalence and genetic basis of the main inherited cardiac conditions in the studied patient cohort.

Phenotype/Disorder	Estimated population prevalence	Main associated genes
HCM	1 in 500	*MYBPC3, MYH7, TNNT2, TNNI3, TPM1, ACTC1, MYL3, MYL2*
DCM	1 in 250	*TTN, LMNA, MYH7, BAG3, DES, FLNC, RBM20, SCN5A, TNNC1, TNNT2, PLN*
ARVC	1 in 1,000-1,500	*PKP2, DSP, DSG2, DSC2, TMEM43, PLN, DES, JUP*
NDLVC	-	*DSP, FLNC, DSM, LMNA, PLN*
RCM	-	*TTN, MYH7, LMNA, FLNC, TTR*
VH	-	-
LQTS	1 in 2,500	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5, CALM1, CALM2, CALM3, TRDN*
BrS	3-5 in 10,000	*SCN5A, SCN4A, SCN10A, KCNH2, CACNA1C, CACNA2D1*
CPVT	1 in 10,000	*RYR2, CASQ2, TRDN, TECRL, KCNJ2, CALM1, CALM2, CALM3, SCN5A, PKP2, ANK2*
CCDs	-	*SCN5A, TRPM4*
SADS	-	Genes associated with LQTS, BrS and CPVT
MFS	1 in 5,000	*FBN1*
vEDS	1 in 100,000	*COL3A1*
LDS	1 in 20,000	*TGFBR1, TGFBR2, SMAD3, TGFB2, TGFB3*

Abbreviations: HCM = hypertrophic cardiomyopathy; DCM = dilated cardiomyopathy; ARVC = arrhythmogenic right ventricular cardiomyopathy; NDLVC = non-dilated left ventricular cardiomyopathy; RCM = restrictive cardiomyopathy; VH = ventricular hypertrabeculation; LQTS = long QT syndrome; BrS = Brugada syndrome; CPVT = catecholaminergic polymorphic ventricular tachycardia; CCDs = cardiac conduction defects; SADS = sudden arrhythmic death syndrome; MFS = Marfan syndrome; vEDS = vascular Ehlers-Danlos syndrome; LDS = Loeys-Dietz syndrome.

Inherited conditions with cardiac involvement

A fourth distinct category can be distinguished with other inherited conditions exhibiting cardiac involvement: a heterogeneous group of disorders, such as **familial hypercholesterolaemia**, various forms of **heart valve disease**, **pulmonary arterial hypertension**, **RASopathies**, and **neuromuscular disorders**.

Familial hypercholesterolaemia (FH) is an AD condition characterised by significantly elevated concentrations of circulating low-density lipoprotein cholesterol (LDL-C). With an estimated prevalence of 1 in 300 individuals, FH is primarily related to P variants in the genes *LDLR*, *APOB*, and *PCSK9* (Gray et al., 2024). Although fundamentally a metabolic disorder, FH is associated with a high risk of developing premature coronary artery disease (Alway et al., 2024). Consequently, the disorder is considered as a subgroup of ICCs by some experts (Alway et al., 2024), even though its management often involves specialists beyond cardiologists, such as endocrinologists.

Heart valve disease refers to any condition where one or more of the heart's four valves fail to function properly, thereby disrupting the normal flow of blood through the heart and into the systemic circulation. While diverse etiologies, including age-related degeneration, other cardiac diseases, or infections, can cause these disorders, some cases arise from congenital heart defects. The most common congenital form is bicuspid aortic valve disease (BAV), which has a prevalence of 0.5-2% and an estimated familial heritability ranging from 47-89% (Gehlen et al., 2023; Yu & Bouatia-Naji, 2024). Although BAV typically follows an AD inheritance pattern with reduced penetrance and variable expressivity, encompassing both syndromic forms (e.g., LDS with *TGFBR1* or *TGFBR2* mutations as well as familial TAA and dissection syndrome with *ACTA2* mutations) and non-syndromic monogenic cases (related to *NOTCH1*, *ADAMTS19*, *SMAD6*, or *ROBO4*), some studies indicate a heterogeneous nature with polygenic inheritance in certain contexts (Galian-Gay et al., 2019; Gehlen et al., 2023; Rashed et al., 2022).

Pulmonary arterial hypertension (PAH) comprises a heterogeneous group of progressive and cardiopulmonary vascular disorders, characterised by elevated pulmonary arterial pressure and vasoconstriction, accompanied by structural changes in the distal pulmonary arteries, including the formation of vaso-occlusive lesions (Aldred et al., 2022; Bousseau et al., 2023). While PAH is a complex multifactorial disease, in some cases it can be inherited through heterozygous mutations following an AD pattern with incomplete penetrance (Aldred et al., 2022). P variants in the *BMPR2* gene are particularly prominent,

found in 70-80% of heritable cases and 10-15% of idiopathic cases and associated with increased disease susceptibility, earlier onset, and increased severity and mortality (Aldred et al., 2022; Bousseau et al., 2023). Other genes implicated in PAH include *EIF2AK4, TBX4, ATP13A3, GDF2, SOX17, AQP1, ACVRL1, SMAD9, ENG, KCNK3* and *CAV1* (Bousseau et al., 2023).

RASopathies represent a group of AD disorders caused by P variants in genes located within the canonical Ras-mitogen-activated protein kinase signaling cascade, with Noonan syndrome being the most representative (Chennappan & Kontaridis, 2025; Hilal et al., 2023). These conditions are frequently associated with diverse cardiac manifestations, including a range of congenital heart defects (e.g., pulmonary valve stenosis, atrial septal defects, ventricular septal defects, atrioventricular canal defects, patent ductus arteriosus, tetralogy of Fallot, etc.) and HCM (Chennappan & Kontaridis, 2025).

Neuromuscular disorders (NMDs) constitute a diverse group of conditions that impair the normal function of nerves and muscles. While their primary impact is on skeletal muscle weakness and function, cardiac muscle can also be significantly affected. Clinically relevant cardiac involvement in NMDs manifests most frequently as cardiomyopathies (commonly HCM and DCM) and various conduction defects with arrhythmias (Feingold et al., 2017; Finsterer & Stöllberger, 2016).

1.3.2. Next generation sequencing in the genetic diagnosis of inherited cardiac conditions

Genetic testing plays an increasingly pivotal role in identifying the molecular basis of ICCs. NGS technologies have become a cornerstone of clinical genetics, enabling the simultaneous analysis of multiple genes associated with cardiomyopathies, CHAN, and aortopathies. This capability provides essential information for accurate risk assessment, genetic counselling, and therapeutic decision-making (Papadopoulou et al., 2023). By facilitating the identification of both disease-causing variants and genetic modifiers that contribute to disease risk, NGS has transformed the diagnosis of hereditary cardiac disorders, significantly advancing our understanding of their underlying pathology and fostering the implementation of precision medicine approaches (Ojeda et al., 2024).

Current diagnostic strategies based on NGS for cardiomyopathies—and broadly applicable to ICCs—include disease-specific gene panels, expanded cardiovascular panels, WES and WGS (Voinescu et al., 2024; R. Walsh & Cook, 2017; Yogasundaram et al., 2021). Disease-focused panels, such as those targeting HCM or DCM, typically contain genes with well-established and clinically actionable associations. In contrast, broader panels—such as the Illumina TruSight Cardio—incorporate both established and emerging candidate genes. The TruSight Cardio panel, for example, includes 174 genes associated with 17 cardiac syndromes, encompassing cardiomyopathies, CHAN, aortopathies, and other conditions such as FH (Chmielewski et al., 2024).

Despite their utility, gene panels may fail to include recently discovered or less-characterised genes. For instance, *FLNC*, associated with both DCM and HCM, may be excluded from some targeted panels (Voinescu et al., 2024). While expanded panels generally offer a higher yield of P/LP variants due to broader genomic coverage, they also increase the probability of encountering VUS and are associated with higher testing costs (Voinescu et al., 2024; Yogasundaram et al., 2021). Ouellette et al. (2018), for example, reported a VUS rate of 87% with a large cardiomyopathy panel, compared to just 30% with a disease-specific panel. Moreover, that study found a lower diagnostic yield (15%) in 46 children tested with a broad panel, compared to a 32% yield in 104 children assessed with a targeted one. These findings underscore the importance of selecting the appropriate testing strategy based on the patient's phenotype: for individuals with a well-defined clinical presentation, a focused panel may be preferable, while a broader panel may be employed in more ambiguous cases—optionally followed by post-analytic filtering of gene content.

WES offers a more comprehensive approach by interrogating all protein-coding regions of the genome. It captures the majority of known disease-causing variants in ICCs and allows for reanalysis as new gene-disease associations emerge (R. Walsh & Cook, 2017). WES is particularly valuable in trio-based studies or small nuclear families, where suspected inheritance patterns—such as recessive or *de novo* variants—may not point to a specific gene a priori. However, WES has limitations related to variable sequencing coverage, which may affect clinically relevant regions. Notably, certain exons, such as exon 1 of *KCNQ1*, are challenging to capture adequately, potentially limiting diagnostic sensitivity (R. Walsh & Cook, 2017).

In contrast, WGS enables the detection of variants across the entire genome, including non-coding and deep intronic regions, that may be P. This expanded scope is particularly beneficial in complex cases or in patients who remain undiagnosed after targeted panel or WES testing (Voinescu et al., 2024).

According to Voinescu et al. (2024), reported diagnostic yields in ICCs cohorts—including those with cardiomyopathies and CHAN—range from 20–61% for targeted gene panels, 22–73% for WES, and 50–57% for WGS.

These evolving sequencing strategies, while crucial for improving diagnostic yield, are complemented by the growing recognition of the importance of reinterpreting existing genetic data over time. As variant classification evolves and analytical tools improve, the reanalysis of prior NGS results has emerged as a valuable approach to increase diagnostic accuracy in ICCs, as detailed in the following section.

1.3.3. Reevaluation of next generation sequencing data in inherited cardiac conditions patients

Publications specifically focused on the reevaluation of NGS data in ICCs remain relatively limited compared to the larger number of studies addressing neurological disorders, paediatric diseases, or other rare conditions (James et al., 2020; Schobers et al., 2022; Van Slobbe et al., 2023; Vorsteveld et al., 2024). Nevertheless, the existing literature highlights the potential diagnostic utility of such approaches in the cardiovascular context (**Table 1.6**). To better understand the practical impact of reevaluation efforts in the field of ICCs, it is useful to distinguish between three main approaches reported in the literature: **1) Retesting studies**, which involve generating new sequencing data; **2) Reanalysis studies**, which revisit previously obtained data using updated analytical tools; and **3) Reinterpretation studies**, which reassesses previously analysed and interpreted variants. The following sections outline representative examples of each strategy and highlight their respective contributions to improving diagnostic yield in ICCs cohorts.

Retesting studies

Some studies have adopted a retesting strategy, which involves new sequencing of patient samples rather than a reanalysis of previously generated data. For example, a 2022 study from the Australian Genomics Cardiovascular Genetic Disorders Flagship reported a diagnostic yield in 6 out of 125 patients (4.8%) with HCM or LQTS who had

previously received negative results from their initial clinical genetic testing (Chang et al., 2022). This study employed WGS for retesting, thereby generating new sequencing data rather than relying on existing datasets. Similarly, Cuesta-Llavona et al. (2024) identified new P/LP variants in 6 of 33 HCM patients (18%) through retesting using a targeted panel of 205 cardiovascular-related genes, focusing on individuals who had no P/LP variants even after variant reclassification.

Reinterpretation studies

Several other investigations have focused exclusively on the reinterpretation of previously identified genetic variants, without generating new sequencing data. These studies underscore the dynamic and evolving nature of variant classification over time. For instance, in a cohort with cardiac CHAN, Campuzano et al. (2020) reported a change in pathogenicity classification in 71.9% (92/128) of variants over a ten-year period. Notably, one VUS was upgraded to LP (0.7%), and ten LP variants were further upgraded to P (7.8%). Sarquella-Brugada et al. (2022), also studying inherited CHAN, observed a reclassification in 18.4% (9/49) of variants across five years, including the upgrade of one LP variant to P (2.0%) and the downgrade of eight VUS to likely benign (16.3%). Similarly, in a HCM cohort, Cuesta-Llavona et al. (2024) found that 22% (71/319) of variants underwent reclassification, with 5.6% (4 variants) being upgraded from VUS to P/LP. In a study specifically addressing VUS reclassification in patients with suspected ICCs, Novelli et al. (2022) reported that 26.6% (26/94) of VUS were reclassified, including 25.5% (24 variants) upgraded to P/LP. Likewise, Martin et al. (2024) observed a reclassification rate of 32% (17/53) in a CHAN cohort, with 6% (3 variants) reclassified as LP. Further, in a five-year study involving 233 patients with cardiomyopathy, Horgan et al. (2025) reported that 4.8% (12/248) of VUS were reclassified, including 1.6% upgraded to P/LP. Neubauer et al. (2021), examining 45 cases of sudden unexplained death, reinterpreted 11 previously reported variants, of which 5, initially classified as probably P or LP, were downgraded to VUS.

Reanalysis studies

Neubauer et al. (2021) provide a representative example of genetic data reanalysis in the context of sudden unexplained death, often linked to ICCs. In this study, WES data from a previous analysis (Neubauer et al., 2018) were available for 35 out of 45 cases. In addition, 15 new cases were sequenced *de novo* for the study and therefore were not included in the reanalysis subset. Among the full cohort of 45 individuals, P/LP SNVs were

identified in 10 individuals (22.2%), totalling 14 variants. Notably, only three of these individuals presented a cardiac phenotype. The study also included SV analysis, identifying 18 SVs in 15 individuals, thereby contributing to a more comprehensive diagnostic approach.

Similarly, Josephs et al. (2024) conducted a reanalysis of 210 undiagnosed paediatric cardiomyopathy cases from the 100,000 Genomes Project. Their updated analytical strategy incorporated revised gene panels, unbiased *de novo* variant detection, and improved variant prioritisation pipelines. This reanalysis led to a potential diagnosis in 49 individuals (23.4%), comprising 31 with a probable diagnosis (at least one P/LP variant) and 18 with a possible diagnosis (at least one VUS of high clinical interest).

A recent study from the Australian Genomics Cardiovascular Genetic Disorders Flagship (Austin et al., 2024) included patients with cardiomyopathy, cardiac CHAN, and CHD who had not previously undergone NGS. Genome sequencing was performed as the primary diagnostic approach. For those with negative initial findings, a second-tier analysis using an expanded gene panel was undertaken shortly thereafter. Although this strategy closely resembled reanalysis, the short time frame distinguished it from classic reinterpretation efforts. This second-tier analysis identified P/LP variants in 15 additional patients, and VUS in another 15, although an overall diagnostic yield for this reanalysis-like phase was not reported.

Finally, Caroselli et al. (2025) reanalysed 142 previously negative HCM cases using an improved *in silico* pipeline for detecting deep intronic variants in the *MYBPC3* gene. This approach enabled the identification of a spliceogenic variant in three individuals (2.1%), illustrating how advances in bioinformatic tools can uncover previously undetected P variants.

Table 1.6. Reevaluation approaches of next generation sequencing data in inherited cardiac conditions patients.

Source (reference)	Type of reevaluation	Cohort size	Cohort phenotype	Findings
Campuzano et al. (2020)	Reinterpretation	128	CHAN	71.87% reclassified; 1 VUS → LP (0.7%), 10 LP → P (7.8%)
Neubauer et al. (2021)	Reanalysis (WES)	45 (35 reanalysed)	Sudden unexplained death	14 P/LP SNVs in 10 cases (22.2%); SVs in 15 individuals
	Reinterpretation	11	Sudden unexplained death	5 P/LP variants downgraded to VUS
Chang et al. (2022)	Retesting (WGS)	125	HCM, LQTS	6/125 diagnostic (4.8%)
Novelli et al. (2022)	Reinterpretation	94	HCM, DCM, ACM, BrS, LQTS	26.6% reclassified; 24 VUS → P/LP (25.53%)
Sarquella-Brugada et al. (2022)	Reinterpretation	49	CHAN	18.36% reclassified; 1 LP → P (2.04%), 8 VUS → LB (16.32%)
Austin et al., 2024	Reanalysis	Not specified	Cardiomyopathies, CHAN and CHD	15 P/LP variants; 15 VUS
Cuesta-Llavona et al. (2024)	Retesting (targeted panel)	33	HCM	6/33 diagnostic (18%)
	Reinterpretation	319	HCM	22% reclassified; 4 VUS → P/LP (5.6%)
Josephs et al. (2024)	Reanalysis	210	Paediatric cardiomyopathy	Potential diagnosis in 23.4%
Martin et al. (2024)	Reinterpretation	53	CHAN	32% reclassified; 3 (6%) → LP
Caroselli et al. (2025)	Reanalysis (intronic regions of *MYBPC3*)	142	HCM	3 spliceogenic *MYBPC3* variants identified (2.1%)
Horgan et al. (2025)	Reinterpretation	233	HCM, DCM and ACM	4.8% reclassified; 1.6% → P/LP

This table summarises key studies exploring retesting, reinterpretation, and reanalysis strategies in inherited cardiac conditions cohorts. Abbreviations: CHAN = channelopathies; VUS = variant of uncertain significance; P/LP = pathogenic/likely pathogenic; WES = whole exome sequencing; SNVs = single nucleotide variants; SVs = structural variants, WGS = whole genome sequencing; HCM = hypertrophic cardiomyopathy; LQTS = long QT syndrome; DCM = dilated cardiomyopathy; ACM = arrhythmogenic cardiomyopathy; BrS = Brugada syndrome; CHD = congenital heart disease; LB = likely benign.

These findings illustrate how the reevaluation of NGS data in ICCs can lead to significant diagnostic refinements, either by identifying novel variants, reclassifying previously ambiguous ones, or applying improved bioinformatic pipelines.

1.4. Inherited bone marrow failure syndromes

Inherited bone marrow failure syndromes (IBMFSs) represent a clinically heterogeneous spectrum of rare monogenic diseases, primarily characterised by significant cytopenia affecting one or more haematopoietic cell lineages, often progressing to bone marrow failure, and caused by germline mutations in genes critical for haematopoiesis, telomere biology, RNA maturation and processing, ribosome biogenesis, DNA repair, and cellular maintenance pathways (Dillahunt et al., 2025; Fiesco-Roa et al., 2022; Kawashima et al., 2023; H.-Y. Kim et al., 2022).

Patients frequently present with extra-haematological features, often manifesting early in life. These can include multiple congenital anomalies, dysmorphism, developmental delays, skeletal dysplasia, short stature, and a significantly increased predisposition to certain cancers, such as acute myeloid leukemia or solid tumors (Dillahunt et al., 2025; Kawashima et al., 2023).

The most common IBMFSs are **Fanconi anaemia**, **Diamond-Blackfan anaemia**, **dyskeratosis congenita**, and **Shwachman-Diamond syndrome** (**Table 1.7**). Other disorders include severe congenital neutropenia, congenital dyserythropoietic anaemia, congenital amegakaryocytic thrombocytopenia, thrombocytopenia-absent radii and *GATA2* deficiency.

Table 1.7. Clinical characteristics of main inherited bone marrow failure syndromes.

Characteristics	FA	DC	DBA	SDS
Estimated incidence	1 in 136,000[1]	<1 in 1,000,000[2]	1 in 500,000[3]	1 in 75,000[4]
Inheritance pattern	AR > AD, XLR	XLR > AR, AD	AR > AD	AR
Typical haematological findings	Pancytopenia	Pancytopenia	Anaemia	Neutropenia
Extra-haematological symptoms	Growth deficiency, skeletal anomalies (radial axis), skin pigmentation, small head/eyes, genitourinary anomaly, reproductive problems	Growth deficiency, abnormal nails, leukoplakia, reticular pigmentation, pulmonary fibrosis, grey hair, cerebellar hypoplasia	Growth deficiency, head/facial anomaly, skeletal anomaly (thumb), kidney/heart anomaly	Growth deficiency, skeletal anomaly (metaphyseal dysostosis), exocrine pancreatic insufficiency
Short telomere	+	++	+	+
MDS/AML predisposition	+	+	+	+
Solid cancer predisposition	Squamous cell carcinoma (head/neck, genitourinary)	Squamous cell carcinoma (head/neck, skin)	Osteosarcoma	Not common

Edited from Kawashima et al. (2023). References: 1. J. Bhandari et al. (2024). 2. Fernández García & and Teruya-Feldstein (2014). 3. Gadhiya & Wills (2025). 4. Farooqui et al. (2025). Abbreviations: FA = Fanconi anaemia; DC = dyskeratosis congenita; DBA = Diamond–Blackfan anaemia; SDS = Shwachman–Diamond syndrome; AR = autosomal recessive; AD = autosomal dominant; XLR = X-linked recessive; MDS = myelodysplastic syndrome; AML = acute myeloid leukaemia. + = sometimes present; ++ = almost always present.

1.4.1. Fanconi anaemia

Fanconi anaemia (FA) is an inherited AR disorder (X-linked in 2% of cases) with an estimated incidence of 1 out of 136,000 newborns and mainly characterised by progressive bone marrow failure (from asymptomatic cytopenia to severe bone marrow aplasia) and an increased predisposition to malignancy, especially acute myeloid leukaemia but also solid tumors (e.g., head and neck squamous cell carcinomas) (J. Bhandari et al., 2024; Dantas & Pereira, 2024; Mead et al., 2025; Ramírez et al., 2025). In addition to haematological manifestations, patients may exhibit various somatic abnormalities, affecting the skin (café-au-lait spots), skeletal system (absent thumbs, radial hypoplasia, scoliosis), genitourinary, gastrointestinal, cardiac, and neurological systems (Mead et al., 2025).

FA-causing genes (see **Table 2.4** in **2. Materials and methods**) encode proteins essential for repairing DNA interstrand cross-links (**Figure 1.4**) via homologous recombination (J. Bhandari et al., 2024; Kawashima et al., 2023). This defective repair leads to significant genomic instability, manifested as chromosomal damage, pancytopenia, and a characteristic hypersensitivity to DNA cross-linking agents (J. Bhandari et al., 2024; Dokal & Vulliamy, 2010). This distinct hypersensitivity forms the basis for the chromosome fragility test, the diagnostic gold standard for suspected FA patients. The test involves exposing lymphocytes to DNA cross-linkers like diepoxybutane (DEB) or mitomycin C to induce chromosomal breakage (J. Bhandari et al., 2024; Dantas & Pereira, 2024). This assay can also detect reverse somatic mosaicism, which is observed in some FA patients (Dantas & Pereira, 2024; Ramírez et al., 2021). A positive test result typically prompts genetic testing to identify the specific causative gene mutation.

1.4.2. Diamond-Blackfan anaemia

Diamond-Blackfan anaemia (DBA) is an AD inherited red blood cell aplasia, with an incidence rate of 1 in 500,000 live births (Gadhiya & Wills, 2025). It typically presents within the first year of life (Gadhiya & Wills, 2025). Key hematological features include normochromic macrocytic anaemia, reticulocytopenia, and a near absence of erythroid progenitors in the bone marrow (Mead et al., 2025). Approximately 30% to 50% of patients exhibit congenital malformations affecting craniofacial, upper limb, cardiac, and urinary systems, in addition to growth retardation (Gadhiya & Wills, 2025). Most patients also present with increased mean corpuscular volume (MCV), elevated erythrocyte adenosine deaminase activity, and persistent fetal hemoglobin (Mead et al., 2025).

DBA is predominantly caused by mutations in ribosomal genes (**Figure 1.4**). These include genes encoding the small (*RPS7, RPS10, RPS15A, RPS17, RPS19*, *RPS24*, *RPS26, RPS27, RPS28,* and *RPS29*) and the large *(RPL5, RPL9, RPL11, RPL15, RPL18, RPL26, RPL27, RPL31, RPL35*, and *RPL35A*) ribosomal subunits (Kawashima et al., 2023). Thus, the primary defect in DBA is impaired ribosome biogenesis, which subsequently leads to other biological anomalies such as increased apoptosis and upregulation of p53 (Mead et al., 2025).

1.4.3. Dyskeratosis congenita

Dyskeratosis congenita (DC) is a telomeric disease with AD, AR or XLR inheritance patterns, with an estimated incidence of <1 out of 1,000,000 newborns (Fernández García & and Teruya-Feldstein, 2014; Mead et al., 2025). DC is characterised by the mucocutaneous triad of abnormal skin pigmentation, nail dystrophy and mucosal leukoplakia (Mead et al., 2025).

DC patients are characterised by shortened telomeres resulting from mutations in genes involved in telomere length maintenance (**Figure 1.4**) such as *DKC1*, *TERC* and *TERT* (Garofola et al., 2025; Kawashima et al., 2023).

1.4.4. Shwachman-Diamond syndrome

Shwachman-Diamond syndrome (SDS) is a rare AR disorder, with a reported incidence of 1 in 75,000 individuals, characterised by a common triad of exocrine pancreatic dysfunction, skeletal abnormalities, and bone marrow dysfunction (Farooqui et al., 2025; Kawashima et al., 2023).

In over 90% of cases, patients carry biallelic variants in the *SBDS* gene, which plays a critical role in the maturation of the 60S ribosomal subunit (**Figure 1.4**) (Farooqui et al., 2025; Kawashima et al., 2023; Mead et al., 2025). *DNAJC21* and *EFL1*, also implicated in ribosome biogenesis, have additionally been associated with SDS.

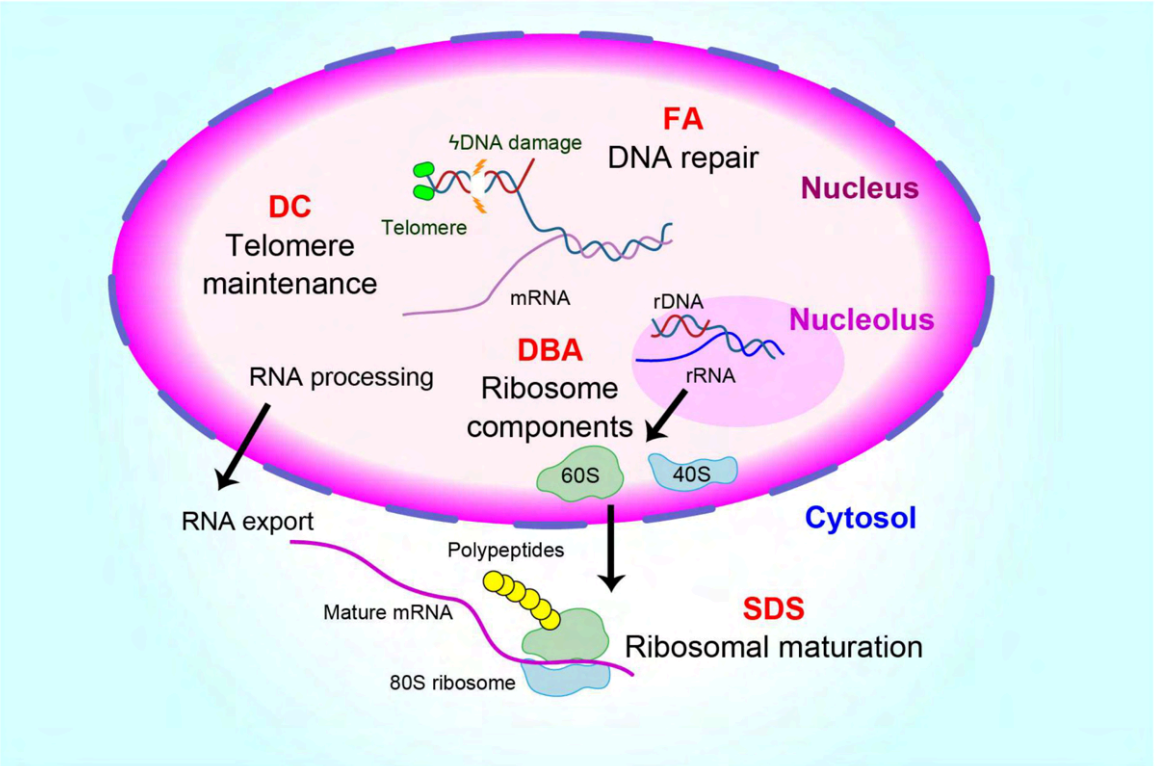

Figure 1.4. Landscape of genetic alterations in inherited bone marrow failure syndromes. Reproduced from Kawashima et al. (2023). Abbreviations: FA = Fanconi anaemia; DC = dyskeratosis congenita; DBA = Diamond-Blackfan anaemia; SDS = Shwachman-Diamond syndrome.

1.4.5. Next generation sequencing in the genetic diagnosis of inherited bone marrow failure syndromes

Prior to the widespread implementation of NGS in diagnostic genetics, the diagnosis of IBMFSs primarily relied on clinical manifestations and functional screening tests, such as the chromosome fragility test for suspected FA patients. Subsequent identification of specific disease-causing variants typically involved Sanger sequencing. However, this approach presented significant diagnostic challenges, particularly in cases of phenotypic overlap with other IBMFSs, in patients lacking a substantial family history or overt physical anomalies, or when no specific screening test was available for the disorder (e.g., *GATA2* haploinsufficiency) (Ghemlas et al., 2015; Kwon et al., 2025; Lin et al., 2024). The inherent genetic heterogeneity of IBMFSs further compounded these diagnostic difficulties.

The advent of NGS technologies has effectively overcome these limitations due to their capacity to simultaneously interrogate numerous genes across large patient cohorts at a comparatively low cost. Indeed, advancements in NGS have facilitated the rapid identification of an expanding number of genes associated with IBMFSs, including more

recently discovered candidates such as *SAMD9, SAMD9L, MECOM,* and *ERCC6L2* (Pallavelangini et al., 2023). The most common NGS approach in this field is targeted gene panel sequencing, with numerous examples demonstrating its utility over the years (Gálvez et al., 2021; Ghemlas et al., 2015; Kwon et al., 2025; Lin et al., 2024; Muramatsu et al., 2017; Pallavelangini et al., 2023). These panels have achieved a diagnostic yield of up to 50% in patients with suspected IBMFSs (Kwon et al., 2025). WES has also been widely employed, particularly valued for its capacity to identify novel genes and variants (Bogliolo et al., 2020; Hamada et al., 2020; Muramatsu et al., 2017; S. Wang et al., 2021).

1.4.6. Reevaluation of next generation sequencing data in inherited bone marrow failure syndromes patients

In contrast to the growing number of reevaluation studies in ICCs, studies specifically focused on the reanalysis of NGS data in large cohorts of patients with IBMFSs remain relatively scarce. Most of the available literature comprises individual case reports, small patient series, or broader studies of Mendelian diseases in which IBMFSs cases are only a subset.

One example resembling a reanalysis study was conducted by Lauhasurayotin et al. (2019). Initially, the authors used an NGS gene panel targeting known IBMFSs-related genes to screen 258 patients for P SNVs and small indels. In cases without conclusive findings, CNV analysis was subsequently performed on 168 individuals using CNV Tools from the NextGene software, which compares coverage ratios between test samples and control datasets. This complementary approach identified P deletions in IBMFSs genes in 10 out of 168 patients (approximately 6%), including five cases of DBA. Although the time interval between initial and subsequent analyses was short, and thus this study does not represent a formal reanalysis, it nonetheless highlights the diagnostic value of re-examining NGS data using alternative computational methods, particularly as a cost-effective alternative to WGS for CNV detection.

Reanalysis of WES data has also proven effective in individual IBMFSs cases. For instance:

- A canonical splice-site variant in *DNAJC21* (NM_001348420.2: c.983+1G>A; p.Gly299Alafs*2) was identified in a five-year-old patient with cytopenia, recurrent infections, and extra-haematopoietic features, consistent with SDS (Durmaz et al., 2024).
- A homozygous missense variant in *EFL1* (NM_024580.5: c.379A>G; p.Thr127Ala) was found in a 14-year-old presenting with an SDS-like phenotype (Tan et al., 2018).
- A heterozygous germline variant in *EIF6* (NM_002212.4: c.100T>C; p.Phe34Leu) was identified in a 23-year-old SDS patient who also carried biallelic *SBDS* P variants (Taha et al., 2022).
- Bandini et al. (2023) reported two truncating variants in *ERCC6L2* (c.1930C>T; p.Arg644Ter and c.2156del; p.Gly719AspfsTer50) in two siblings diagnosed with bone marrow failure syndrome-2.

Additionally, two WES reanalysis studies involving broader undiagnosed cohorts (not specifically focused on IBMFSs) provide further examples. In one case, a P variant in *FANCA* led to the diagnosis of FA, enabling timely haematopoietic stem cell transplantation and preventing clinical deterioration. In another, a heterozygous frameshift variant in *RPS26* (NM_001029.3: c.131_132del; p.Ile44Serfs*11) was found in a patient presenting with anaemia and vitamin B12 deficiency, ultimately confirming a diagnosis of DBA (Ewans et al., 2018).

Finally, a WGS-based reanalysis performed by Wen et al. (2025) identified non-coding variants in two DBA patients: a promoter variant in *RPS7* (NM_001011.4: c.-19G>C) and an intronic variant in *RPS19* (NM_001022.4: c.172+350C>T), further illustrating how non-coding regions may harbor clinically relevant alterations missed by conventional exome analyses.

Together, these findings underline the emerging diagnostic value of reevaluating NGS data in IBMFSs, particularly in unsolved cases. Although current evidence is largely limited to individual reports, the growing use of reanalysis in rare genetic disorders suggests that broader, systematic efforts in IBMFSs cohorts could substantially improve diagnostic outcomes.

2. Materials and methods

2.1. Patient cohorts

Two patient cohorts have been analysed: The first one included patients with clinical diagnoses of different cardiomyopathies, CHAN or aortic pathologies (cohort 1), while the second one consisted in patients exhibiting a suspected FA clinical phenotype (cohort 2).

Cohort 1 details - Inherited Cardiac Conditions (ICCs) cohort: 933 patients (70% males, 30% females) with suspected cardiomyopathy, channelopathy or aortic pathology were analysed. Patients were referred to the Genetics Department of *Hospital de la Santa Creu i Sant Pau* (HSCSP) between January of 2019 and October of 2024. Among these, 742 patients (79.5%) had a negative genetic test examining a panel of genes according to their clinical phenotypes and were included in the reanalysis study. Patients provided written informed consent approved by the Ethics Committee of the hospital to study cardiovascular genes (CIGNT00201, CAS-V01-2019-01). Demographic and clinical information were available as provided by the referring cardiologist. Phenotypic classification was established according to the 2023 ESC Guidelines (Arbelo et al., 2023), and included HCM, DCM, ARVC, NDLVC, RCM, patients referred due to VH (previously considered left ventricular non-compaction cardiomyopathy), CHAN such as LQTS, BrS, and CPVT. Other phenotype groups included CDDs and SADS. Finally, FSA (e.g., Marfan, Ehlers-Danlos, Loeys-Dietz syndromes) were also included (see **Table 2.1** for phenotype distribution). This classification was based on a comprehensive diagnostic work-up combining ECG, echocardiography, CMR, coronary angiography, electrophysiological studies (EPS), and, in cases of suspected aortopathy, assessment of systemic features. A detailed three-generation family history was also obtained to identify potential inheritance patterns and at-risk relatives.

Table 2.1. Phenotypic distribution of the 933-patient cohort with suspected cardiomyopathy, channelopathy or aortic disease.

Phenotype	Total
ARVC	35
CDDs	2
CHAN	71
DCM	329
FSA	126
HCM	279
NDLVC	48
RCM	1
SADS	15
VH	27
Total	**933**

Abbreviations: ARVC = arrhythmogenic right ventricular cardiomyopathy; CCDs = cardiac conduction diseases; CHAN = channelopathies; DCM = dilated cardiomyopathy; FSA = familial syndromic aortopathies; HCM = hypertrophic cardiomyopathy; NDLVC = non-dilated left ventricular cardiomyopathy; RCM = restrictive cardiomyopathy; SADS = sudden arrhythmic death syndrome; VH = ventricular hypertrabeculation.

Cohort 2 details - Suspected FA (SFA) cohort: This group included clinical cases, who were referred for genetic testing based on initial clinical suspicion of FA, with a negative chromosome fragility test conducted between 2005 and 2009 (**Figure 4.4**), and the absence of FA-related gene candidate causing variants after initial WES test. Six patients met these criteria and were recruited for the study: one male (16.7%) and five females (83.3%). Two patients were siblings (patients 5 and 6). The relevant clinical histories for the six patients are summarised below:

- Patient 1 was diagnosed with immune thrombocytopenic purpura (ITP) at the age of 10 (2001). In the following years, the patient developed bilateral interstitial pneumonia with respiratory failure, as well as recurrent aphthous ulcers affecting the lips and oral mucosa. Chronic diarrhoea led to a diagnosis of irritable bowel syndrome. In late 2003, haematological evaluation revealed complete bone marrow aplasia.
- Patient 2 was diagnosed in 2010 with mild leukopenia–neutropenia and anaemia, with a MCV at the upper limit of normal for age.
- Patient 3 was referred in 2007 for genetic testing to exclude FA. However, clinical information from the time of the chromosome fragility test was not available, and further clinical data could not be retrieved due to the inability to contact the referring physician.

- Patient 4 presented with moderate thrombocytopenia at the age of two. Additional clinical features included microcephaly, café-au-lait spots, epileptic seizures, and developmental delay. The patient's mother also had microcephaly, and there was a family history of epilepsy; the father was reported to be healthy. Thrombocytopenia progressed over time, and although a haematopoietic stem cell transplant was recommended, the family declined the procedure. The patient died shortly thereafter.
- Patients 5 and 6 were siblings presenting with macrocytic anaemia, short stature, and syndromic features. Their father had a childhood diagnosis of FA. Patient 6 was diagnosed with FA in 1988, whereas the diagnosis in patient 5 remained inconclusive. Patient 6 displayed a more severe phenotype, including left renal agenesis, bilateral cryptorchidism, hypertelorism, microcephaly, prominent ears, and psychomotor retardation.

Clinical and demographic data, as well as chromosome fragility test results for all patients, are summarised in **Table 2.2**.

Table 2.2. Demographic and chromosome fragility data and clinical features of suspected Fanconi anaemia reanalysed patients.

Pt	Birth date	Sex	Ethnicity	Year of first diagnosis	Fragility study date	% Aberrant cells (DEB)	First clinical phenotype	Disease course	Family history
1	06/08/1991	F	Caucasian	2001	25/01/2006	0	Immune thrombocytopenic purpura	Bone marrow aplasia in 2003 (allogeneic transplant from a family donor), bilateral interstitial pneumonias with associated respiratory failure (lung transplant in 2021), aphthosis, irritable bowel syndrome, severe neutropenia in the most recent clinical report	-
2	04/04/2001	F	Caucasian	2010	01/03/2006	2	Mild leukopenia-neutropenia anaemia	Anaemia was normalised, leukopenia maintained, medical discharge in 2015	-
3	26/12/2006	F	Caucasian	-	31/07/2007	4	Referred for 'FA exclusion in 2007'	Not available	-
4	10/02/2001	F	Gypsy	2003	06/11/2007	9	Moderate thrombocytopenia, café-au-lait spots, microcephaly, epileptic episodes and mild mental retardation	Severe megakaryocytic thrombocytopenia in 2014 (transplant required, but the family refused, and the patient died shortly afterward)	Healthy father, mother with microcephaly and family history of epilepsy
5*	04/12/1979	F	Caucasian	1988	29/01/2009	11	Macrocytic anaemia, short stature and a syndromic appearance	Not available	Paternal family history: FA diagnosed in childhood and subsequently ruled out

Pt	Birth date	Sex	Ethnicity	Year of first diagnosis	Fragility study date	% Aberrant cells (DEB)	First clinical phenotype	Disease course	Family history
6*	21/06/1981	M	Caucasian	1988	29/01/2009	13	Macrocytic anaemia, short stature and syndromic appearance, agenesis of the left kidney, bilateral cryptorchidism, hypertelorism, microcephaly, winged auricles, and psychomotor retardation	Not available	Paternal family history: FA diagnosed in childhood and subsequently ruled out

*Siblings. Abbreviations: Pt = patient; F = female; M = male; DEB = diepoxybutane; FA = Fanconi anaemia.

Chromosome fragility was assessed in all 6 patients using DEB induction, as previously described (Auerbach, 2015; Castella et al., 2011). Telomere length was measured as previously described by Cawthon (2009) with some modifications. Briefly, genomic DNA was extracted from whole blood, purified using the DNeasy® PowerClean® Pro Cleanup Kit (Qiagen, 12997-50), and quality controlled. Telomere length was measured using the monochrome multiplex qPCR on a 7900HT Applied Biosystems instrument. Reactions included telomere and albumin primers and PowerUp™ SYBR™ Green Master Mix (Applied Biosystems). Each sample was analysed in triplicate wells on two separate plates. A five-point standard curve of control DNA was used to calculate the T/S ratio. Data analysis was performed using the Design and Analysis software (v.2.6.0; Thermo Fisher).

2.2. Standard genetic testing

Genomic DNA was extracted from whole blood samples of both patient cohorts using standard laboratory protocols. Genetic testing was carried out differently depending on the patients' cohort: **1) ICCs patients**: Samples were processed at the Genetics Department of HSCSP. Briefly, DNA libraries were prepared using SeqCap or Kapa reagents (Roche). Custom CES probes (Roche) were used to capture coding exons and flanking regions (approximately +/-50 bp) of ~5,000 genes associated with human diseases according to OMIM (http://omim.org/) and selected literature supporting gene-disease association, known deep intronic P variants present in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) at the moment of design were also targeted. Sequencing was carried out to obtain 2x150 (NextSeq500, Illumina) or 2x100 (NextSeq1000, Illumina) base paired-end reads. **2) SFA patients**: WES for four of these individuals (designated as patients 1 to 4) was carried out at Sistema Genómicos (Valencia, Spain) during July 2013, following their standard protocols. Subsequently, in early 2014, samples from the remaining two patients (patients 5 and 6) were sent to the GATC Biotech sequencing center (Konstanz, Germany).

Processing of raw sequencing data (fastq files) from both cohorts was subjected to an automated bioinformatics data analysis pipeline. Overall, sequence reads were aligned to the GRCh37/hg19 human reference genome with the use of the BWA algorithm (Li & Durbin, 2009). Read quality assessment was performed using FastQC (Andrews, S., 2010). SNVs and small indels were called using GATK (McKenna et al., 2010), followed by functional annotation with the use of ANNOVAR (Wang et al., 2010) and custom annotation scripts. CNVs were identified through coverage analysis of the resulting sequence alignment files using ExomeDepth (Plagnol et al., 2012), GenomicRanges and

IRanges (Lawrence et al., 2013) R packages. BAM files of all candidate SNV, indel and CNV calls were manually inspected using IGV (Robinson et al., 2011, 2017, 2023; Thorvaldsdóttir et al., 2013) to assess coverage patterns, determine zygosity, and identify potential sequencing artifacts. This pipeline, partially or completely, has been used in several publications (Bogliolo et al., 2020; Cavestro et al., 2024; Dols-Icardo et al., 2024; Esmel-Vilomara et al., 2023; Garcia-Melendo et al., 2021; Sierra-Marcos et al., 2024)

Sequencing quality was assessed using Picard tools from Broad Institute (http://broadinstitute.github.io/picard/) and custom scripts. Coverage metrics, including mean coverage, median coverage and percentage of accumulated coverage at least at 20X were determined for both cohorts (**Table 2.3**).

Table 2.3. Coverage metrics of the sequencing process for studied groups.

Cohort	NGS approach	Mean coverage	Median coverage	Percentage of accumulated coverage at least at 20X (%)
ICCs	CES	111.3X	106.6X	98.6
SFA	WES	86.1X	70.3X	87

Abbreviations: ICCs = inherited cardiac conditions; SFA = suspected Fanconi anaemia; CES = clinical exome sequencing; WES = whole exome sequencing.

The initial routine diagnostic genetic testing in the ICCs cohort utilised a virtual gene panel tailored to the individual patient's cardiac disease subtype (see **1. Introduction** for subtype associated genes). WES variants from the SFA patient group were filtered using a defined set of FA-related genes (**Table 2.4**).

Table 2.4. Fanconi anaemia related genes studied in the initial routine diagnostic test.

Gene/Locus	Gene/Locus MIM number	Location	FA complementation group	Inheritance	Phenotype MIM number
MAD2L2	604094	1p36.22	V	AR	617243
UBE2T	610538	1q32.1	T	AR	616435
PHF9	608111	2p16.1	L	AR	614083
FANCD2	613984	3p25.3	D2	AR	227646
FANCE	613976	6p21.31	E	AR	600901
XRCC2	600375	7q36.1	U	AR	617247
XRCC9	602956	9p13.3	G	AR	614082
FANCC	613899	9q22.32	C	AR	227645
FANCF	613897	11p14.3	F	AR	603467
BRCA2	600185	13q13.1	D1	AR	605724
RAD51	179617	15q15.1	R	AD	617244

Gene/Locus	Gene/Locus MIM number	Location	FA complementation group	Inheritance	Phenotype MIM number
FANCI	611360	15q26.1	I	AR	609053
SLX4	613278	16p13.3	P	AR	613951
ERCC4	133520	16p13.12	Q	AR	615272
PALB2	610355	16p12.2	N	AR	610832
RFWD3	614151	16q23.1	W	AR	617784
FANCA	607139	16q24.3	A	AR	227650
BRCA1	113705	17q21.31	S’	AR	617883
RAD51C	602774	17q22	O	AR	613390
BRIP1	605882	17q23.2	J	-	609054
FANCB	300515	Xp22.2	B	XLR	300514
FANCM	609644	14q21.2	M	AR	618096

Abbreviations: AR = autosomal recessive; AD = autosomal dominant; XLR = X-Linked Recessive.

2.3. Data reanalysis

After standard analysis performed as described above, undiagnosed patients underwent a systematic process of existing data reanalysis (**Figures 2.1 and 2.2**). This bioinformatics process used VCF files as an input and utilised updated variant databases and programs included in an automated pipeline detailed above. The ICCs cohort underwent aditional variant filtering using a 433-gene *in silico* panel, specifically designed to cover genes associated with a wide range of cardiovascular diseases and syndromes that include cardiac manifestations (**Table 2.5**, **Figure 2.1**). To elaborate this ICCs gene panel it was taken into account several resources including scientific literature, ClinVar, OMIM, and Orphanet. The SFA cohort underwent a two-stage analysis approach: 1) Analysis of FA related genes, and 2) If 1) gave no candidate causing variants, the rest of the genes included in the whole exome capture kit were analysed with the goal of finding a new candidate gene associated with FA phenotype. Details about the reanalysis process are described in next paragraphs.

Table 2.5. Comprehensive list of cardiac associated genes analysed.

Gene symbol	HGNC ID	Location	Associated disease/s	Source
A2ML1	HGNC:23336	12p13.31	Neuro-cardio-facio-cutaneous syndrome	PMID: 24896146
AARS2	HGNC:21022	6p21.1	Leukoencephalopathy, progressive, with ovarian failure, 615889, Autosomal recessive; Combined oxidative phosphorylation deficiency 8, 614096, Autosomal recessive	OMIM
ABCA1	HGNC:29	9q31.1	Tangier disease, 205400, Autosomal recessive; HDL deficiency, familial, 1, 604091, Autosomal dominant	OMIM
ABCB1	HGNC:40	7q21.12	Myocardial Infarction; Acute Coronary Syndrome	PMID: 25118983
ABCC6	HGNC:57	16p13.11	Pseudoxanthoma elasticum, 264800, Autosomal recessive; Arterial calcification, generalized, of infancy, 2, 614473, Autosomal recessive; Pseudoxanthoma elasticum, forme fruste, 177850, Autosomal dominant	OMIM
ABCC9	HGNC:60	12p12.1	Cardiomyopathy, dilated, 1O, 608569, Autosomal dominant; Hypertrichotic osteochondrodysplasia (Cantu syndrome), 239850, Autosomal dominant; Atrial fibrillation, familial, 12, 614050, Autosomal dominant; Intellectual disability and myopathy syndrome, 619719, Autosomal recessive	OMIM
ABCG5	HGNC:13886	2p21	Sitosterolemia 2, 618666, Autosomal recessive	OMIM
ABCG8	HGNC:13887	2p21	Sitosterolemia 1, 210250, Autosomal recessive; {Gallbladder disease 4}, 611465	OMIM
ACAD9	HGNC:21497	3q21.3	Mitochondrial complex I deficiency, nuclear type 20, 611126, Autosomal recessive	OMIM
ACADVL	HGNC:92	17p13.1	VLCAD deficiency, 201475, Autosomal recessive	OMIM
ACTA1	HGNC:129	1q42.13	Congenital myopathy 2B, severe infantile, autosomal recessive, 620265, Autosomal recessive; Myopathy, scapulohumeroperoneal, 616852, Autosomal dominant; Congenital myopathy 2C, severe infantile, autosomal dominant, 620278, Autosomal dominant; Congenital myopathy 2A, typical, autosomal dominant, 161800, Autosomal dominant	OMIM
ACTA2	HGNC:130	10q23.31	Smooth muscle dysfunction syndrome, 613834, Autosomal dominant; Aortic aneurysm, familial thoracic 6, 611788, Autosomal dominant; Moyamoya disease 5, 614042	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
ACTB	HGNC:132	7p22.1	Baraitser-Winter syndrome 1, 243310, Autosomal dominant; Becker nevus, syndromic or isolated, somatic mosaic, 604919; Thrombocytopenia 8, with dysmorphic features and developmental delay, 620475, Autosomal dominant; Dystonia-deafness syndrome 1, 607371, Autosomal dominant; Congenital smooth muscle hamartoma with or without hemihypertrophy, somatic mosaic, 620479	OMIM
ACTC1	HGNC:143	15q14	Left ventricular noncompaction 4, 613424, Autosomal dominant; Cardiomyopathy, hypertrophic, 11, 612098, Autosomal dominant; Atrial septal defect 5, 612794, Autosomal dominant; Cardiomyopathy, dilated, 1R, 613424, Autosomal dominant	OMIM
ACTG1	HGNC:144	17q25.3	Deafness, autosomal dominant 20/26, 604717, Autosomal dominant; Baraitser-Winter syndrome 2, 614583, Autosomal dominant	OMIM
ACTN2	HGNC:164	1q43	Myopathy, distal, 6, adult onset, 618655, Autosomal dominant; Cardiomyopathy, hypertrophic, 23, with or without LVNC, 612158, Autosomal dominant; Congenital myopathy 8, 618654, Autosomal dominant; Cardiomyopathy, dilated, 1AA, with or without LVNC, 612158, Autosomal dominant	OMIM
ACVR1	HGNC:171	2q24.1	Calcific Aortic Valve Disease	PMID: 36005436
ACVR2B	HGNC:174	3p22.2	Heterotaxy, visceral, 4, autosomal, 613751	OMIM
ACVRL1	HGNC:175	12q13.13	Telangiectasia, hereditary hemorrhagic, type 2, 600376, Autosomal dominant	OMIM
ADAMTS2	HGNC:218	5q35.3	Ehlers-Danlos syndrome, dermatosparaxis type, 225410, Autosomal recessive	OMIM
ADAMTSL4	HGNC:19706	1q21.2	Coronary Artery Disease	PMID: 38324186
AGK	HGNC:21869	7q34	Cataract 38, autosomal recessive, 614691, Autosomal recessive; Sengers syndrome, 212350, Autosomal recessive	OMIM
AGL	HGNC:321	1p21.2	Glycogen storage disease IIIa, 232400, Autosomal recessive; Glycogen storage disease IIIb, 232400, Autosomal recessive	OMIM
AGPAT2	HGNC:325	9q34.3	Lipodystrophy, congenital generalized, type 1, 608594, Autosomal recessive	OMIM
AKAP9	HGNC:379	7q21.2	Long QT syndrome 11, 611820, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
AKT1	HGNC:391	14q32.33	Breast cancer, somatic, 114480; Cowden syndrome 6, 615109; Colorectal cancer, somatic, 114500; Proteus syndrome, somatic, 176920; Ovarian cancer, somatic, 167000	OMIM
AKT2	HGNC:392	19q13.2	Akt2-Related Familial Partial Lipodystrophy	ORPHA:79085
ALMS1	HGNC:428	2p13.1	Alstrom syndrome, 203800, Autosomal recessive	OMIM
ALPK3	HGNC:17574	15q25.3	Cardiomyopathy, familial hypertrophic 27, 618052, Autosomal recessive	OMIM
AMPD1	HGNC:468	1p13.2	Dilated Cardiomyopathy	PMID: 10086958
ANGPTL3	HGNC:491	1p31.3	Hypobetalipoproteinemia, familial, 2, 605019, Autosomal recessive	OMIM
ANK2	HGNC:493	4q25-q26	Long QT syndrome 4, 600919, Autosomal dominant; Cardiac arrhythmia, ankyrin-B-related, 600919, Autosomal dominant	OMIM
ANK3	HGNC:494	10q21.2	Intellectual developmental disorder, autosomal recessive 37, 615493, Autosomal recessive	OMIM
ANKRD1	HGNC:15819	10q23.31	Familial Isolated Dilated Cardiomyopathy	PMID: 19525294
ANO5	HGNC:27337	11p14.3	Muscular dystrophy, limb-girdle, autosomal recessive 12, 611307, Autosomal recessive; Miyoshi muscular dystrophy 3, 613319, Autosomal recessive; Gnathodiaphyseal dysplasia, 166260, Autosomal dominant	OMIM
APOA1	HGNC:600	11q23.3	Hypoalphalipoproteinemia, primary, 2, 618463, Autosomal recessive; Amyloidosis, 3 or more types, 105200, Autosomal dominant; Hypoalphalipoproteinemia, primary, 2, intermediate, 619836, Autosomal dominant	OMIM
APOA5	HGNC:17288	11q23.3	Hyperchylomicronemia, late-onset, 144650, Autosomal dominant; {Hypertriglyceridemia, susceptibility to}, 145750, Autosomal dominant	OMIM
APOB	HGNC:603	2p24.1	Hypercholesterolemia, familial, 2, 144010, Autosomal dominant; Hypobetalipoproteinemia, 615558, Autosomal recessive	OMIM
APOC2	HGNC:609	19q13.32	Hyperlipoproteinemia, type Ib, 207750, Autosomal recessive	OMIM
APOC3	HGNC:610	11q23.3	Familial Hyperalphalipoproteinemia	ORPHA:181428

Gene symbol	HGNC ID	Location	Associated disease/s	Source
APOE	HGNC:613	19q13.32	Alzheimer disease 2, 104310, Autosomal dominant; Sea-blue histiocyte disease, 269600, Autosomal recessive; { Alzheimer disease, protection against, due to APOE3-Christchurch}, 607822, Autosomal dominant; {Coronary artery disease, severe, susceptibility to}, 617347; Lipoprotein glomerulopathy, 611771; { Macular degeneration, age-related}, 603075, Autosomal dominant; Hyperlipoproteinemia, type III, 617347	OMIM
ASPH	HGNC:757	8q12.3	Atrioventricular Nodal Reentry Tachycardia	PMID: 32508047
ATP5F1E	HGNC:838	20q13.32	Mitochondrial complex V (ATP synthase) deficiency, nuclear type 3, 614053, Autosomal recessive	OMIM
ATP7A	HGNC:869	Xq21.1	Occipital horn syndrome, 304150, X-linked recessive; Neuronopathy, distal hereditary motor, X-linked, 300489, X-linked recessive; Menkes disease, 309400, X-linked recessive	OMIM
ATPAF2	HGNC:18802	17p11.2	Mitochondrial complex V (ATP synthase) deficiency, nuclear type 1, 604273, Autosomal recessive	OMIM
B3GAT3	HGNC:923	11q12.3	Multiple joint dislocations, short stature, craniofacial dysmorphism, with or without congenital heart defects, 245600, Autosomal recessive	OMIM
B4GALT7	HGNC:930	5q35.3	Ehlers-Danlos syndrome, spondylodysplastic type, 1, 130070, Autosomal recessive	OMIM
BAG3	HGNC:939	10q26.11	Cardiomyopathy, dilated, 1HH, 613881, Autosomal dominant; Myopathy, myofibrillar, 6, 612954, Autosomal dominant	OMIM
BCOR	HGNC:20893	Xp11.4	Microphthalmia, syndromic 2, 300166, X-linked dominant	OMIM
BGN	HGNC:1044	Xq28	Meester-Loeys syndrome, 300989, X-linked; Spondyloepimetaphyseal dysplasia, X-linked, 300106, X-linked recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
BMP10	HGNC:20869	2p13.3	Pulmonary Arterial Hypertension; Congenital Heart Disease	PMIDs: 26056270; 35737725; 15073151; 17068149; 29843651; 30578383
BMPR1A	HGNC:1076	10q23.2	Pulmonary Arterial Hypertension	PMID: 30029678
BMPR1B	HGNC:1077	4q22.3	Pulmonary Arterial Hypertension	PMID: 30029678
BMPR2	HGNC:1078	2q33.1-q33.2	Pulmonary hypertension, familial primary, 1, with or without HHT, 178600, Autosomal dominant; Pulmonary hypertension, primary, fenfluramine or dexfenfluramine-associated, 178600, Autosomal dominant; Pulmonary venoocclusive disease 1, 265450, Autosomal dominant	OMIM
BRAF	HGNC:1097	7q34	Melanoma, malignant, somatic, 155600; LEOPARD syndrome 3, 613707, Autosomal dominant; Cardiofaciocutaneous syndrome, 115150, Autosomal dominant; Adenocarcinoma of lung, somatic, 211980; Noonan syndrome 7, 613706, Autosomal dominant; Colorectal cancer, somatic, 114500; Nonsmall cell lung cancer, somatic, 211980	OMIM
BSCL2	HGNC:15832	11q12.3	Lipodystrophy, congenital generalized, type 2, 269700, Autosomal recessive; Neuronopathy, distal hereditary motor, autosomal dominant 13, 619112, Autosomal dominant; Silver spastic paraplegia syndrome, 270685, Autosomal dominant; Encephalopathy, progressive, with or without lipodystrophy, 615924, Autosomal recessive	OMIM
C1S	HGNC:1247	12p13.31	C1s deficiency, 613783; Ehlers-Danlos syndrome, periodontal type, 2, 617174, Autosomal dominant	OMIM
CACNA1C	HGNC:1390	12p13.33	Timothy syndrome, 601005, Autosomal dominant; Long QT syndrome 8, 618447, Autosomal dominant; Neurodevelopmental disorder with hypotonia, language delay, and skeletal defects with or without seizures, 620029, Autosomal dominant; Brugada syndrome 3, 611875, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
CACNA1D	HGNC:1391	3p21.1	Primary aldosteronism, seizures, and neurologic abnormalities, 615474, Autosomal dominant; Sinoatrial node dysfunction and deafness, 614896, Autosomal recessive	OMIM
CACNA1H	HGNC:1395	16p13.3	{Epilepsy, childhood absence, susceptibility to, 6}, 611942; Hyperaldosteronism, familial, type IV, 617027, Autosomal dominant; {Epilepsy, idiopathic generalized, susceptibility to, 6}, 611942	OMIM
CACNA2D1	HGNC:1399	7q21.11	Brugada Syndrome; Short Qt Syndrome	ORPHA:130; ORPHA:51083
CACNB2	HGNC:1402	10p12	Brugada syndrome 4, 611876, Autosomal dominant	OMIM
CALM1	HGNC:1442	14q32.11	Ventricular tachycardia, catecholaminergic polymorphic, 4, 614916, Autosomal dominant; Long QT syndrome 14, 616247, Autosomal dominant	OMIM
CALM2	HGNC:1445	2p21	Long QT syndrome 15, 616249, Autosomal dominant	OMIM
CALM3	HGNC:1449	19q13.32	Long QT syndrome 16, 618782, Autosomal dominant; Ventricular tachycardia, catecholaminergic polymorphic 6, 618782, Autosomal dominant	OMIM
CALR	HGNC:1455	19p13.13	Myelofibrosis, somatic, 254450; Thrombocythemia, somatic, 187950	OMIM
CALR3	HGNC:20407	19p13.11	Hypertrophic Cardiomyopathy	PMIDs: 17655857; 29988065
CAPN3	HGNC:1480	15q15.1	Muscular dystrophy, limb-girdle, autosomal recessive 1, 253600, Autosomal recessive; Muscular dystrophy, limb-girdle, autosomal dominant 4, 618129, Autosomal dominant	OMIM
CASQ2	HGNC:1513	1p13.1	Ventricular tachycardia, catecholaminergic polymorphic, 2, 611938, Autosomal recessive	OMIM
CAV1	HGNC:1527	7q31.2	Lipodystrophy, congenital generalized, type 3, 612526, Autosomal recessive; Pulmonary hypertension, primary, 3, 615343, Autosomal dominant; Lipodystrophy, familial partial, type 7, 606721, Autosomal dominant	OMIM
CAV3	HGNC:1529	3p25.3	Myopathy, distal, Tateyama type, 614321, Autosomal dominant; Creatine phosphokinase, elevated serum, 123320, Autosomal dominant; Cardiomyopathy, familial hypertrophic, 192600, Autosomal dominant, Digenic dominant; Rippling	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			muscle disease 2, 606072, Autosomal dominant; Long QT syndrome 9, 611818, Autosomal dominant	
CAVIN1	HGNC:9688	17q21.2	Lipodystrophy, congenital generalized, type 4, 613327, Autosomal recessive	OMIM
CAVIN4	HGNC:33742	9q31.1	Dilated Cardiomyopathy	PMID: 21642240
CBL	HGNC:1541	11q23.3	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia, 613563, Autosomal dominant; Juvenile myelomonocytic leukemia, 607785, Somatic mutation, Autosomal dominant	OMIM
CBS	HGNC:1550	21q22.3	Thrombosis, hyperhomocysteinemic, 236200, Autosomal recessive; Homocystinuria, B6-responsive and nonresponsive types, 236200, Autosomal recessive	OMIM
CCDC39	HGNC:25244	3q26.33	Ciliary dyskinesia, primary, 14, 613807, Autosomal recessive	OMIM
CCDC40	HGNC:26090	17q25.3	Ciliary dyskinesia, primary, 15, 613808, Autosomal recessive	OMIM
CETP	HGNC:1869	16q13	[High density lipoprotein cholesterol level QTL 10], 143470, Autosomal dominant; Hyperalphalipoproteinemia, 143470, Autosomal dominant	OMIM
CFC1	HGNC:18292	2q21.1	Heterotaxy, visceral, 2, autosomal, 605376, Autosomal dominant	OMIM
CHD7	HGNC:20626	8q12.2	Hypogonadotropic hypogonadism 5 with or without anosmia, 612370, Autosomal dominant; CHARGE syndrome, 214800, Autosomal dominant	OMIM
CHRM2	HGNC:1951	7q33	Dilated Cardiomyopathy	PMIDs: 15231368; 9711178; 11908781; 18451336; 10904849; 8973765
CHST14	HGNC:24464	15q15.1	Ehlers-Danlos syndrome, musculocontractural type 1, 601776, Autosomal recessive	OMIM
CIDEC	HGNC:24229	3p25.3	Lipodystrophy, familial partial, type 5, 615238, Autosomal recessive	OMIM
CITED2	HGNC:1987	6q24.1	Atrial septal defect 8, 614433, Autosomal dominant; Ventricular septal defect 2, 614431, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
COA5	HGNC:33848	2q11.2	Mitochondrial complex IV, deficiency, nuclear type 9, 616500, Autosomal recessive	OMIM
COA6	HGNC:18025	1q42.2	Mitochondrial complex IV deficiency, nuclear type 13, 616501, Autosomal recessive	OMIM
COL1A1	HGNC:2197	17q21.33	Osteogenesis imperfecta, type II, 166210, Autosomal dominant; Caffey disease, 114000, Autosomal dominant; Ehlers-Danlos syndrome, arthrochalasia type, 1, 130060, Autosomal dominant; Osteogenesis imperfecta, type I, 166200, Autosomal dominant; {Bone mineral density variation QTL, osteoporosis}, 166710, Autosomal dominant; Combined osteogenesis imperfecta and Ehlers-Danlos syndrome 1, 619115, Autosomal dominant; Osteogenesis imperfecta, type IV, 166220, Autosomal dominant; Osteogenesis imperfecta, type III, 259420, Autosomal dominant	OMIM
COL1A2	HGNC:2198	7q21.3	Osteogenesis imperfecta, type III, 259420, Autosomal dominant; {Osteoporosis, postmenopausal}, 166710, Autosomal dominant; Ehlers-Danlos syndrome, arthrochalasia type, 2, 617821, Autosomal dominant; Combined osteogenesis imperfecta and Ehlers-Danlos syndrome 2, 619120, Autosomal dominant; Ehlers-Danlos syndrome, cardiac valvular type, 225320, Autosomal recessive; Osteogenesis imperfecta, type IV, 166220, Autosomal dominant; Osteogenesis imperfecta, type II, 166210, Autosomal dominant	OMIM
COL3A1	HGNC:2201	2q32.2	Ehlers-Danlos syndrome, vascular type, 130050, Autosomal dominant; Polymicrogyria with or without vascular-type EDS, 618343, Autosomal recessive	OMIM
COL4A5	HGNC:2207	Xq22.3	Alport syndrome 1, X-linked, 301050, X-linked dominant	OMIM
COL5A1	HGNC:2209	9q34.3	Ehlers-Danlos syndrome, classic type, 1, 130000, Autosomal dominant; Fibromuscular dysplasia, multifocal, 619329, Autosomal dominant	OMIM
COL5A2	HGNC:2210	2q32.2	Ehlers-Danlos syndrome, classic type, 2, 130010, Autosomal dominant	OMIM
COL7A1	HGNC:2214	3p21.31	Epidermolysis bullosa (related to Dilated Cardiomyopathy)	PMIDs: 36660025; 10869001; 20447501

Gene symbol	HGNC ID	Location	Associated disease/s	Source
COQ2	HGNC:25223	4q21.23	{Multiple system atrophy, susceptibility to}, 146500, Autosomal dominant, Autosomal recessive; Coenzyme Q10 deficiency, primary, 1, 607426, Autosomal recessive	OMIM
COX15	HGNC:2263	10q24.2	Mitochondrial complex IV deficiency, nuclear type 6, 615119, Autosomal recessive	OMIM
COX6B1	HGNC:2280	19q13.12	Mitochondrial complex IV deficiency, nuclear type 7, 619051, Autosomal recessive	OMIM
CPT1A	HGNC:2328	11q13.3	CPT deficiency, hepatic, type IA, 255120, Autosomal recessive	OMIM
CPT2	HGNC:2330	1p32.3	{Encephalopathy, acute, infection-induced, 4, susceptibility to}, 614212, Autosomal dominant, Autosomal recessive; CPT II deficiency, infantile, 600649, Autosomal recessive; CPT II deficiency, lethal neonatal, 608836, Autosomal recessive; CPT II deficiency, myopathic, stress-induced, 255110, Autosomal dominant, Autosomal recessive	OMIM
CREBBP	HGNC:2348	16p13.3	Menke-Hennekam syndrome 1, 618332, Autosomal dominant; Rubinstein-Taybi syndrome 1, 180849, Autosomal dominant	OMIM
CRELD1	HGNC:14630	3p25.3	Atrioventricular septal defect, partial, with heterotaxy syndrome, 606217, Autosomal dominant; {Atrioventricular septal defect, susceptibility to, 2}, 606217, Autosomal dominant	OMIM
CRPPA	HGNC:37276	7p21.2	Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 7, 616052, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 7, 614643, Autosomal recessive	OMIM
CRYAB	HGNC:2389	11q23.1	Myopathy, myofibrillar, fatal infantile hypertonic, alpha-B crystallin-related, 613869, Autosomal recessive; Myopathy, myofibrillar, 2, 608810, Autosomal dominant; Cataract 16, multiple types, 613763, Autosomal dominant, Autosomal recessive; Cardiomyopathy, dilated, 1II, 615184, Autosomal dominant	OMIM
CSRP3	HGNC:2472	11p15.1	Cardiomyopathy, dilated, 1M, 607482; Cardiomyopathy, hypertrophic, 12, 612124, Autosomal dominant	OMIM
CTF1	HGNC:2499	16p11.2	Dilated Cardiomyopathy	PMIDs: 11058912; 11691527

Gene symbol	HGNC ID	Location	Associated disease/s	Source
CTNNA1	HGNC:2509	5q31.2	Macular dystrophy, patterned, 2, 608970, Autosomal dominant	OMIM
CTNNA3	HGNC:2511	10q21.3	Arrhythmogenic right ventricular dysplasia 13, 615616, Autosomal dominant	OMIM
CTNNB1	HGNC:2514	3p22.1	Exudative vitreoretinopathy 7, 617572, Autosomal dominant; Pilomatricoma, somatic, 132600; Colorectal cancer, somatic, 114500; Neurodevelopmental disorder with spastic diplegia and visual defects, 615075, Autosomal dominant; Medulloblastoma, somatic, 155255; Ovarian cancer, somatic, 167000; Hepatocellular carcinoma, somatic, 114550	OMIM
CYP3A4	HGNC:2637	7q22.1	Vitamin D-dependent rickets, type 3, 619073, Autosomal dominant	OMIM
CYP3A5	HGNC:2638	7q22.1	{Hypertension, salt-sensitive essential, susceptibility to}, 145500, Multifactorial	OMIM
DBH	HGNC:2689	9q34.2	Orthostatic hypotension 1, due to DBH deficiency, 223360, Autosomal recessive	OMIM
DES	HGNC:2770	2q35	Scapuloperoneal syndrome, neurogenic, Kaeser type, 181400, Autosomal dominant; Cardiomyopathy, dilated, 1I, 604765, Autosomal dominant; Myopathy, myofibrillar, 1, 601419, Autosomal dominant, Autosomal recessive	OMIM
DLD	HGNC:2898	7q31.1	Dihydrolipoamide dehydrogenase deficiency, 246900, Autosomal recessive	OMIM
DMD	HGNC:2928	Xp21.2-p21.1	Becker muscular dystrophy, 300376, X-linked recessive; Cardiomyopathy, dilated, 3B, 302045, X-linked; Duchenne muscular dystrophy, 310200, X-linked recessive	OMIM
DMPK	HGNC:2933	19q13.32	Myotonic dystrophy 1, 160900, Autosomal dominant	OMIM
DNAAF1	HGNC:30539	16q24.1	Ciliary dyskinesia, primary, 13, 613193, Autosomal recessive	OMIM
DNAAF19	HGNC:32700	17q21.31	Ciliary dyskinesia, primary, 17, 614679, Autosomal recessive	OMIM
DNAAF3	HGNC:30492	19q13.42	Ciliary dyskinesia, primary, 2, 606763, Autosomal recessive	OMIM
DNAAF4	HGNC:21493	15q21.3	{Dyslexia, susceptibility to, 1}, 127700, Autosomal dominant; Ciliary dyskinesia, primary, 25, 615482, Autosomal recessive	OMIM
DNAI1	HGNC:2954	9p13.3	Ciliary dyskinesia, primary, 1, with or without situs inversus, 244400, Autosomal recessive	OMIM
DNAJC19	HGNC:30528	3q26.33	3-methylglutaconic aciduria, type V, 610198, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
DNM1L	HGNC:2973	12p11.21	Optic atrophy 5, 610708, Autosomal dominant; Encephalopathy, lethal, due to defective mitochondrial peroxisomal fission 1, 614388, Autosomal dominant, Autosomal recessive	OMIM
DOLK	HGNC:23406	9q34.11	Congenital disorder of glycosylation, type Im, 610768, Autosomal recessive	OMIM
DPP6	HGNC:3010	7q36.2	Intellectual developmental disorder, autosomal dominant 33, 616311, Autosomal dominant; {Ventricular fibrillation, paroxysmal familial, 2}, 612956, Autosomal dominant	OMIM
DSC2	HGNC:3036	18q12.1	Arrhythmogenic right ventricular dysplasia 11 with mild palmoplantar keratoderma and woolly hair, 610476, Autosomal dominant, Autosomal recessive; Arrhythmogenic right ventricular dysplasia 11, 610476, Autosomal dominant, Autosomal recessive	OMIM
DSG2	HGNC:3049	18q12.1	Cardiomyopathy, dilated, 1BB, 612877, Autosomal recessive; Arrhythmogenic right ventricular dysplasia 10, 610193, Autosomal dominant	OMIM
DSP	HGNC:3052	6p24.3	Arrhythmogenic right ventricular dysplasia 8, 607450, Autosomal dominant; Epidermolysis bullosa, lethal acantholytic, 609638, Autosomal recessive; Keratosis palmoplantaris striata II, 612908, Autosomal dominant; Dilated cardiomyopathy with woolly hair, keratoderma, and tooth agenesis, 615821, Autosomal dominant; Cardiomyopathy, dilated, with woolly hair and keratoderma, 605676, Autosomal recessive	OMIM
DTNA	HGNC:3057	18q12.1	Left ventricular noncompaction 1, with or without congenital heart defects, 604169, Autosomal dominant	OMIM
DYSF	HGNC:3097	2p13.2	Muscular dystrophy, limb-girdle, autosomal recessive 2, 253601, Autosomal recessive; Miyoshi muscular dystrophy 1, 254130, Autosomal recessive; Myopathy, distal, with anterior tibial onset, 606768, Autosomal recessive	OMIM
ECE1	HGNC:3146	1p36.12	{Hypertension, essential, susceptibility to}, 145500, Multifactorial; Hirschsprung disease, cardiac defects, and autonomic dysfunction, 613870, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
EEF1A2	HGNC:3192	20q13.33	Developmental and epileptic encephalopathy 33, 616409, Autosomal dominant; Intellectual developmental disorder, autosomal dominant 38, 616393, Autosomal dominant	OMIM
EFEMP2	HGNC:3219	11q13.1	Cutis laxa, autosomal recessive, type IB, 614437, Autosomal recessive	OMIM
EHMT1	HGNC:24650	9q34.3	Kleefstra syndrome 1, 610253, Autosomal dominant	OMIM
EIF2AK3	HGNC:3255	2p11.2	Wolcott-Rallison Syndrome	PMID: 10932183
EIF2AK4	HGNC:19687	15q15.1	Pulmonary venoocclusive disease 2, 234810, Autosomal recessive	OMIM
ELAC2	HGNC:14198	17p12	{Prostate cancer, hereditary, 2, susceptibility to}, 614731; Combined oxidative phosphorylation deficiency 17, 615440, Autosomal recessive	OMIM
ELN	HGNC:3327	7q11.23	Cutis laxa, autosomal dominant, 123700, Autosomal dominant; Supravalvar aortic stenosis, 185500, Autosomal dominant	OMIM
EMD	HGNC:3331	Xq28	Emery-Dreifuss muscular dystrophy 1, X-linked, 310300, X-linked recessive	OMIM
ENG	HGNC:3349	9q34.11	Telangiectasia, hereditary hemorrhagic, type 1, 187300, Autosomal dominant	OMIM
ENPP1	HGNC:3356	6q23.2	{Obesity, susceptibility to}, 601665, Autosomal dominant, Multifactorial, Autosomal recessive; Hypophosphatemic rickets, autosomal recessive, 2, 613312, Autosomal recessive; {Diabetes mellitus, non-insulin-dependent, susceptibility to}, 125853, Autosomal dominant; Arterial calcification, generalized, of infancy, 1, 208000, Autosomal recessive; Cole disease, 615522, Autosomal dominant	OMIM
EP300	HGNC:3373	22q13.2	Rubinstein-Taybi Syndrome	PMID: 20301699
EPG5	HGNC:29331	18q12.3-q21.1	Vici syndrome, 242840, Autosomal recessive	OMIM
EVC	HGNC:3497	4p16.2	Ellis-van Creveld syndrome, 225500, Autosomal recessive; Weyers acrofacial dysostosis, 193530, Autosomal dominant	OMIM
EYA4	HGNC:3522	6q23.2	Cardiomyopathy, dilated, 1J, 605362, Autosomal dominant; Deafness, autosomal dominant 10, 601316, Autosomal dominant	OMIM
FAH	HGNC:3579	15q25.1	Tyrosinemia, type I, 276700, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
FBN1	HGNC:3603	15q21.1	Geleophysic dysplasia 2, 614185, Autosomal dominant; Weill-Marchesani syndrome 2, dominant, 608328, Autosomal dominant; Ectopia lentis, familial, 129600, Autosomal dominant; MASS syndrome, 604308, Autosomal dominant; Marfan lipodystrophy syndrome, 616914, Autosomal dominant; Acromicric dysplasia, 102370, Autosomal dominant; Marfan syndrome, 154700, Autosomal dominant; Stiff skin syndrome, 184900, Autosomal dominant	OMIM
FBN2	HGNC:3604	5q23.3	Macular degeneration, early-onset, 616118, Autosomal dominant; Contractural arachnodactyly, congenital, 121050, Autosomal dominant	OMIM
FGF12	HGNC:3668	3q28-q29	Brugada Syndrome	PMID: 20301690
FHL1	HGNC:3702	Xq26.3	Myopathy, X-linked, with postural muscle atrophy, 300696, X-linked recessive; Emery-Dreifuss muscular dystrophy 6, X-linked, 300696, X-linked recessive; Uruguay faciocardiomusculoskeletal syndrome, 300280, X-linked recessive; Scapuloperoneal myopathy, X-linked dominant, 300695, X-linked dominant; Reducing body myopathy, X-linked 1b, with late childhood or adult onset, 300718, X-linked; Reducing body myopathy, X-linked 1a, severe, infantile or early childhood onset, 300717, X-linked dominant	OMIM
FHL2	HGNC:3703	2q12.2	Familial Isolated Dilated Cardiomyopathy	PMID: 17416352
FHOD3	HGNC:26178	18q12.2	Cardiomyopathy, familial hypertrophic, 28, 619402, Autosomal dominant	OMIM
FKBP14	HGNC:18625	7p14.3	Ehlers-Danlos syndrome, kyphoscoliotic type, 2, 614557, Autosomal recessive	OMIM
FKRP	HGNC:17997	19q13.32	Muscular dystrophy-dystroglycanopathy (congenital with or without impaired intellectual development), type B, 5, 606612, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 5, 607155, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 5, 613153, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
FKTN	HGNC:3622	9q31.2	Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 4, 611588, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4, 253800, Autosomal recessive; Cardiomyopathy, dilated, 1X, 611615, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital without impaired intellectual development), type B, 4, 613152, Autosomal recessive	OMIM
FLNA	HGNC:3754	Xq28	Chromosome Xq27.3-q28 duplication syndrome, 300869, X-linked recessive,Chromosome Xq28 duplication syndrome, 300815,Moyamoya disease 4, 300845, X-linked recessive,Otopalatodigital syndrome, type II, 304120, X-linked dominant; Intestinal pseudoobstruction, neuronal, 300048, X-linked recessive; Cardiac valvular dysplasia, X-linked, 314400, X-linked; FG syndrome 2, 300321, X-linked; Melnick-Needles syndrome, 309350, X-linked dominant; Terminal osseous dysplasia, 300244, X-linked dominant; Congenital short bowel syndrome, 300048, X-linked recessive; Otopalatodigital syndrome, type I, 311300, X-linked dominant; Heterotopia, periventricular, 1, 300049, X-linked dominant; Frontometaphyseal dysplasia 1, 305620, X-linked recessive	OMIM
FLNC	HGNC:3756	7q32.1	Cardiomyopathy, familial hypertrophic, 26, 617047, Autosomal dominant; Arrhythmogenic right ventricular dysplasia, familial, 617047, Autosomal dominant; Cardiomyopathy, familial restrictive 5, 617047, Autosomal dominant; Myopathy, distal, 4, 614065, Autosomal dominant; Myopathy, myofibrillar, 5, 609524, Autosomal dominant	OMIM
FOXC1	HGNC:3800	6p25.3	Axenfeld-Rieger syndrome, type 3, 602482, Autosomal dominant; Anterior segment dysgenesis 3, multiple subtypes, 601631, Autosomal dominant	OMIM
FOXE3	HGNC:3808	1p33	Anterior segment dysgenesis 2, multiple subtypes, 610256, Autosomal recessive; {Aortic aneurysm, familial thoracic 11, susceptibility to}, 617349, Autosomal dominant; Cataract 34, multiple types, 612968	OMIM
FOXF1	HGNC:3809	16q24.1	Alveolar capillary dysplasia with misalignment of pulmonary veins, 265380, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
FOXH1	HGNC:3814	8q24.3	Sporadic Conotruncal Heart Defect	PMID: 32003456
FOXP1	HGNC:3823	3p13	Atrial Septal Defect 1	ClinVar: RCV001528148.1
FOXP3	HGNC:6106	Xp11.23	Centronuclear Myopathy	PMID: 38982518
FOXRED1	HGNC:26927	11q24.2	Mitochondrial complex I deficiency, nuclear type 19, 618241, Autosomal recessive	OMIM
FXN	HGNC:3951	9q21.11	Friedreich ataxia with retained reflexes, 229300, Autosomal recessive; Friedreich ataxia, 229300, Autosomal recessive	OMIM
GAA	HGNC:4065	17q25.3	Glycogen storage disease II, 232300, Autosomal recessive	OMIM
GATA4	HGNC:4173	8p23.1	Tetralogy of Fallot, 187500, Autosomal dominant; Atrial septal defect 2, 607941, Autosomal dominant; Ventricular septal defect 1, 614429, Autosomal dominant; Atrioventricular septal defect 4, 614430, Autosomal dominant; Testicular anomalies with or without congenital heart disease, 615542, Autosomal dominant	OMIM
GATA5	HGNC:15802	20q13.33	Congenital heart defects, multiple types, 5, 617912, Autosomal dominant, Autosomal recessive	OMIM
GATA6	HGNC:4174	18q11.2	Atrial septal defect 9, 614475, Autosomal dominant; Persistent truncus arteriosus, 217095; Pancreatic agenesis and congenital heart defects, 600001, Autosomal dominant; Atrioventricular septal defect 5, 614474, Autosomal dominant; Tetralogy of Fallot, 187500, Autosomal dominant	OMIM
GATAD1	HGNC:29941	7q21.2	Cardiomyopathy, dilated, 2B, 614672, Autosomal recessive	OMIM
GBE1	HGNC:4180	3p12.2	Glycogen storage disease IV, 232500, Autosomal recessive; Polyglucosan body disease, adult form, 263570, Autosomal recessive	OMIM
GDF1	HGNC:4214	19p13.11	Congenital heart defects, multiple types, 6, 613854, Autosomal dominant; Right atrial isomerism (Ivemark), 208530, Autosomal recessive	OMIM
GDF2	HGNC:4217	10q11.22	Telangiectasia, hereditary hemorrhagic, type 5, 615506, Autosomal dominant	OMIM
GFM1	HGNC:13780	3q25.32	Combined oxidative phosphorylation deficiency 1, 609060, Autosomal recessive	OMIM
GJA1	HGNC:4274	6q22.31	Erythrokeratodermia variabilis et progressiva 3, 617525, Autosomal dominant; Craniometaphyseal dysplasia, autosomal recessive, 218400, Autosomal recessive;	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			Oculodentodigital dysplasia, 164200, Autosomal dominant; Palmoplantar keratoderma with congenital alopecia, 104100, Autosomal dominant; Syndactyly, type III, 186100, Autosomal dominant; Oculodentodigital dysplasia, autosomal recessive, 257850, Autosomal recessive	
GJA5	HGNC:4279	1q21.2	Atrial fibrillation, familial, 11, 614049, Autosomal dominant; Atrial standstill, digenic (GJA5/SCN5A), 108770, Autosomal dominant	OMIM
GLA	HGNC:4296	Xq22.1	Fabry disease, cardiac variant, 301500, X-linked; Fabry disease, 301500, X-linked	OMIM
GLB1	HGNC:4298	3p22.3	GM1-gangliosidosis, type I, 230500, Autosomal recessive; GM1-gangliosidosis, type III, 230650, Autosomal recessive; Mucopolysaccharidosis type IVB (Morquio), 253010, Autosomal recessive; GM1-gangliosidosis, type II, 230600, Autosomal recessive	OMIM
GLIS3	HGNC:28510	9p24.2	Diabetes mellitus, neonatal, with congenital hypothyroidism, 610199, Autosomal recessive	OMIM
GMPPB	HGNC:22932	3p21.31	Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 14, 615352, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with impaired intellectual development), type B, 14, 615351, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 14, 615350, Autosomal recessive	OMIM
GNPTAB	HGNC:29670	12q23.2	Mucolipidosis III alpha/beta, 252600, Autosomal recessive; Mucolipidosis II alpha/beta, 252500, Autosomal recessive	OMIM
GPC3	HGNC:4451	Xq26.2	Wilms tumor, somatic, 194070; Simpson-Golabi-Behmel syndrome, type 1, 312870, X-linked recessive	OMIM
GPD1	HGNC:4455	12q13.12	Early Repolarization Syndrome	PMID: 31922248
GPD1L	HGNC:28956	3p22.3	Brugada syndrome 2, 611777, Autosomal dominant	OMIM
GPIHBP1	HGNC:24945	8q24.3	Hyperlipoproteinemia, type 1D, 615947, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
GREM2	HGNC:17655	1q43	Atrial fibrillation	PMIDs: 24648383; 23223679
GTPBP3	HGNC:14880	19p13.11	Combined oxidative phosphorylation deficiency 23, 616198, Autosomal recessive	OMIM
GUSB	HGNC:4696	7q11.21	Mucopolysaccharidosis VII, 253220, Autosomal recessive	OMIM
GYG1	HGNC:4699	3q24	Glycogen storage disease XV, 613507, Autosomal recessive; Polyglucosan body myopathy 2, 616199, Autosomal recessive	OMIM
HADHA	HGNC:4801	2p23.3	HELLP syndrome, maternal, of pregnancy, 609016, Autosomal recessive; LCHAD deficiency, 609016, Autosomal recessive; Mitochondrial trifunctional protein deficiency 1, 609015, Autosomal recessive; Fatty liver, acute, of pregnancy, 609016, Autosomal recessive	OMIM
HAMP	HGNC:15598	19q13.12	Hemochromatosis, type 2B, 613313, Autosomal recessive	OMIM
HAND2	HGNC:4808	4q34.1	Familial Isolated Dilated Cardiomyopathy	PMID: 30217752
HCN4	HGNC:16882	15q24.1	Sick sinus syndrome 2, 163800, Autosomal dominant; {Epilepsy, idiopathic generalized, susceptibility to, 18}, 619521, Autosomal dominant; Brugada syndrome 8, 613123	OMIM
HFE	HGNC:4886	6p22.2	Hemochromatosis, type 1, 235200, Autosomal recessive	OMIM
HRAS	HGNC:5173	11p15.5	Bladder cancer, somatic, 109800; Thyroid carcinoma, follicular, somatic, 188470; Congenital myopathy with excess of muscle spindles, 218040, Autosomal dominant; Nevus sebaceous or woolly hair nevus, somatic, 162900; Schimmelpenning-Feuerstein-Mims syndrome, somatic mosaic, 163200; Spitz nevus or nevus spilus, somatic, 137550; Costello syndrome, 218040, Autosomal dominant	OMIM
IDH2	HGNC:5383	15q26.1	D-2-hydroxyglutaric aciduria 2, 613657	OMIM
ILK	HGNC:6040	11p15.4	Dilated Cardiomyopathy	PMIDs: 17646580; 17131835
INS	HGNC:6081	11p15.5	Diabetes mellitus, insulin-dependent, 2, 125852, Autosomal dominant; Maturity-onset diabetes of the young, type 10, 613370, Autosomal dominant;	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			Hyperproinsulinemia, 616214, Autosomal dominant; Diabetes mellitus, permanent neonatal 4, 618858, Autosomal dominant, Autosomal recessive	
INVS	HGNC:17870	9q31.1	Nephronophthisis 2, infantile, 602088, Autosomal recessive	OMIM
IRX3	HGNC:14360	16q12.2	Cardiac Conduction Disease; Atrial Fibrillation	PMID: 22992950
IRX4	HGNC:6129	5p15.33	Congenital Heart Disease	PMID: 21544582
ISL1	HGNC:6132	5q11.1	Congenital Heart Disease	PMIDs: 14667410; 30390123; 36170181
JAG1	HGNC:6188	20p12.2	Deafness, congenital heart defects, and posterior embryotoxon, 617992, Autosomal dominant; Charcot-Marie-Tooth disease, axonal, type 2HH, 619574, Autosomal dominant; Alagille syndrome 1, 118450, Autosomal dominant; Tetralogy of Fallot, 187500, Autosomal dominant	OMIM
JPH2	HGNC:14202	20q13.12	Cardiomyopathy, dilated, 2E, 619492, Autosomal recessive; Cardiomyopathy, hypertrophic, 17, 613873, Autosomal dominant	OMIM
JUP	HGNC:6207	17q21.2	Naxos disease, 601214, Autosomal recessive; Arrhythmogenic right ventricular dysplasia 12, 611528, Autosomal dominant	OMIM
KANSL1	HGNC:24565	17q21.31	Koolen-De Vries syndrome, 610443, Autosomal dominant	OMIM
KAT6B	HGNC:17582	10q22.2	SBBYSS syndrome, 603736, Autosomal dominant; Genitopatellar syndrome, 606170, Autosomal dominant	OMIM
KCNA5	HGNC:6224	12p13.32	Atrial fibrillation, familial, 7, 612240, Autosomal dominant	OMIM
KCND2	HGNC:6238	7q31.31	Brugada Syndrome; Atrial Fibrillation, Long QT Syndrome	PMIDs: 20301690; 30571183; 15563876
KCND3	HGNC:6239	1p13.2	Spinocerebellar ataxia 19, 607346, Autosomal dominant; Brugada syndrome 9, 616399, Autosomal dominant	OMIM
KCNE1	HGNC:6240	21q22.12	Jervell and Lange-Nielsen syndrome 2, 612347, Autosomal recessive; Long QT syndrome 5, 613695, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
KCNE2	HGNC:6242	21q22.11	Long QT syndrome 6, 613693, Autosomal dominant; Atrial fibrillation, familial, 4, 611493	OMIM
KCNE3	HGNC:6243	11q13.4	Brugada syndrome 6, 613119	OMIM
KCNE4	HGNC:6244	2q36.1	Hereditary Arrhythmia	PMID: 27484720
KCNE5	HGNC:6241	Xq23	Brugada Syndrome	PMID: 21493962
KCNH2	HGNC:6251	7q36.1	Short QT syndrome 1, 609620; Long QT syndrome 2, 613688, Autosomal dominant	OMIM
KCNJ2	HGNC:6263	17q24.3	Atrial fibrillation, familial, 9, 613980, Autosomal dominant; Andersen syndrome, 170390, Autosomal dominant; Short QT syndrome 3, 609622	OMIM
KCNJ5	HGNC:6266	11q24.3	Long QT syndrome 13, 613485, Autosomal dominant; Hyperaldosteronism, familial, type III, 613677, Autosomal dominant	OMIM
KCNJ8	HGNC:6269	12p12.1	Brugada Syndrome	PMID: 22056721
KCNK17	HGNC:14465	6p21.2	Cardiac conduction disease	PMID: 25426816
KCNK3	HGNC:6278	2p23.3	Pulmonary hypertension, primary, 4, 615344, Autosomal dominant	OMIM
KCNQ1	HGNC:6294	11p15.5-p15.4	Short QT syndrome 2, 609621, Autosomal dominant; Atrial fibrillation, familial, 3, 607554, Autosomal dominant; Long QT syndrome 1, 192500, Autosomal dominant; {Long QT syndrome 1, acquired, susceptibility to}, 192500, Autosomal dominant; Jervell and Lange-Nielsen syndrome, 220400, Autosomal recessive	OMIM
KLF10	HGNC:11810	8q22.3	Hypertrophic Cardiomyopathy	PMIDs: 16888812; 22234868
KMT2D	HGNC:7133	12q13.12	Branchial arch abnormalities, choanal atresia, athelia, hearing loss, and hypothyroidism syndrome, 620186, Autosomal dominant; Kabuki syndrome 1, 147920, Autosomal dominant	OMIM
KRAS	HGNC:6407	12p12.1	Gastric cancer, somatic, 613659; Oculoectodermal syndrome, somatic, 600268; Breast cancer, somatic, 114480; Noonan syndrome 3, 609942, Autosomal dominant; RAS-associated autoimmune leukoproliferative disorder, 614470, Autosomal dominant; Arteriovenous malformation of the brain, somatic, 108010; Lung cancer, somatic, 211980; Pancreatic carcinoma, somatic, 260350; Leukemia,	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			acute myeloid, somatic, 601626; Schimmelpenning-Feuerstein-Mims syndrome, somatic mosaic, 163200; Cardiofaciocutaneous syndrome 2, 615278, Autosomal dominant; Bladder cancer, somatic, 109800	
LAMA2	HGNC:6482	6q22.33	Muscular dystrophy, limb-girdle, autosomal recessive 23, 618138, Autosomal recessive; Muscular dystrophy, congenital, merosin deficient or partially deficient, 607855, Autosomal recessive	OMIM
LAMA4	HGNC:6484	6q21	Cardiomyopathy, dilated, 1JJ, 615235, Autosomal dominant	OMIM
LAMP2	HGNC:6501	Xq24	Danon disease, 300257, X-linked dominant	OMIM
LDB3	HGNC:15710	10q23.2	Left ventricular noncompaction 3, 601493, Autosomal dominant; Cardiomyopathy, hypertrophic, 24, 601493, Autosomal dominant; Myopathy, myofibrillar, 4, 609452, Autosomal dominant; Cardiomyopathy, dilated, 1C, with or without LVNC, 601493, Autosomal dominant	OMIM
LDLR	HGNC:6547	19p13.2	LDL cholesterol level QTL2, 143890, Autosomal dominant, Autosomal recessive; Hypercholesterolemia, familial, 1, 143890, Autosomal dominant, Autosomal recessive	OMIM
LDLRAP1	HGNC:18640	1p36.11	Hypercholesterolemia, familial, 4, 603813, Autosomal recessive	OMIM
LEFTY2	HGNC:3122	1q42.12	Congenital Heart Disease	PMID: 25111179
LIAS	HGNC:16429	4p14	Hyperglycinemia, lactic acidosis, and seizures, 614462, Autosomal recessive	OMIM
LIPA	HGNC:6617	10q23.31	Wolman disease, 620151, Autosomal recessive; Cholesteryl ester storage disease, 278000, Autosomal recessive	OMIM
LIPC	HGNC:6619	15q21.3	{Diabetes mellitus, noninsulin-dependent}, 125853, Autosomal dominant; Hepatic lipase deficiency, 614025, Autosomal recessive; [High density lipoprotein cholesterol level QTL 12], 612797	OMIM
LMF1	HGNC:14154	16p13.3	Hypertriglyceridemia	PMID: 32793115

Gene symbol	HGNC ID	Location	Associated disease/s	Source
LMNA	HGNC:6636	1q22	Mandibuloacral dysplasia, 248370, Autosomal recessive; Heart-hand syndrome, Slovenian type, 610140, Autosomal dominant; Cardiomyopathy, dilated, 1A, 115200, Autosomal dominant; Emery-Dreifuss muscular dystrophy 3, autosomal recessive, 616516, Autosomal recessive; Restrictive dermopathy 2, 619793; Charcot-Marie-Tooth disease, type 2B1, 605588, Autosomal recessive; Emery-Dreifuss muscular dystrophy 2, autosomal dominant, 181350, Autosomal dominant; Hutchinson-Gilford progeria, 176670, Autosomal dominant; Lipodystrophy, familial partial, type 2, 151660, Autosomal dominant; Muscular dystrophy, congenital, 613205, Autosomal dominant; Malouf syndrome, 212112, Autosomal dominant	OMIM
LOX	HGNC:6664	5q23.1	Aortic aneurysm, familial thoracic 10, 617168, Autosomal dominant	OMIM
LPA	HGNC:6667	6q25.3-q26	[LPA deficiency, congenital], 618807, Autosomal dominant; {Coronary artery disease, susceptibility to}, 618807, Autosomal dominant	OMIM
LPL	HGNC:6677	8p21.3	Familial lipoprotein lipase deficiency	ORPHA:309015
LRP6	HGNC:6698	12p13.2	{Coronary artery disease, autosomal dominant, 2}, 610947, Autosomal dominant; Tooth agenesis, selective, 7, 616724, Autosomal dominant	OMIM
LZTR1	HGNC:6742	22q11.21	Noonan syndrome 2, 605275, Autosomal recessive; Noonan syndrome 10, 616564, Autosomal dominant; {Schwannomatosis-2, susceptibility to}, 615670, Autosomal dominant	OMIM
MAP2K1	HGNC:6840	15q22.31	Cardiofaciocutaneous syndrome 3, 615279, Autosomal dominant; Melorheostosis, isolated, somatic mosaic, 155950	OMIM
MAP2K2	HGNC:6842	19p13.3	Cardiofaciocutaneous syndrome 4, 615280, Autosomal dominant	OMIM
MAP3K8	HGNC:6860	10p11.23	Noonan Syndrome	PMID: 37568403
MCTP2	HGNC:25636	15q26.2	Chromosome 15Q26-Qter Deletion Syndrome	PMID: 23773997

Gene symbol	HGNC ID	Location	Associated disease/s	Source
MED12	HGNC:11957	Xq13.1	Lujan-Fryns syndrome, 309520, X-linked recessive; Ohdo syndrome, X-linked, 300895, X-linked recessive; Hardikar syndrome, 301068, X-linked dominant; Opitz-Kaveggia syndrome, 305450, X-linked recessive	OMIM
MED13L	HGNC:22962	12q24.21	Impaired intellectual development and distinctive facial features with or without cardiac defects, 616789, Autosomal dominant	OMIM
MEF2A	HGNC:6993	15q26.3	{Coronary artery disease, autosomal dominant, 1}, 608320, Autosomal dominant	OMIM
MEF2C	HGNC:6996	5q14.3	Dilated cardiomyopathy; Hypertrophic Cardiomyopathy	PMIDs: 37315521; 28902616; 22718505
MFAP5	HGNC:29673	12p13.31	Aortic aneurysm, familial thoracic 9, 616166, Autosomal dominant	OMIM
MIB1	HGNC:21086	18q11.2	Left ventricular noncompaction 7, 615092, Autosomal dominant	OMIM
MKS1	HGNC:7121	17q22	Bardet-Biedl syndrome 13, 615990, Autosomal recessive; Meckel syndrome 1, 249000, Autosomal recessive; Joubert syndrome 28, 617121, Autosomal recessive	OMIM
MLYCD	HGNC:7150	16q23.3	Malonyl-CoA decarboxylase deficiency, 248360, Autosomal recessive	OMIM
MMP21	HGNC:14357	10q26.2	Heterotaxy, visceral, 7, autosomal, 616749, Autosomal recessive	OMIM
MRPL3	HGNC:10379	3q22.1	Combined oxidative phosphorylation deficiency 9, 614582, Autosomal recessive	OMIM
MRPL44	HGNC:16650	2q36.1	Combined oxidative phosphorylation deficiency 16, 615395, Autosomal recessive	OMIM
MRPS22	HGNC:14508	3q23	Ovarian dysgenesis 7, 618117, Autosomal recessive; Combined oxidative phosphorylation deficiency 5, 611719, Autosomal recessive	OMIM
MTO1	HGNC:19261	6q13	Combined oxidative phosphorylation deficiency 10, 614702, Autosomal recessive	OMIM
MTTP	HGNC:7467	4q23	Hypercholesterolemia, Familial, 1	PMID: 36685950
MYBPC3	HGNC:7551	11p11.2	Cardiomyopathy, hypertrophic, 4, 115197, Autosomal dominant, Autosomal recessive; Cardiomyopathy, dilated, 1MM, 615396, Autosomal dominant; Left ventricular noncompaction 10, 615396, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
MYH11	HGNC:7569	16p13.11	Megacystis-microcolon-intestinal hypoperistalsis syndrome 2, 619351, Autosomal recessive; Aortic aneurysm, familial thoracic 4, 132900, Autosomal dominant; Visceral myopathy 2, 619350, Autosomal dominant	OMIM
MYH6	HGNC:7576	14q11.2	{Sick sinus syndrome 3}, 614090; Atrial septal defect 3, 614089; Cardiomyopathy, dilated, 1EE, 613252, Autosomal dominant; Cardiomyopathy, hypertrophic, 14, 613251, Autosomal dominant	OMIM
MYH7	HGNC:7577	14q11.2	Laing distal myopathy, 160500, Autosomal dominant; Cardiomyopathy, hypertrophic, 1, 192600, Autosomal dominant, Digenic dominant; Left ventricular noncompaction 5, 613426, Autosomal dominant; Cardiomyopathy, dilated, 1S, 613426, Autosomal dominant; Congenital myopathy 7B, myosin storage, autosomal recessive, 255160, Autosomal recessive; Congenital myopathy 7A, myosin storage, autosomal dominant, 608358, Autosomal dominant	OMIM
MYL2	HGNC:7583	12q24.11	Cardiomyopathy, hypertrophic, 10, 608758, Autosomal dominant; Myopathy, myofibrillar, 12, infantile-onset, with cardiomyopathy, 619424, Autosomal recessive	OMIM
MYL3	HGNC:7584	3p21.31	Cardiomyopathy, hypertrophic, 8, 608751, Autosomal dominant, Autosomal recessive	OMIM
MYL4	HGNC:7585	17q21.32	Atrial fibrillation, familial, 18, 617280, Autosomal dominant	OMIM
MYLIP	HGNC:21155	6p22.3	Hypolipemia	PMID: 21765216
MYLK	HGNC:7590	3q21.1	Megacystis-microcolon-intestinal hypoperistalsis syndrome 1, 249210, Autosomal recessive; Aortic aneurysm, familial thoracic 7, 613780, Autosomal dominant	OMIM
MYLK2	HGNC:16243	20q11.21	Cardiomyopathy, hypertrophic, 1, digenic, 192600, Autosomal dominant, Digenic dominant	OMIM
MYOM1	HGNC:7613	18p11.31	Hypertrophic Cardiomyopathy	PMID: 33452765
MYOT	HGNC:12399	5q31.2	Myopathy, myofibrillar, 3, 609200, Autosomal dominant	OMIM
MYOZ2	HGNC:1330	4q26	Cardiomyopathy, hypertrophic, 16, 613838, Autosomal dominant	OMIM
MYPN	HGNC:23246	10q21.3	Cardiomyopathy, hypertrophic, 22, 615248, Autosomal dominant; Congenital myopathy 24, 617336, Autosomal recessive; Cardiomyopathy, familial restrictive, 4,	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			615248, Autosomal dominant; Cardiomyopathy, dilated, 1KK, 615248, Autosomal dominant	
NDUFAF1	HGNC:18828	15q15.1	Mitochondrial complex I deficiency, nuclear type 11, 618234, Autosomal recessive	OMIM
NDUFAF2	HGNC:28086	5q12.1	Mitochondrial Complex I Deficiency, Nuclear Type 1	PMID: 19384974
NDUFB11	HGNC:20372	Xp11.3	Linear skin defects with multiple congenital anomalies 3, 300952, X-linked dominant; Mitochondrial complex I deficiency, nuclear type 30, 301021, X-linked	OMIM
NEBL	HGNC:16932	10p12.31	Dilated Cardiomyopathy	ClinVar: SCV000992363.1
NEK8	HGNC:13387	17q11.2	Renal-hepatic-pancreatic dysplasia 2, 615415, Autosomal recessive; Nephronophthisis 9, 613824	OMIM
NEXN	HGNC:29557	1p31.1	Cardiomyopathy, dilated, 1CC, 613122, Autosomal dominant; Cardiomyopathy, hypertrophic, 20, 613876, Autosomal dominant	OMIM
NF1	HGNC:7765	17q11.2	Watson syndrome, 193520, Autosomal dominant; Leukemia, juvenile myelomonocytic, 607785, Somatic mutation, Autosomal dominant; Neurofibromatosis, familial spinal, 162210, Autosomal dominant; Neurofibromatosis, type 1, 162200, Autosomal dominant; Neurofibromatosis-Noonan syndrome, 601321, Autosomal dominant	OMIM
NKX2-5	HGNC:2488	5q35.1	Hypoplastic left heart syndrome 2, 614435, Autosomal dominant; Tetralogy of Fallot, 187500, Autosomal dominant; Hypothyroidism, congenital nongoitrous, 5, 225250, Autosomal dominant; Conotruncal heart malformations, variable, 217095; Ventricular septal defect 3, 614432, Autosomal dominant; Atrial septal defect 7, with or without AV conduction defects, 108900, Autosomal dominant	OMIM
NKX2-6	HGNC:32940	8p21.2	Persistent truncus arteriosus, 217095; Conotruncal heart malformations, 217095	OMIM
NME8	HGNC:16473	7p14.1	Ciliary dyskinesia, primary, 6, 610852, Autosomal recessive	OMIM
NNT	HGNC:7863	5p12	Abdominal Obesity-Metabolic Syndrome 1	PMID: 32629517
NODAL	HGNC:7865	10q22.1	Heterotaxy, visceral, 5, 270100, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
NONO	HGNC:7871	Xq13.1	Intellectual developmental disorder, X-linked syndromic 34, 300967, X-linked	OMIM
NOS1AP	HGNC:16859	1q23.3	Long Qt Syndrome 1	PMIDs: 20538168; 19822806
NOTCH1	HGNC:7881	9q34.3	Adams-Oliver syndrome 5, 616028, Autosomal dominant; Aortic valve disease 1, 109730, Autosomal dominant	OMIM
NOTCH2	HGNC:7882	1p12	Alagille syndrome 2, 610205, Autosomal dominant; Hajdu-Cheney syndrome, 102500, Autosomal dominant	OMIM
NPC1L1	HGNC:7898	7p13	[Ezetimibe, nonresponse to], 617966; [Low density lipoprotein cholesterol level QTL 7], 617966	OMIM
NPHP3	HGNC:7907	3q22.1	Nephronophthisis 3, 604387, Autosomal recessive; Renal-hepatic-pancreatic dysplasia 1, 208540, Autosomal recessive; Meckel syndrome 7, 267010, Autosomal recessive	OMIM
NPHP4	HGNC:19104	1p36.31	Congenital heart disease	PMID: 22550138
NPPA	HGNC:7939	1p36.22	Atrial standstill 2, 615745, Autosomal recessive; Atrial fibrillation, familial, 6, 612201, Autosomal dominant	OMIM
NR2F2	HGNC:7976	15q26.2	46XX sex reversal 5, 618901, Autosomal dominant; Congenital heart defects, multiple types, 4, 615779, Autosomal dominant	OMIM
NRAS	HGNC:7989	1p13.2	Noonan syndrome 6, 613224, Autosomal dominant; RAS-associated autoimmune lymphoproliferative syndrome type IV, somatic, 614470; Melanocytic nevus syndrome, congenital, somatic, 137550; Epidermal nevus, somatic, 162900; Schimmelpenning-Feuerstein-Mims syndrome, somatic mosaic, 163200; Thyroid carcinoma, follicular, somatic, 188470; Neurocutaneous melanosis, somatic, 249400; Colorectal cancer, somatic, 114500	OMIM
NSD1	HGNC:14234	5q35.3	Sotos syndrome, 117550, Autosomal dominant	OMIM
NUP155	HGNC:8063	5p13.2	Atrial fibrillation 15, 615770, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
OFD1	HGNC:2567	Xp22.2	Simpson-Golabi-Behmel syndrome, type 2, 300209, X-linked recessive; Retinitis pigmentosa 23, 300424, X-linked recessive; Orofaciodigital syndrome I, 311200, X-linked dominant; Joubert syndrome 10, 300804, X-linked recessive	OMIM
PCCA	HGNC:8653	13q32.3	Propionicacidemia, 606054, Autosomal recessive	OMIM
PCCB	HGNC:8654	3q22.3	Propionicacidemia, 606054, Autosomal recessive	OMIM
PCDH15	HGNC:14674	10q21.1	Usher syndrome, type 1D/F digenic, 601067, Digenic recessive, Autosomal recessive; Deafness, autosomal recessive 23, 609533, Autosomal recessive; Usher syndrome, type 1F, 602083, Autosomal recessive	OMIM
PCSK9	HGNC:20001	1p32.3	{Low density lipoprotein cholesterol level QTL 1}, 603776, Autosomal dominant; Hypercholesterolemia, familial, 3, 603776, Autosomal dominant	OMIM
PDGFRA	HGNC:8803	4q12	Gastrointestinal stromal tumor/GIST-plus syndrome, somatic or familial, 175510; Hypereosinophilic syndrome, idiopathic, resistant to imatinib, 607685, Isolated cases, Somatic mutation	OMIM
PDHA1	HGNC:8806	Xp22.12	Hypertrophic Cardiomyopathy	PMID: 39920986
PDLIM3	HGNC:20767	4q35.1	Atrial Standstill 1	PMID: 11329061
PERP	HGNC:17637	6q23.3	Arrhythmogenic cardiomyopathy	DOI: 10.1016/j.rccar.2018.01.004
PHKA1	HGNC:8925	Xq13.1	Glycogen storage disease due to muscle phosphorylase kinase deficiency	ORPHA:715
PITX2	HGNC:9005	4q25	Ring dermoid of cornea, 180550, Autosomal dominant; Axenfeld-Rieger syndrome, type 1, 180500, Autosomal dominant; Anterior segment dysgenesis 4, 137600, Autosomal dominant	OMIM
PKD1	HGNC:9008	16p13.3	Polycystic kidney disease 1, 173900, Autosomal dominant	OMIM
PKD1L1	HGNC:18053	7p12.3	Heterotaxy, visceral, 8, autosomal, 617205, Autosomal recessive	OMIM
PKD2	HGNC:9009	4q22.1	Polycystic kidney disease 2, 613095, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
PKP2	HGNC:9024	12p11.21	Arrhythmogenic right ventricular dysplasia 9, 609040, Autosomal dominant	OMIM
PKP4	HGNC:9026	2q24.1	Arrhythmogenic Right Ventricular Cardiomyopathy	DOI: 10.1016/j.rccar.2018.01.004
PLEC	HGNC:9069	8q24.3	Epidermolysis bullosa simplex 5D, generalized intermediate, autosomal recessive, 616487, Autosomal recessive; Epidermolysis bullosa simplex 5B, with muscular dystrophy, 226670, Autosomal recessive; Epidermolysis bullosa simplex 5C, with pyloric atresia, 612138, Autosomal recessive; Epidermolysis bullosa simplex 5A, Ogna type, 131950, Autosomal dominant; Muscular dystrophy, limb-girdle, autosomal recessive 17, 613723, Autosomal recessive	OMIM
PLIN1	HGNC:9076	15q26.1	Lipodystrophy, familial partial, type 4, 613877, Autosomal dominant	OMIM
PLN	HGNC:9080	6q22.31	Cardiomyopathy, dilated, 1P, 609909; Cardiomyopathy, hypertrophic, 18, 613874, Autosomal dominant	OMIM
PLOD1	HGNC:9081	1p36.22	Ehlers-Danlos syndrome, kyphoscoliotic type, 1, 225400, Autosomal recessive	OMIM
PLTP	HGNC:9093	20q13.12	Glycogen storage disease due to muscle phosphorylase kinase deficiency	ORPHA:715
PMM2	HGNC:9115	16p13.2	Congenital disorder of glycosylation, type Ia, 212065, Autosomal recessive	OMIM
PNPLA2	HGNC:30802	11p15.5	Neutral lipid storage disease with myopathy, 610717, Autosomal recessive	OMIM
POMT1	HGNC:9202	9q34.13	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 1, 236670, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (limb-girdle), type C, 1, 609308, Autosomal recessive; Muscular dystrophy-dystroglycanopathy (congenital with impaired intellectual development), type B, 1, 613155, Autosomal recessive	OMIM
PPA2	HGNC:28883	4q24	Sudden cardiac failure, alcohol-induced, 617223, Autosomal recessive; Sudden cardiac failure, infantile, 617222, Autosomal recessive	OMIM
PPARA	HGNC:9232	22q13.31	Hyperapobetalipoproteinemia	PMID: 10828087
PPARG	HGNC:9236	3p25.2	{Diabetes, type 2}, 125853, Autosomal dominant; Insulin resistance, severe, digenic, 604367, Autosomal dominant; Lipodystrophy, familial partial, type 3,	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
			604367, Autosomal dominant; [Obesity, resistance to]; Obesity, severe, 601665, Autosomal dominant, Multifactorial, Autosomal recessive; Carotid intimal medial thickness 1, 609338	
PPP1CB	HGNC:9282	2p23.2	Noonan syndrome-like disorder with loose anagen hair 2, 617506, Autosomal dominant	OMIM
PPP1R13L	HGNC:18838	19q13.32	Arrhythmogenic cardiomyopathy with variable ectodermal abnormalities, 620519, Autosomal recessive	OMIM
PRDM16	HGNC:14000	1p36.32	Left ventricular noncompaction 8, 615373, Autosomal dominant; Cardiomyopathy, dilated, 1LL, 615373, Autosomal dominant	OMIM
PRKAG2	HGNC:9386	7q36.1	Glycogen storage disease of heart, lethal congenital, 261740, Autosomal dominant; Wolff-Parkinson-White syndrome, 194200, Autosomal dominant; Cardiomyopathy, hypertrophic 6, 600858, Autosomal dominant	OMIM
PRKG1	HGNC:9414	10q11.23-q21.1	Aortic aneurysm, familial thoracic 8, 615436, Autosomal dominant	OMIM
PSEN1	HGNC:9508	14q24.2	Pick disease, 172700, Autosomal dominant; Alzheimer disease, type 3, with spastic paraparesis and apraxia, 607822, Autosomal dominant; Dementia, frontotemporal, 600274, Autosomal dominant; Acne inversa, familial, 3, 613737, Autosomal dominant; Cardiomyopathy, dilated, 1U, 613694, Autosomal dominant; Alzheimer disease, type 3, with spastic paraparesis and unusual plaques, 607822, Autosomal dominant; Alzheimer disease, type 3, 607822, Autosomal dominant	OMIM
PSEN2	HGNC:9509	1q42.13	Alzheimer disease-4, 606889, Autosomal dominant; Cardiomyopathy, dilated, 1V, 613697, Autosomal dominant	OMIM
PTF1A	HGNC:23734	10p12.2	Pancreatic and cerebellar agenesis, 609069, Autosomal recessive; Pancreatic agenesis 2, 615935, Autosomal recessive	OMIM
PTPN11	HGNC:9644	12q24.13	Noonan syndrome 1, 163950, Autosomal dominant; LEOPARD syndrome 1, 151100, Autosomal dominant; Metachondromatosis, 156250, Autosomal dominant; Leukemia, juvenile myelomonocytic, somatic, 607785	OMIM
PYGM	HGNC:9726	11q13.1	McArdle disease, 232600, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
RAF1	HGNC:9829	3p25.2	Cardiomyopathy, dilated, 1NN, 615916, Autosomal dominant; Noonan syndrome 5, 611553, Autosomal dominant; LEOPARD syndrome 2, 611554, Autosomal dominant	OMIM
RANGRF	HGNC:17679	17p13.1	Brugada Syndrome	PMID: 24142675
RASA1	HGNC:9871	5q14.3	Capillary malformation-arteriovenous malformation 1, 608354, Autosomal dominant; Basal cell carcinoma, somatic, 605462	OMIM
RASA2	HGNC:9872	3q23	Noonan syndrome	PMID: 25049390
RBCK1	HGNC:15864	20p13	Polyglucosan body myopathy 1 with or without immunodeficiency, 615895, Autosomal recessive	OMIM
RBM20	HGNC:27424	10q25.2	Cardiomyopathy, dilated, 1DD, 613172, Autosomal dominant	OMIM
RET	HGNC:9967	10q11.21	{Hirschsprung disease, susceptibility to, 1}, 142623, Autosomal dominant; Multiple endocrine neoplasia IIA, 171400, Autosomal dominant; {Hirschsprung disease, protection against}, 142623, Autosomal dominant; Medullary thyroid carcinoma, 155240, Autosomal dominant; Pheochromocytoma, 171300, Autosomal dominant; Multiple endocrine neoplasia IIB, 162300, Autosomal dominant	OMIM
RIT1	HGNC:10023	1q22	Noonan syndrome 8, 615355, Autosomal dominant	OMIM
RMND1	HGNC:21176	6q25.1	Combined oxidative phosphorylation deficiency 11, 614922, Autosomal recessive	OMIM
RRAS	HGNC:10447	19q13.33	Noonan syndrome	PMID: 24705357
RYR2	HGNC:10484	1q43	Ventricular tachycardia, catecholaminergic polymorphic, 1, 604772, Autosomal dominant; Ventricular arrhythmias due to cardiac ryanodine receptor calcium release deficiency syndrome, 115000, Autosomal dominant	OMIM
SALL4	HGNC:15924	20q13.2	IVIC syndrome, 147750, Autosomal dominant; Duane-radial ray syndrome, 607323, Autosomal dominant	OMIM
SAR1B	HGNC:10535	5q31.1	Hypercholesterolemia, Familial, 1	PMID: 26546829
SCARB1	HGNC:1664	12q24.31	Hypercholesterolemia, Familial, 1; Hypoalphalipoproteinemia, Primary, 1	PMID: 33975440

Gene symbol	HGNC ID	Location	Associated disease/s	Source
SCN10A	HGNC:10582	3p22.2	Brugada Syndrome	PMIDs: 24998131; 28407228; 25691538
SCN1B	HGNC:10586	19q13.11	Generalized epilepsy with febrile seizures plus, type 1, 604233, Autosomal dominant; Developmental and epileptic encephalopathy 52, 617350, Autosomal recessive; Cardiac conduction defect, nonspecific, 612838; Atrial fibrillation, familial, 13, 615377, Autosomal dominant; Brugada syndrome 5, 612838	OMIM
SCN2B	HGNC:10589	11q23.3	Atrial fibrillation, familial, 14, 615378, Autosomal dominant	OMIM
SCN3B	HGNC:20665	11q24.1	Atrial fibrillation, familial, 16, 613120, Autosomal dominant; Brugada syndrome 7, 613120, Autosomal dominant	OMIM
SCN4B	HGNC:10592	11q23.3	Atrial fibrillation, familial, 17, 611819, Autosomal dominant; Long QT syndrome 10, 611819, Autosomal dominant	OMIM
SCN5A	HGNC:10593	3p22.2	Ventricular fibrillation, familial, 1, 603829; Heart block, progressive, type IA, 113900, Autosomal dominant; Cardiomyopathy, dilated, 1E, 601154, Autosomal dominant; Heart block, nonprogressive, 113900, Autosomal dominant; Long QT syndrome 3, 603830, Autosomal dominant; Sick sinus syndrome 1, 608567, Autosomal recessive; Brugada syndrome 1, 601144, Autosomal dominant; Atrial fibrillation, familial, 10, 614022, Autosomal dominant; {Sudden infant death syndrome, susceptibility to}, 272120, Autosomal recessive	OMIM
SCNN1B	HGNC:10600	16p12.2	Bronchiectasis with or without elevated sweat chloride 1, 211400, Autosomal dominant; Pseudohypoaldosteronism, type IB2, autosomal recessive, 620125, Autosomal recessive; Liddle syndrome 1, 177200, Autosomal dominant	OMIM
SCNN1G	HGNC:10602	16p12.2	Bronchiectasis with or without elevated sweat chloride 3, 613071, Autosomal dominant; Pseudohypoaldosteronism, type IB3, autosomal recessive, 620126, Autosomal recessive; Liddle syndrome 2, 618114, Autosomal dominant	OMIM
SCO2	HGNC:10604	22q13.33	Myopia 6, 608908, Autosomal dominant; Mitochondrial complex IV deficiency, nuclear type 2, 604377, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
SDHA	HGNC:10680	5p15.33	Cardiomyopathy, dilated, 1GG, 613642, Autosomal recessive; Mitochondrial complex II deficiency, nuclear type 1, 252011, Autosomal recessive; Neurodegeneration with ataxia and late-onset optic atrophy, 619259, Autosomal dominant; Pheochromocytoma/paraganglioma syndrome 5, 614165, Autosomal dominant	OMIM
SELENON	HGNC:15999	1p36.11	Congenital myopathy 3 with rigid spine, 602771, Autosomal recessive	OMIM
SGCA	HGNC:10805	17q21.33	Muscular dystrophy, limb-girdle, autosomal recessive 3, 608099, Autosomal recessive	OMIM
SGCB	HGNC:10806	4q12	Muscular dystrophy, limb-girdle, autosomal recessive 4, 604286, Autosomal recessive	OMIM
SGCD	HGNC:10807	5q33.2-q33.3	Cardiomyopathy, dilated, 1L, 606685; Muscular dystrophy, limb-girdle, autosomal recessive 6, 601287, Autosomal recessive	OMIM
SGCG	HGNC:10809	13q12.12	Muscular dystrophy, limb-girdle, autosomal recessive 5, 253700, Autosomal recessive	OMIM
SHOC2	HGNC:15454	10q25.2	Noonan syndrome-like with loose anagen hair 1, 607721, Autosomal dominant	OMIM
SKI	HGNC:10896	1p36.33-p36.32	Shprintzen-Goldberg syndrome, 182212, Autosomal dominant	OMIM
SLC22A5	HGNC:10969	5q31.1	Carnitine deficiency, systemic primary, 212140, Autosomal recessive	OMIM
SLC22A8	HGNC:10972	11q12.3	Hypercholesterolemia, Familial, 1	PMID: 37732779
SLC25A20	HGNC:1421	3p21.31	Carnitine-acylcarnitine translocase deficiency, 212138, Autosomal recessive	OMIM
SLC25A3	HGNC:10989	12q23.1	Mitochondrial phosphate carrier deficiency, 610773, Autosomal recessive	OMIM
SLC25A4	HGNC:10990	4q35.1	Mitochondrial DNA depletion syndrome 12B (cardiomyopathic type) AR, 615418, Autosomal recessive; Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant 2, 609283, Autosomal dominant; Mitochondrial DNA depletion syndrome 12A (cardiomyopathic type) AD, 617184, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
SLC25A40	HGNC:29680	7q21.12	Combined Oxidative Phosphorylation Deficiency 8	DOI: 10.1016/j.arteri.2020.12.013
SLC2A10	HGNC:13444	20q13.12	Arterial tortuosity syndrome, 208050, Autosomal recessive	OMIM
SLC39A13	HGNC:20859	11p11.2	Ehlers-Danlos syndrome, spondylodysplastic type, 3, 612350, Autosomal recessive	OMIM
SLC40A1	HGNC:10909	2q32.2	Hemochromatosis, type 4, 606069, Autosomal dominant	OMIM
SLC8A1	HGNC:11068	2p22.1	Cardiovascular System Disease	PMIDs: 20109173; 17435337
SLCO1B1	HGNC:10959	12p12.1	Hypercholesterolemia, Familial, 1; Alagille Syndrome	PMID: 31981411
SLMAP	HGNC:16643	3p14.3	Brugada Syndrome	PMID: 23064965
SMAD1	HGNC:6767	4q31.21	Pulmonary Arterial Hypertension; Congenital Heart Disease	PMIDs: 35281600; 21898662; 23478097
SMAD3	HGNC:6769	15q22.33	Loeys-Dietz syndrome 3, 613795, Autosomal dominant	OMIM
SMAD4	HGNC:6770	18q21.2	Pancreatic cancer, somatic, 260350; Myhre syndrome, 139210, Autosomal dominant; Polyposis, juvenile intestinal, 174900, Autosomal dominant; Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, 175050, Autosomal dominant	OMIM
SMAD6	HGNC:6772	15q22.31	Aortic valve disease 2, 614823, Autosomal dominant; {Radioulnar synostosis, nonsyndromic}, 179300, Autosomal dominant; {Craniosynostosis 7, susceptibility to}, 617439, Autosomal dominant	OMIM
SMAD9	HGNC:6774	13q13.3	Pulmonary hypertension, primary, 2, 615342, Autosomal dominant	OMIM
SNTA1	HGNC:11167	20q11.21	Long QT syndrome 12, 612955, Autosomal dominant	OMIM
SOS1	HGNC:11187	2p22.1	Noonan syndrome 4, 610733, Autosomal dominant; Fibromatosis, gingival, 1, 135300, Autosomal dominant	OMIM
SOS2	HGNC:11188	14q21.3	Noonan syndrome 9, 616559, Autosomal dominant	OMIM
SPEG	HGNC:16901	2q35	Centronuclear myopathy 5, 615959, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
SPRED1	HGNC:20249	15q14	Noonan Syndrome And Noonan-Related Syndrome	ClinVar: RCV001813180.3
SURF1	HGNC:11474	9q34.2	Charcot-Marie-Tooth disease, type 4K, 616684, Autosomal recessive; Mitochondrial complex IV deficiency, nuclear type 1, 220110, Autosomal recessive	OMIM
SYNE1	HGNC:17089	6q25.2	Arthrogryposis multiplex congenita 3, myogenic type, 618484, Autosomal recessive; Emery-Dreifuss muscular dystrophy 4, autosomal dominant, 612998, Autosomal dominant; Spinocerebellar ataxia, autosomal recessive 8, 610743, Autosomal recessive	OMIM
SYNE2	HGNC:17084	14q23.2	Emery-Dreifuss muscular dystrophy 5, autosomal dominant, 612999, Autosomal dominant	OMIM
SYNGAP1	HGNC:11497	6p21.32	Intellectual developmental disorder, autosomal dominant 5, 612621, Autosomal dominant	OMIM
TAB2	HGNC:17075	6q25.1	Congenital heart defects, nonsyndromic, 2, 614980, Autosomal dominant	OMIM
TAFAZZIN	HGNC:11577	Xq28	Barth syndrome, 302060, X-linked recessive	OMIM
TBC1D4	HGNC:19165	13q22.2	Hypercholesterolemia, Familial, 1	PMID: 36707786
TBX1	HGNC:11592	22q11.21	Tetralogy of Fallot, 187500, Autosomal dominant; DiGeorge syndrome, 188400, Autosomal dominant; Conotruncal anomaly face syndrome, 217095; Velocardiofacial syndrome, 192430, Autosomal dominant	OMIM
TBX20	HGNC:11598	7p14.2	Atrial septal defect 4, 611363	OMIM
TBX5	HGNC:11604	12q24.21	Holt-Oram syndrome, 142900, Autosomal dominant	OMIM
TCAP	HGNC:11610	17q12	Cardiomyopathy, hypertrophic, 25, 607487, Autosomal dominant; Muscular dystrophy, limb-girdle, autosomal recessive 7, 601954, Autosomal recessive	OMIM
TECRL	HGNC:27365	4q13.1	Ventricular tachycardia, catecholaminergic polymorphic, 3, 614021, Autosomal recessive	OMIM
TFAP2B	HGNC:11743	6p12.3	Patent ductus arteriosus 2, 617035, Autosomal dominant; Char syndrome, 169100, Autosomal dominant	OMIM
TFR2	HGNC:11762	7q22.1	Hemochromatosis, type 3, 604250, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
TGFB2	HGNC:11768	1q41	Loeys-Dietz syndrome 4, 614816, Autosomal dominant	OMIM
TGFB3	HGNC:11769	14q24.3	Arrhythmogenic right ventricular dysplasia 1, 107970, Autosomal dominant; Loeys-Dietz syndrome 5, 615582, Autosomal dominant	OMIM
TGFBR1	HGNC:11772	9q22.33	{Multiple self-healing squamous epithelioma, susceptibility to}, 132800, Autosomal dominant; Loeys-Dietz syndrome 1, 609192, Autosomal dominant	OMIM
TGFBR2	HGNC:11773	3p24.1	Loeys-Dietz syndrome 2, 610168, Autosomal dominant; Colorectal cancer, hereditary nonpolyposis, type 6, 614331; Esophageal cancer, somatic, 133239	OMIM
TGFBR3	HGNC:11774	1p22.1	Aortic Root Dilation	ORPHA:231160
TMEM43	HGNC:28472	3p25.1	Arrhythmogenic right ventricular dysplasia 5, 604400, Autosomal dominant; Auditory neuropathy, autosomal dominant 3, 619832, Autosomal dominant; Emery-Dreifuss muscular dystrophy 7, AD, 614302, Autosomal dominant	OMIM
TMEM70	HGNC:26050	8q21.11	Mitochondrial complex V (ATP synthase) deficiency, nuclear type 2, 614052, Autosomal recessive	OMIM
TMPO	HGNC:11875	12q23.1	Familial Isolated Dilated Cardiomyopathy	PMID: 16247757
TNNI3	HGNC:11947	19q13.42	Cardiomyopathy, dilated, 2A, 611880, Autosomal recessive; Cardiomyopathy, hypertrophic, 7, 613690, Autosomal dominant; Cardiomyopathy, familial restrictive, 1, 115210, Autosomal dominant; Cardiomyopathy, dilated, 1FF, 613286	OMIM
TNNI3K	HGNC:19661	1p31.1	Cardiac conduction disease with or without dilated cardiomyopathy, 616117, Autosomal dominant	OMIM
TNNT2	HGNC:11949	1q32.1	Cardiomyopathy, dilated, 1D, 601494, Autosomal dominant; Cardiomyopathy, hypertrophic, 2, 115195, Autosomal dominant; Cardiomyopathy, familial restrictive, 3, 612422, Autosomal dominant; Left ventricular noncompaction 6, 601494, Autosomal dominant	OMIM
TOPBP1	HGNC:17008	3q22.1	Pulmonary Arterial Hypertension	PMID: 24702692
TOR1AIP1	HGNC:29456	1q25.2	Muscular dystrophy, autosomal recessive, with rigid spine and distal joint contractures, 617072, Autosomal recessive	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
TP63	HGNC:15979	3q28	Premature ovarian failure 21, 620311, Autosomal dominant; Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3, 604292, Autosomal dominant; Hay-Wells syndrome, 106260, Autosomal dominant; Split-hand/foot malformation 4, 605289, Autosomal dominant; Orofacial cleft 8, 618149; Rapp-Hodgkin syndrome, 129400, Autosomal dominant; ADULT syndrome, 103285, Autosomal dominant; Limb-mammary syndrome, 603543, Autosomal dominant	OMIM
TPM1	HGNC:12010	15q22.2	Left ventricular noncompaction 9, 611878, Autosomal dominant; Cardiomyopathy, hypertrophic, 3, 115196, Autosomal dominant; Cardiomyopathy, dilated, 1Y, 611878, Autosomal dominant	OMIM
TRDN	HGNC:12261	6q22.31	Cardiac arrhythmia syndrome, with or without skeletal muscle weakness, 615441, Autosomal recessive	OMIM
TRIB1	HGNC:16891	8q24.13	Hypercholesterolemia, Familial	PMIDs: 20692138; 37078290
TRIM32	HGNC:16380	9q33.1	Bardet-Biedl syndrome 11, 615988, Autosomal recessive; Muscular dystrophy, limb-girdle, autosomal recessive 8, 254110, Autosomal recessive	OMIM
TRPM4	HGNC:17993	19q13.33	Progressive familial heart block, type IB, 604559, Autosomal dominant; Erythrokeratodermia variabilis et progressiva 6, 618531, Autosomal dominant	OMIM
TSFM	HGNC:12367	12q14.1	Combined oxidative phosphorylation deficiency 3, 610505, Autosomal recessive	OMIM
TTN	HGNC:12403	2q31.2	Muscular dystrophy, limb-girdle, autosomal recessive 10, 608807, Autosomal recessive; Cardiomyopathy, familial hypertrophic, 9, 613765, Autosomal dominant; Congenital myopathy 5 with cardiomyopathy, 611705, Autosomal recessive; Tibial muscular dystrophy, tardive, 600334, Autosomal dominant; Cardiomyopathy, dilated, 1G, 604145, Autosomal dominant; Myopathy, myofibrillar, 9, with early respiratory failure, 603689, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
TTR	HGNC:12405	18q12.1	Amyloidosis, hereditary, transthyretin-related, 105210, Autosomal dominant; Carpal tunnel syndrome, familial, 115430, Autosomal dominant; [Dystransthyretinemic hyperthyroxinemia], 145680, Autosomal dominant	OMIM
TXNRD2	HGNC:18155	22q11.21	Familial Isolated Dilated Cardiomyopathy	PMID: 21247928
UPF3B	HGNC:20439	Xq24	Intellectual developmental disorder, X-linked syndromic 14, 300676, X-linked recessive	OMIM
VCL	HGNC:12665	10q22.2	Cardiomyopathy, dilated, 1W, 611407; Cardiomyopathy, hypertrophic, 15, 613255, Autosomal dominant	OMIM
WFS1	HGNC:12762	4p16.1	Deafness, autosomal dominant 6/14/38, 600965, Autosomal dominant; Cataract 41, 116400, Autosomal dominant; Wolfram-like syndrome, autosomal dominant, 614296, Autosomal dominant; {Diabetes mellitus, noninsulin-dependent, association with}, 125853, Autosomal dominant; Wolfram syndrome 1, 222300, Autosomal recessive	OMIM
WT1	HGNC:12796	11p13	Mesothelioma, somatic, 156240; Meacham syndrome, 608978, Autosomal dominant; Frasier syndrome, 136680, Somatic mutation, Autosomal dominant; Nephrotic syndrome, type 4, 256370, Autosomal dominant; Denys-Drash syndrome, 194080, Somatic mutation, Autosomal dominant; Wilms tumor, type 1, 194070, Somatic mutation, Autosomal dominant	OMIM
XK	HGNC:12811	Xp21.1	McLeod syndrome with or without chronic granulomatous disease, 300842, X-linked	OMIM
ZDHHC9	HGNC:18475	Xq26.1	Congenital Heart Disease	DOI: 10.1016/j.rccar.2018.01.004; PMID: 37325411
ZFHX3	HGNC:777	16q22.2-q22.3	Prostate cancer, somatic, 176807; {Atrial fibrillation 8, susceptibility to}, 613055, Autosomal dominant; Spinocerebellar ataxia 4, 600223, Autosomal dominant	OMIM
ZFPM2	HGNC:16700	8q23	Diaphragmatic hernia 3, 610187; 46XY sex reversal 9, 616067, Autosomal dominant; Tetralogy of Fallot, 187500, Autosomal dominant	OMIM

Gene symbol	HGNC ID	Location	Associated disease/s	Source
ZIC3	HGNC:12874	Xq26.3	Congenital heart defects, nonsyndromic, 1, X-linked, 306955, X-linked recessive; Heterotaxy, visceral, 1, X-linked, 306955, X-linked recessive; VACTERL association, X-linked, 314390, X-linked recessive	OMIM
ZMPSTE24	HGNC:12877	1p34.2	Mandibuloacral dysplasia with type B lipodystrophy, 608612, Autosomal recessive; Restrictive dermopathy 1, 275210, Autosomal recessive	OMIM

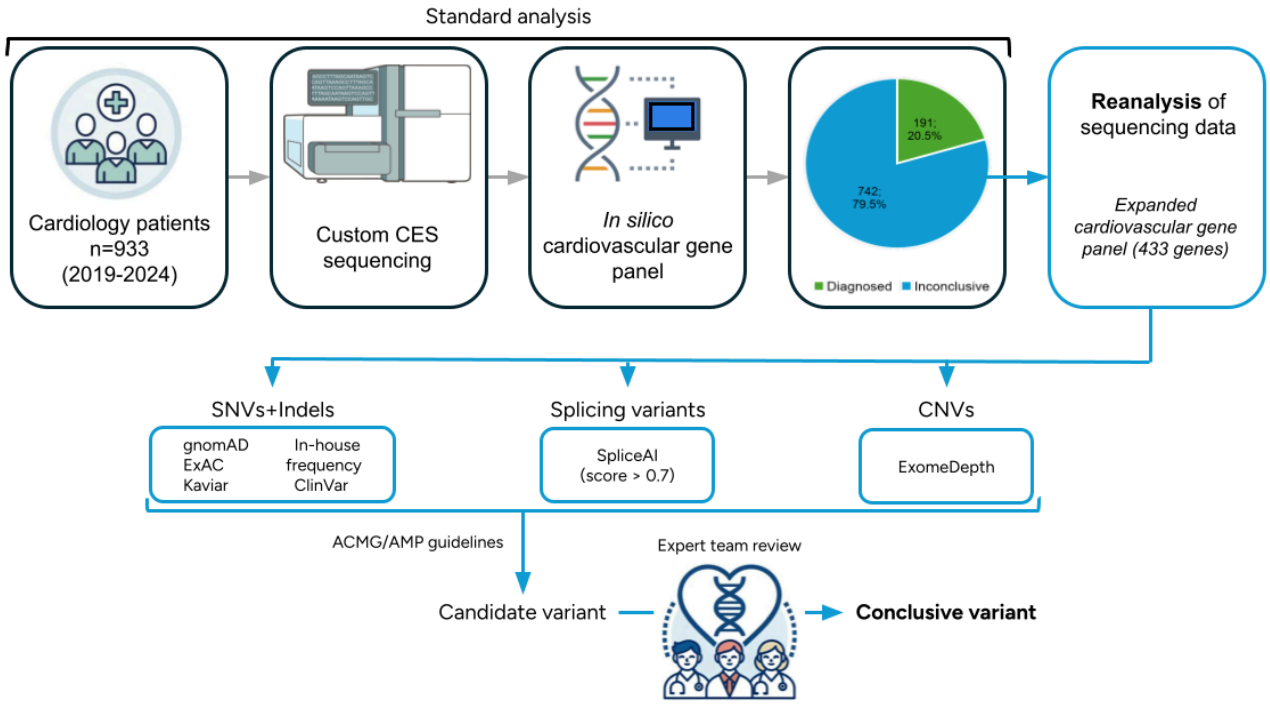

Figure 2.1. Workflow of the reanalysis of the Inherited Cardiac Conditions (ICCs) cohort. Abbreviations: CES = clinical exome sequencing; SNVs = single nucleotide variants; Indels = insertion-deletions; CNVs = copy number variants; ACMG/AMP = American College of Medical Genetics and Genomics and Association for Molecular Pathology.

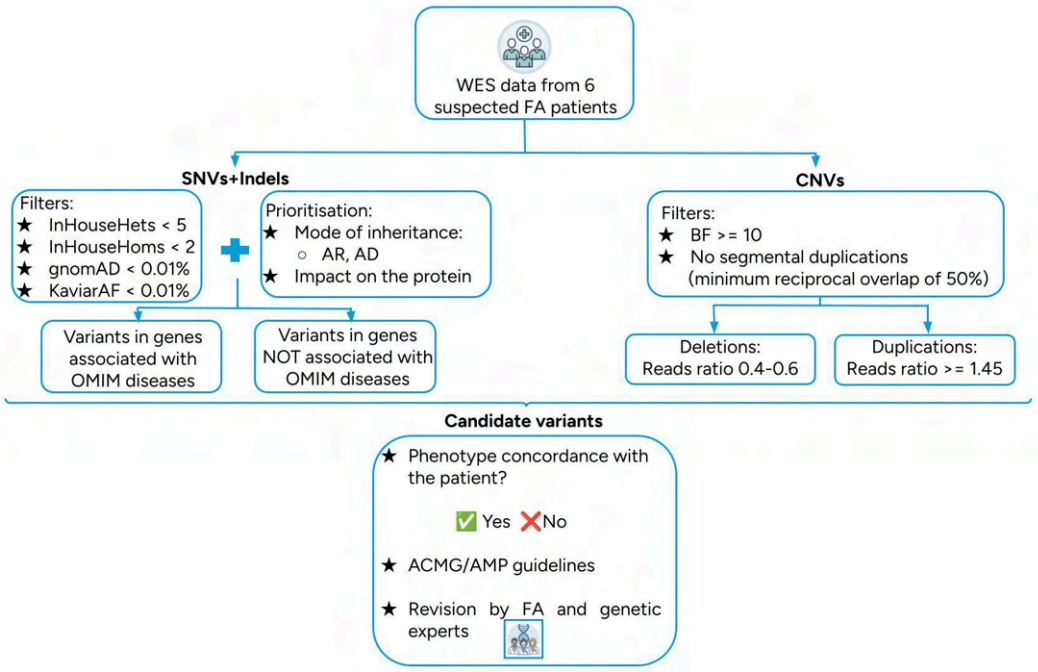

Figure 2.2. Workflow of the reanalysis of the Suspected Fanconi anaemia (SFA) cohort. Abbreviations: WES = whole exome sequencing; FA = Fanconi anaemia; SNVs = single nucleotide variants; indels = insertion-deletions; CNVs = copy number variants; InHouseHets = inhouse heterozygous individuals; InHouseHoms = inhouse homozygous individuals; AR = autosomal recessive; AD = autosomal dominant; BF = Bayes factor; ACMG/AMP = American College of Medical Genetics and Genomics and Association for Molecular Pathology.

2.3.1. Variant filtering and prioritisation

After the bioinformatics pipeline provided a variant dataset with updated information, variant prioritisation using a comprehensive approach, integrating public and in-house resources, as well as gene, variant, and patient-specific information was carried out to identify potential disease-causing variants. Specifically, this phase of variant filtering and prioritisation encompassed the assessment of the following evidence:

- **Allele frequency:** Variant frequency was evaluated using population data from public databases, including gnomAD (Karczewski et al., 2020), ExAC (Karczewski et al., 2017), and Kaviar (Glusman et al., 2011), and the inhouse database of individuals from the same geographical area of the Genetics Department of HSCSP (~3000 individuals: 75% CES data, 25% WES).
- **Variant type:** The functional consequence of each variant type (missense, in-frame, splicing, frameshift, nonsense, CNV).
- **Disease mode of inheritance of the suspected disease:** AR, AD, XLR and XLD.
- ***In silico* prediction:** Rare Exome Variant Ensemble Learner (REVEL) program (Ioannidis et al., 2016) was utilised to predict the potential impact of variants on protein function.

- **Clinical information of variant databases:** Updated information from ClinVar (Landrum et al., 2018) and Human Gene Mutation Database (HGMD) (Stenson et al., 2003) including literature review was considered.
- **Clinical data integration:** Patient-specific clinical and genetic data were considered together to assess the concordance between genotype and phenotype.
- **Variants of uncertain significance** in the initial genetic report of ICCs patients were again reassessed in view of the new evidence and corresponding clinical context of the patient.
- **Variants with a minor allele frequency (MAF) greater than 5% in population variant databases were excluded** from the reanalysis of the ICCs cohort, in line with a conservative filtering approach. This threshold is justified by the estimated prevalence of ICCs, which is approximately 1 in 200 individuals—corresponding to a carrier frequency of around 0.1%—as reported by Ormondroyd et al. (2020). It is important to note that this prevalence estimate reflects the aggregated frequency of P variants across multiple genes and phenotypes, meaning that the expected frequency of any single causative variant is likely to be substantially lower.

Deep intronic variants analysis

SpliceAI software was employed to assess deep intronic variants and canonical splicing signals with the aim of identifying potential splicing events. The obtained VCF files were processed by the program to generate a predictive splicing effect score, ranging from 0 to 1, for each intronic variant. Given that a score of 0.5 indicates equal expression of wild-type and aberrant transcripts, and it is the software developers recommended cutoff while 0.8 is the high-precision threshold, variants with scores of 0.7 or greater were selected for further evaluation. Additionally, exonic variants classified as benign or likely benign were discarded. A quality filter was applied, removing variants with an allele balance (AB) below 0.2.

Single nucleotide variants and short insertion-deletions analysis

In the case of the ICCs cohort, variants with AB below 0.2 were discarded, and between 0.2 and 0.29 were manually inspected using BAM files in the IGV software. Subsequently, remaining variants were selected according to <0.01% allele frequency in gnomAD, ExAC and Kaviar and the presence of fewer than 10 heterozygous or 2 homozygous individuals in our in-house database to retain rare events. Two main groups of variants were considered: 1) Bin 1, encompassing variants classified as P/LP in ClinVar, together with those with a predicted LOF consequence; and 2) Bin 2, containing rare non-synonymous and non-frameshift variants classified as VUS, with conflicting interpretations of

pathogenicity, or absent from ClinVar. Variants of the first group were analysed and in case no candidate variants were found, the second group was examined considering available literature to elucidate if a gene could explain the clinical phenotype of the patient. To further assess bin 2 variants, REVEL, which includes multiple bioinformatic tools and methods: SIFT (Ng & Henikoff, Steven, 2003), PolyPhen-2 (Adzhubei et al., 2010), LRT (Likelihood Ratio Test) (Chun & Fay, 2009), MutationTaster (Schwarz et al., 2010), MutationAssessor (Reva et al., 2011), FATHMM (Rogers et al., 2018), PROVEAN (Y. Choi et al., 2012), MetaSVM (S. Kim et al., 2017), MetaLR (Dong et al., 2015) and M-CAP (Jagadeesh et al., 2016), was used to predict *in silico* their pathogenicity.

For the reanalysis of the SFA cohort (**Figure 2.2**), rare variants not previously described as P/LP were selected using stringent frequency thresholds: a MAF below 0.01% in public databases (gnomAD, ExAC, and Kaviar), or observed in fewer than two homozygous or five heterozygous individuals in our in-house variant database. The analysis was initially restricted to genes with an established disease association in OMIM. In cases where no P variants were identified, the remaining sequenced genes not listed in OMIM were also examined to explore the possibility of novel gene candidates.

Variant prioritisation was based on multiple criteria: predicted protein impact (with emphasis on the most deleterious variants), consistency with the expected mode of inheritance (prioritising recessive models), and the presence of multiple variants within the same gene. In the case of the sibling pair (patients 5 and 6), who exhibited overlapping phenotypes, the analysis specifically focused on variants shared between both individuals.

Copy number variants analysis

CNV analysis was conducted using the ExomeDepth R package (Plagnol et al., 2012), specifically designed to mitigate biases inherent to sequence capture and high-throughput sequencing. This tool employs a Bayesian framework and provides a Bayes Factor (BF) for each CNV call, defined as the base-10 logarithm of the likelihood ratio comparing the CNV model against the null hypothesis of normal copy number. A higher BF indicates stronger statistical support for the presence of a CNV. ExomeDepth also compares read counts in the test sample to an optimised aggregate reference set to compute a read ratio (observed vs. expected coverage). A ratio close to 1 suggests a normal copy number, whereas values near 0.5 or 1.5 are consistent with heterozygous deletions or duplications, respectively.

Called variants that overlapped with segmental duplications (with a minimum reciprocal overlap of 50%) were excluded from the reanalysis. Their high sequence identity (Abdullaev et al., 2021) combined with short read sequencing strategy used hinders the differentiation of potential alterations from a copy of one of these regions. Variants with a BF below 10 were not considered. Only deletions with a reads ratio between 0.4 and 0.6 and duplications with a reads ratio equal or higher than 1.45 were retained.

Reciprocal overlapping intervals between CNVs of the six SFA patients were analysed using the intersect feature of BEDTools (Quinlan & Hall, 2010), considering a minimum overlap of 30%. As two of these patients were siblings, the CNV analysis for this pair was focused on shared variants between them. Hence, the two siblings were analysed separately with minimum overlaps of 80% and 50% to further refine variant calls.

2.3.2. Phenotype and variant classification

Final candidate variants identified after prioritisation were subsequently discussed with cardiologists or FA specialists to assess their clinical relevance in the context of each patient's current phenotype.

In the reanalysis of the ICCs cohort, variants of interest selected for expert review were categorised into three main groups:

1) Clinically relevant variants, defined as those associated with the patient's condition. This group was further subdivided into:
   a) Reclassified variants, previously identified in the initial clinical analysis but now reassessed under updated criteria, and
   b) Novel variants, detected in genes that had not been included in the original diagnostic workflow.
2) Variants initially considered of interest, but subsequently excluded following multidisciplinary review.
3) Variants classified as P/LP according to ClinVar or the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines, but deemed unrelated to the patient's phenotype and therefore not clinically actionable in the present context.

This collaborative review process aimed to clarify the clinical significance of each variant in relation to the patient's presentation and to compile a final, curated variant list (**Figures 2.1 and 2.2**). Final variant classification followed the standardised criteria established by ACMG/AMP guidelines (Richards et al., 2015).

In cases where discrepancies arose, for instance variants classified as VUS by ACMG/AMP criteria but labelled as P/LP in ClinVar or the other way around, classified as P/LP but as VUS in ClinVar or other resource, further evaluation was conducted. All ACMG/AMP evidence codes were critically reassessed in the context of the individual patient's phenotype and the specific molecular and clinical data available.

3. Aims

This study aims to investigate the diagnostic utility of systematically reanalysing existing NGS data in patients with previously inconclusive genetic findings. Specifically, our objectives are:

1) Global Objective: To quantify the increase in molecular diagnostic yield achieved through reanalysis and identify the primary factors contributing to successful rediagnoses, ultimately underscoring the value of iterative genomic data interpretation in closing the diagnostic gap.
2) Cohort-Specific Objectives:
    a) For ICCs, to evaluate how reanalysis, through the reclassification of VUS and the inclusion of additional gene-disease associations, improves diagnostic outcomes and clinical utility.
    b) For SFA, to determine the diagnostic yield of reanalysis in cases where initial targeted genetic testing was inconclusive, exploring the role of broader gene analysis in identifying underlying genetic causes

4. Results

4.1. Inherited Cardiac Conditions cohort

4.1.1. Standard analysis results

The standard analysis was conducted prior to the reanalysis performed in this thesis work. The distribution of genetic testing results across the ICCs cohort is summarised in **Figure 4.1**. Genetic testing outcomes were classified as **inconclusive**, **conclusive** (P/LP variant), or **VUS**. The total number of patients for each of the nine phenotypes and the corresponding distribution across the genetic result categories are detailed, with the fraction of patients within each phenotype category provided in parentheses. Overall, the most frequent genetic finding was a VUS (n=522, 56.01% of the 933 patients), followed by inconclusive (n=220, 23.60%) and conclusive variant (n=191, 20.39%). The proportion of conclusive findings exhibited variability across the analysed phenotypes, ranging from 7% in SADS (n=1/15) to 44% in NDLVC (n=21/48). Similarly, the fraction of inconclusive results varied from 7% in SADS (n=1/15) to 50% in CDDs (n=1/2). Excluding the single RCM case due to its limited representation, VUS were the most prevalent finding in the majority of phenotypes, with notable frequencies observed in DCM (66%), HCM (57%), and ARVC (71%). These data illustrate the heterogeneous diagnostic yield of genetic testing across different cardiac phenotypes and highlight the substantial proportion of VUS identified, underscoring the continued need for research to determine their clinical relevance. Therefore, 742 patients (inconclusive and VUS cases) were subjected to reanalysis.

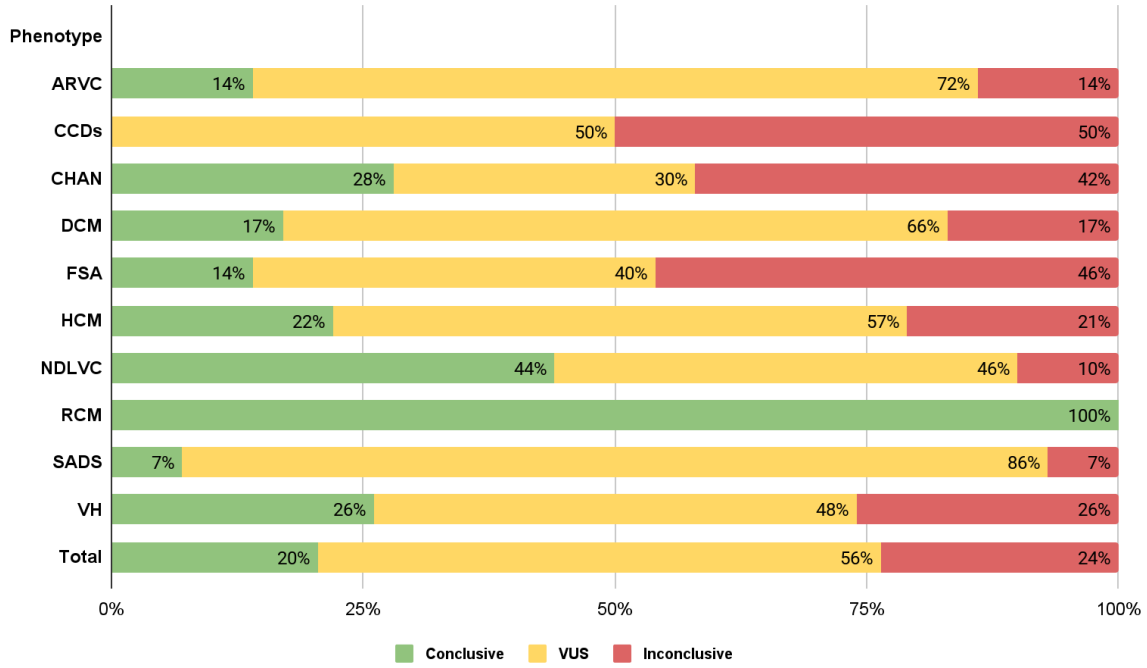

Figure 4.1. Distribution of the Inherited Cardiac Conditions (ICCs) cohort of patients among phenotypes and results of standard testing. The plot summarises the total number of patients per variant category and phenotype. Abbreviations: ARVC = arrhythmogenic right ventricular cardiomyopathy; CDDs = cardiac conduction diseases; CHAN = channelopathies; DCM = dilated cardiomyopathy; FSA = familial syndromic aortopathies; HCM = hypertrophic cardiomyopathy; NDLVC = non-dilated left ventricular cardiomyopathy; RCM = restrictive cardiomyopathy; SADS = sudden arrhythmic death syndrome; VH = ventricular hypertrabeculation; VUS = variant of uncertain significance.

4.1.2. Reanalysis results

In total, 25 heterozygous variants of interest (**Table 4.1**) were identified in the 742 reanalysed cases (3.4%, 25/742). The majority of identified candidate variants were found in genes already analysed at the initial analysis but not contemplated as clinically relevant, and a small subset were variants detected in genes not examined in the genetic analysis at the time when the patient was initially remitted for study.

Table 4.1. Relevant variants identified in the Inherited Cardiac Conditions (ICCs) cohort after reanalysing exome sequencing data.

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
1	M	*TBX1*	NM_001379200.1	c.1149_1150insA p.Gly384Argfs*242	Frameshift	LP	PVS1, PM2	Tetralogy of Fallot, # 187500, AD; DiGeorge syndrome, # 188400, AD; Conotruncal anomaly face syndrome, # 217095; Velocardiofacial syndrome, # 192430, AD	Cardiomyopathy	No	No
2	M	*CACNA1C*	NM_000719.7	c.2927T>C p.Val976Ala	Missense	LP	PP3, PM2, PP2	Timothy syndrome, # 601005, AD; Long QT syndrome 8, # 618447, AD; Neurodevelopmental disorder with hypotonia, language delay, and skeletal defects with or without seizures, # 620029, AD; Brugada syndrome 3, # 611875, AD	Aortic disease	No	No
3	M	*LRP6*	NM_002336.3	c.3277C>T p.Arg1093Trp	Missense	LP	PP3, PM2	{Coronary artery disease, AD, 2}, # 610947, AD; Tooth agenesis, selective, 7, # 616724, AD	HCM	No	No

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
4	M	*SCN5A*	NM_000335.5	c.1100G>A p.Arg367His	Missense	P	PS4, PP3, PM2, PM5, PM1, PS3, PP1, PP5	Ventricular fibrillation, familial, 1, # 603829; Heart block, progressive, type IA, # 113900, AD; Cardiomyopathy, dilated, 1E, # 601154, AD; Heart block, nonprogressive, # 113900, AD; Long QT syndrome 3 #, 603830, AD; Sick sinus syndrome 1, # 608567, AR; Brugada syndrome 1, # 601144, AD; Atrial fibrillation, familial, 10, # 614022, AD; {Sudden infant death syndrome, susceptibility to} , # 272120, AR	Aortic pathology + Brugada type 2 pattern in 2019	No	Yes
5	M	*GDF1*	NM_001492.6	c.952G>A p.Ala318Thr	Missense	LP	PP3, PM2, PS4, PP5	Congenital heart defects, multiple types, 6, # 613854, AD; Right atrial isomerism (Ivemark), # 208530, AR	BrS	No	Yes

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
6	M	*KCNQ1*	NM_000218.3	c.775_776insT p.Arg259Leufs*26	Frameshift	LP	PVS1, PM2	Short QT syndrome 2, # 609621, AD; Atrial fibrillation, familial, 3, # 607554, AD; Long QT syndrome 1, # 192500, AD; {Long QT syndrome 1, acquired, susceptibility to}, # 192500, AD; Jervell and Lange-Nielsen syndrome, # 220400, AR	Aortic pathology	No	No
7	F	*TTN*	NM_001267550.2	c.104413C>T p.Arg34805*	Nonsense	P	PVS1, PM2, PS4, PP5	Muscular dystrophy, limb-girdle, AR 10, # 608807, AR; Cardiomyopathy, familial hypertrophic, 9, # 613765, AD; Congenital myopathy 5 with cardiomyopathy, # 611705, AR; Tibial muscular dystrophy, tardive, # 600334, AD; Cardiomyopathy, dilated, 1G, # 604145, AD; Myopathy, myofibrillar, 9, with early respiratory failure, # 603689, AD	LQTS	No	Yes
8	M	*KCNH2*	NM_000238.4	c.2452T>G p.Ser818Ala	Missense	LP	PM2, PM5, PM1,	Short QT syndrome 1, # 609620; Long QT syndrome 2, #	HCM (no obstructive)	No	Yes

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
							PP3, PP2	613688, AD			
9	M	*MEF2A*	NM_001319206.2	c.830C>T p.Pro277Leu	Missense	VUS	PM2, PP5	{Coronary artery disease, AD, 1}, # 608320, AD	HCM (obstructive)	No	Yes
10	F	*PLN*	NM_002667.5	c.73C>T p.Arg25Cys	Missense	LP	PP1. PM2, PM1, PS3, PP5	Cardiomyopathy, dilated, 1P, # 609909; Cardiomyopathy, hypertrophic, 18, # 613874, AD	Aortic dissection	No	Yes
11	M	*GDF1*	NM_001492.6	c.371G>T p.Cys124Phe	Missense	LP	PS4, PM2, PP5	Congenital heart defects, multiple types, 6, # 613854, AD; Right atrial isomerism (Ivemark), # 208530, AR	DCM	No	Yes
12	M	*RBM20*	NM_001134363.3	c.1910G>T p.Ser637Ile	Missense	LP	PM2, PM5, PM1	Cardiomyopathy, dilated, 1DD, # 613172, AD	DCM	Yes	No
13	M	*ACTC1*	NM_005159.5	c.28C>A p.Leu10Met	Missense	LP	PP3, PM2, PS4, PP2, PP5	Left ventricular noncompaction 4, # 613424, AD; Cardiomyopathy, hypertrophic, 11, # 612098, AD; Atrial septal defect 5, # 612794, AD; Cardiomyopathy, dilated, 1R, # 613424, AD	HCM	Yes	Yes

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
14	F	*NEXN*	NM_144573.4	c.201G>A p.Trp67*	Nonsense	P	PVS1, PM2, PP5	Cardiomyopathy, dilated, 1CC, # 613122, AD; Cardiomyopathy, hypertrophic, 20, # 613876, AD	Coronary aneurysm	No	Yes
15	F	*MYH7*	NM_000257.4	c.2312G>C p.Gly771Ala	Missense	LP	PM2, PM1, PP3, PP2	Laing distal myopathy, # 160500, AD; Cardiomyopathy, hypertrophic, 1, # 192600, AD, Digenic dominant; Left ventricular noncompaction 5, # 613426, AD; Cardiomyopathy, dilated, 1S, # 613426, AD; Congenital myopathy 7B, myosin storage, AR, # 255160, AR; Congenital myopathy 7A, myosin storage, AD, # 608358, AD	HCM	Yes	No
16	M	*ACTC1*	NM_005159.5	c.644A>G p.Lys215Arg	Missense	LP	PP3, PM2, PP2	Left ventricular noncompaction 4, # 613424, AD; Cardiomyopathy, hypertrophic, 11, # 612098, AD; Atrial septal defect 5, # 612794, AD;	DCM	Yes	No

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
								Cardiomyopathy, dilated, 1R, # 613424, AD			
17	M	*KCNQ1*	NM_000218.3	c.898G>A p.Ala300Thr	Missense	P	PS4, PM2, PM5, PM1, PP3, PP2, PS3, PP5	Short QT syndrome 2, # 609621, AD; Atrial fibrillation, familial, 3, # 607554, AD; Long QT syndrome 1, # 192500, AD; {Long QT syndrome 1, acquired, susceptibility to}, # 192500, AD; Jervell and Lange-Nielsen syndrome, # 220400, AR	DCM	No	Yes
18	M	*ACTC1*	NM_005159.5	c.496C>T p.Pro166Ser	Missense	LP	PP3, PM2, PM5, PP2	Left ventricular noncompaction 4, 613424 , AD; Cardiomyopathy, hypertrophic, 11, 612098 , AD; Atrial septal defect 5, 612794 , AD; Cardiomyopathy, dilated, 1R, 613424 , AD	Left ventricular non-compaction cardiomyopathy	Yes	Yes
19	M	*SCN10A*	NM_006514.4	c.2408T>G p.Leu803Arg	Missense	LP	PP3, PM2	Non OMIM Brugada syndrome (PMIDs: 24998131; 28407228; 25691538)	DCM	No	Yes

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
20	M	*SMAD9*	NM_001127217.3	c.781+2T>A	Splicing	LP	PVS1, PM2, PP5	Pulmonary hypertension, primary, 2, 615342 , AD	Arrhythmia	No	Yes
21	M	*PLIN1*	NM_002666.5	c.770delT p.Leu257Argfs*66	Frameshift	LP	PVS1, PM2	Lipodystrophy, familial partial, type 4, 613877 , AD	DCM	No	No
22	M	*ACVR1*	NM_001111067.4	c.616C>T p.Arg206Cys	Missense	LP	PM2, PM5, PP3	Non OMIM Calcific Aortic Valve Disease PMID: 36005436	DCM	No	No
23	M	*TTN*	NM_001267550 (exon 335 A-band)	c.89812_89813insTGCC p.Gln29938Leufs*7	Frameshift	LP	PVS1, PM2	Muscular dystrophy, limb-girdle, AR 10, 608807 , AR; Cardiomyopathy, familial hypertrophic, 9, 613765 , AD; Congenital myopathy 5 with cardiomyopathy, 611705 , AR; Tibial muscular dystrophy, tardive, 600334 , AD; Cardiomyopathy, dilated, 1G, 604145 , AD; Myopathy, myofibrillar, 9, with early respiratory failure, 603689 , AD	HCM	Yes	No

Pt	S	Gene	Transcript	Variant	Type	C. (ACMG)	ACMG evs.	OMIM phenotype	Patient phenotype	P.C	ClinVar
24	M	*ACTA2*	NM_001613.4	c.289C>T p.Arg97Cys	Missense	LP	PP3, PM2, PP2	Smooth muscle dysfunction syndrome, 613834 , AD; Aortic aneurysm, familial thoracic 6, 611788 , AD; Moyamoya disease 5, 614042	Myocarditis	No	No
25	M	*MIB1*	NM_020774	C.2596_2599del p.Cys866Trpfs*27	Frameshift	LP	PVS1, PM2	Left ventricular noncompaction 7, 615092 , AD	Aortic disease	No	Yes

Abbreviations: Pt = Patient; S = Sex; C. = Classification; evs. = evidences; P.C = Phenotype Concordance; M = male; F = female; P = pathogenic; LP = likely pathogenic; VUS = variant of uncertain significance; AD = autosomal dominant..

Overall, 6 out of the 25 variants (0.8%, 6/742) were confirmed as concordant with their respective patient's clinical phenotype after being reviewed by the cardiologists team (**Figure 4.2**). These 6 clinically relevant variants consisted in 5 heterozygous missense types identified in genes already studied but classified as VUS at the time of the first report, and one heterozygous variant detected in a gene not analysed previously (*TTN*) in a specific patient.

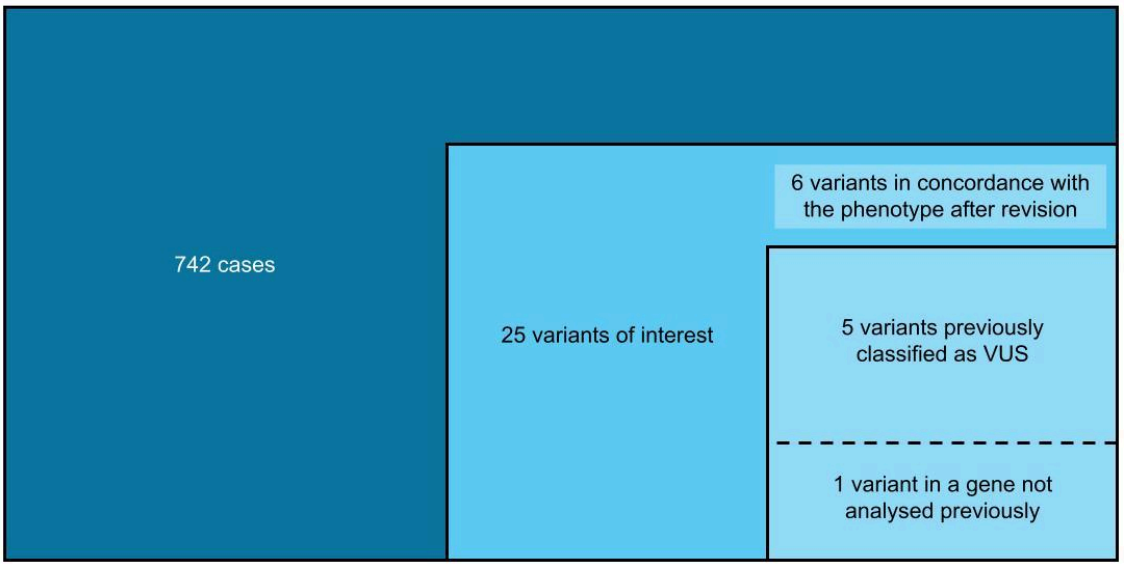

Figure 4.2. Summary of reanalysis outcomes across the cohort. Of the 742 cases reanalysed, 25 variants of interest were identified. Among these, 6 variants were found to be in concordance with the patients' phenotypes after clinical revision. Abbreviations: VUS = variant of uncertain significance.

Four variants of interest that particularly drew attention (genes *SCN5A, NEXN, ACTA2,* and *MIB1*) were reviewed in the context of the patient' phenotype but they were finally classified as VUS due to the lack of correlation between current gene associated disease knowledge with the patient's symptoms.

Reclassified variants

As mentioned before, 5 of the 8 variants related to their respective patient's clinical phenotype were upgraded from VUS to LP (**Figures 4.2 and 4.3**), thus they have been reclassified since the time of the first genetic test.

The upgraded variants were: 1) c.1910G>T in *RBM20* gene (NM_001134363.3: p.Ser637Ile) in patient 12; 2) c.28C>A in *ACTC1* (NM_005159.5: p.Leu10Met) in patient 13; 3) c.2312G>C in *MYH7* (NM_000257.4: p.Gly771Ala) in patient 15; 4) c.644A>G in *ACTC1* (NM_005159.5: p.Lys215Arg) in patient 16; 5) c.496C>T in *ACTC1* gene (NM_005159.5: p.Pro166Ser) in patient 18 (**Table 4.1**). All of them were missense changes.

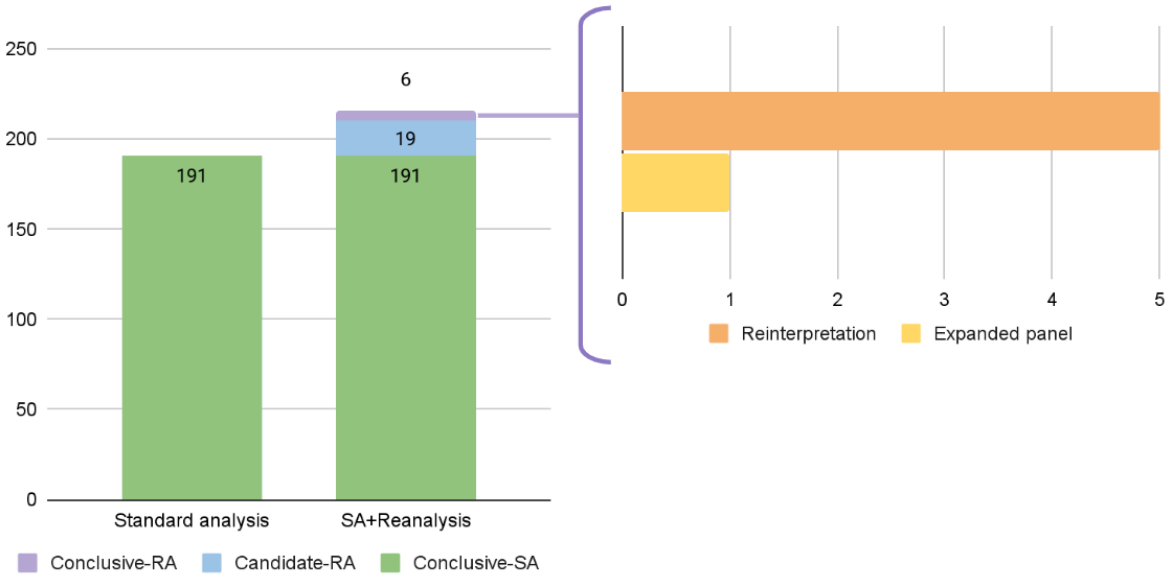

Figure 4.3. Impact of reanalysis on diagnostic yield. The left bar chart compares diagnostic outcomes from standard analysis (SA) alone versus the combined approach of standard analysis and reanalysis (SA + Reanalysis). Reanalysis led to the identification of 19 additional candidate variants (blue) and 6 conclusive variants (purple). The right bar chart breaks down the reasons for these conclusive findings: 5 were due to reinterpretation of previously identified variants (orange), and 1 was due to panel expansion (yellow).

Variants in genes not analysed previously

One P variant was identified in a gene not analysed in the standard analysis of a specific patient: a nonsense variant in the gene *TTN* (NM_001267550.2: c.104413C>T; p.Arg34805*) in patient 7 (**Table 4.1**).

4.2. Suspected Fanconi anaemia cohort

4.2.1. Chromosome fragility test results

Chromosome fragility and G2 arrest data (G2 data not shown) were incompatible with a classical FA phenotype (**Figure 4.4**), disregarding FA as initial diagnosis. Patients (1-6) showed values overlapping with those from non-Fanconi patients' group (**Figure 4.4**).

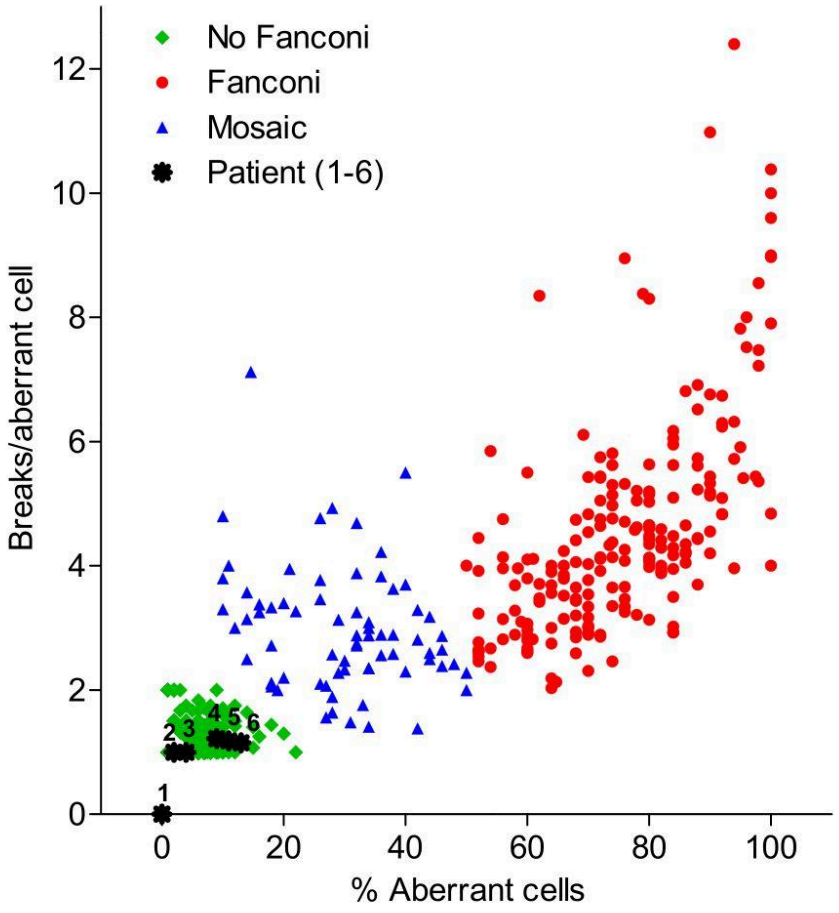

Figure 4.4. Chromosome fragility test results. The plot shows a graphical representation of the relative position of cases (suspected Fanconi anaemia patients 1-6, with numbers over asterisk symbols in the plot) when considering the distribution of historical data from the laboratory in terms of percentage of aberrant cells and mean number of breaks per cell (all induced by diepoxybutane at 0,1 g/ml). Green signals correspond to non-Fanconi patients, red are Fanconi anaemia patients, blue represent mosaic Fanconi anaemia patients, asterisk symbols correspond to reanalysed patients in this work. Mosaic patients are those Fanconi anaemia patients with a percentage of aberrant cells below 50%.

4.2.2. Reanalysis results

Consistently with negative chromosome fragility test, no variants were identified in none of the known FA associated genes (**Table 2.4**) in the standard analysis using WES. The performed reanalysis of sequencing data targeting other genes revealed three clinically significant variants in two out of six patients (33.4%) (**Table 4.2**).

Table 4.2. Clinically relevant variants identified in the Suspected Fanconi anaemia (SFA) cohort after reanalysing exome sequencing data.

Patient	Sex	Gene	Transcript	Variant	Type	Class	ACMG evidences
1	F	*TERT*	NM_198253.3	c.337G>T p.Glu113*	Nonsense	LP	PVS1, PM2
				c.193C>A p.Pro65Thr	Missense	LP	PM2, PM3, PP2
2	F	*RPL5*	NM_000969.5	C.175_176del p.Asp59Tyrfs*53	Frameshift	P	PVS1, PS4, PM2, PP5

Abbreviations: F = female; LP = likely pathogenic; P = pathogenic.

Patient 1 carried two variants in the *TERT* gene. The first one was a nonsense mutation resulting in a premature stop codon, likely leading to a LOF of the protein codified by the allele (NM_198253.3: c.337G>T; p.Glu113). The second variant consisted in a missense change (c.193C>A; p.Pro65Thr). Sanger sequencing confirmed both variants, and the topoisomerase-based cloning analysis demonstrated that they were in trans. Given the association between *TERT* mutations and telomere shortening, telomere length was assessed in patient 1. The results indicated a telomeropathy, with markedly shortened telomeres and a z score considerably lower than the control mean and under the 1st percentile.

Patient 2 presented a frameshift variant in the *RPL5* gene (NM_000969.5: c.175_176del; p.Asp59Tyrfs53*), which introduces a premature stop codon. This is expected to cause LOF, either through production of a truncated, non-functional protein or via nonsense-mediated mRNA decay. Telomere length analysis in patient 2 revealed shortened telomeres with a z score below percentile 10 but above percentile 1 (**Figure 4.5**); thus, telomeropathy in this patient based on this parameter was discarded.

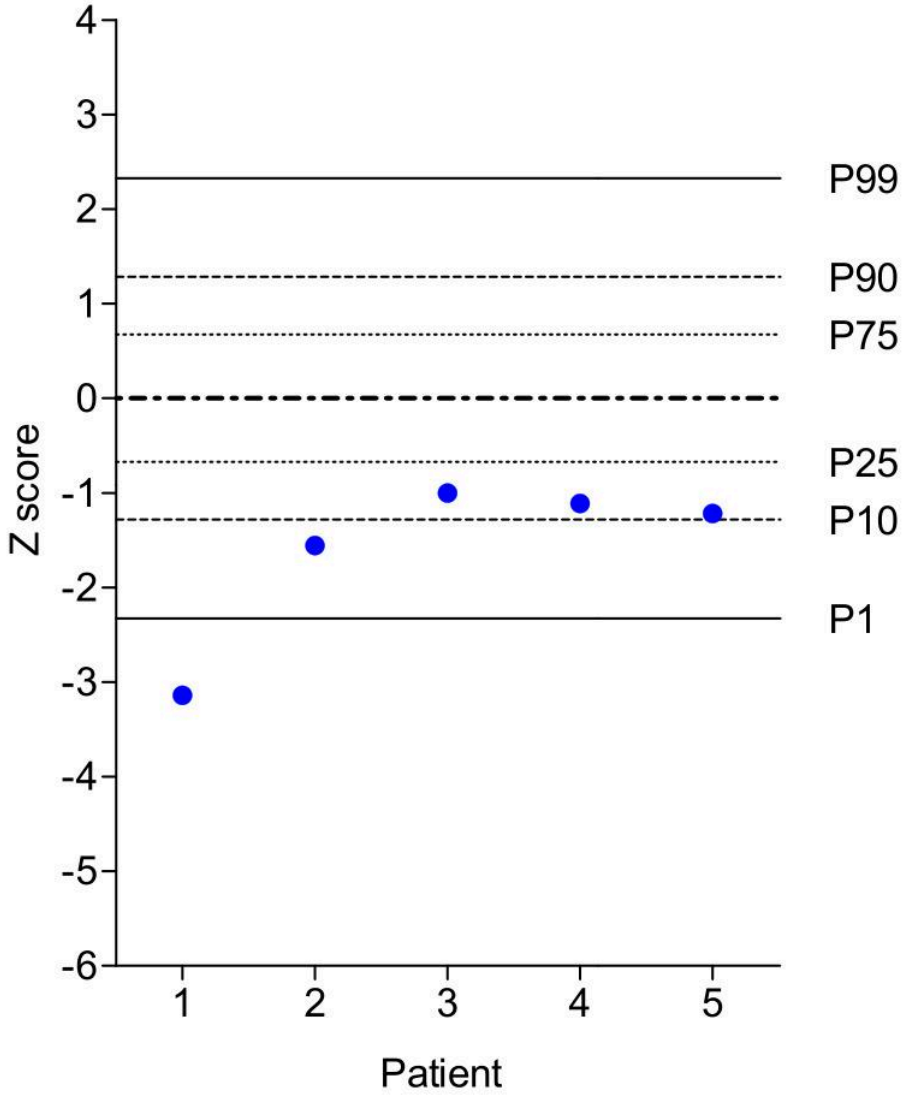

Figure 4.5. Telomere length in suspected Fanconi anaemia patients. Patients 1-5 with suspected FA represented in x-axe in 'Patient' group. Patient 6 not analysed due to technical issues. Horizontal lines represent the mean (0), and the values -2.326, -1.282, -0.674, +0.674, +1.282, and +2.326 correspond to the standard deviations from the mean, which align with the 1st, 10th, 25th, 75th, 90th, and 99th percentiles (P1-P99). The telomere length z score of a control population of 117 individuals (green dots) and 5 patients with a diagnosed telomeropathy (red dots) are shown. Comparisons between the groups were performed using the Mann-Whitney non-parametric test. Non-Fanconi patients, patients with a telomeropathy and individuals in the control population are in the same age range (0-29 years).

In the remaining four patients, no clinically significant variants were found in genes with known disease associations that matched the observed phenotypes. Although patients 3 to 5 had telomere lengths below the 25th percentile (**Figure 4.5**), their z scores remained above the 1st percentile, suggesting incompatibility with a telomeropathy.. Furthermore, analysis of non-OMIM genes did not yield any novel candidate genes, according to

existing evidence and knowledge. Some rare VUS were identified, characterised by extremely low population frequency, high predicted pathogenicity, and occurrence in both siblings, and residing in genes associated to clinical phenotypes or functions partially overlapping patients' features (**Table 4.3**). However, these lacked sufficient evidence to be classified as P/LP following ACMG/AMP guidelines.

Table 4.3. Summary of variants of uncertain significance identified across the patient cohort after reanalysing exome sequencing data.

Patient/s	Gene	Transcript	c.DNA p.change	gnomAD	REVEL	MIM Number
3	*PIEZO1*	NM_001142864.4	c.7184C>T p.Ala2395Val	1.075	196	611184
3	*EPAS1*	NM_001430.5	c.2259C>A p.Asp753Glu	18	39	603349
3	*THPO*	NM_000460.4	c.914C>T p.Thr305Met	1.998	49	600044
3	*CFB*	NM_001710.6	c.1133A>C p.Asn378Thr	271	405	138470
3	*MUC15*	NM_024690.2	c.37019A>G p.Asn12340Ser	0	19	171860
3	*MUC16*	NM_024690.2	c.4181C>T p.Thr1394Ile	0	86	606154
3	*CEBPZ*	NM_005760.3	c.61del p.Ala21Glnfs*2	0	-	612828
3	*CEBPZ*	NM_005760.3	c.60G>C p.Glu20Asp	0	109	612828
3	*ZDXA*	NM_007156.5	c.403_404del p.Cys135Leufs*42	3.536	-	300235
3	*ZDXA*	NM_007156.5	c.401_402del p.Gly134Valfs*43	0	-	300235
4	*RAD50*	NM_005732.4	c.2492A>G p.Glu831Gly	2.787	361	604040
5	*HRCT1*	NM_001039792	c.317_318insCA p.H107Tfs*52	0	-	-
5	*ANKLE1*	NM_001278444.2	c.1772del p.Leu591Cysfs*18	0	-	619348
6	*ABCA8*	NM_001288985.2	c.1181del p.Gly394Alafs*6	4.088	-	612505
5, 6	*PFKL*	NM_001002021.3	c.106G>A p.Gly36Ser	4.008	0.2	171860
5, 6	*GTDC1*	NM_001284238.3	c.26G>A p.Trp9*	9.865	-	610165
5, 6	*CRY2*	NM_021117.5	c.1505G>A p.Arg502Gln	0	506	603732

Patient/s	Gene	Transcript	c.DNA p.change	gnomAD	REVEL	MIM Number
5, 6	*FCGBP*	NM_003890.2	c.753C>A p.His251Gln	0	7	617553
5, 6	*KIF20B*	NM_001284259.2	c.2135G>C p.Cys712Ser	0	84	605498
5, 6	*NCAM1*	NM_001242607.2	c.1118G>A p.Gly373Asp	297	-	116930
5, 6	*TPM4*	NM_003290.3	c.163C>G p.Arg55Gly	0	788	600317

5. Discussion

The reanalysis approach applied in this study for cardiology patients differs notably from strategies employed in IBMFSs and other rare disease contexts. Specifically, our study utilised a CES strategy targeting a comprehensive set of cardiovascular disease genes, rather than broader WES or WGS approaches commonly used in other investigations. Unlike retesting studies that generate new sequencing data, we focused on reinterpreting existing data through an improved analytical pipeline and updated variant databases. Moreover, our gene panel extended beyond genes directly linked to the patients' presenting phenotypes to encompass a wider spectrum of cardiovascular-associated genes, thereby aiming to maximise the identification of clinically relevant variants, including those with pleiotropic or atypical presentations. This broader and more targeted approach provides a unique perspective on the utility of reanalysis in ICCs and contrasts with strategies applied to other patient groups, such as those with SFA. In the following sections, we discuss the distinct outcomes and implications of reanalysis in these two clinical contexts.

5.1. Reanalysis of the Inherited Cardiac Conditions cohort

In the analysed cohort of 742 patients, 25 variants of interest were identified upon reanalysis (3.4% of patients), of which 6 were finally classified as P/LP and concordant with the patients' clinical phenotypes. This represents a diagnostic yield increase of 0.8% beyond the initial analyses. The rest of the identified P/LP variants could not be related to the phenotype of the corresponding cases (a total of 19 out of 742 patients, around 2.5%) but were associated with other cardiac disorders. Five out of the six reclassified variants were initially reported as VUS. The revision of new evidence since the first analysis in combination of the clinical phenotype review, allowed the upgrade to LP. For example:

1) *RBM20* (NM_001134363: c.1910G>T; p.Ser637Ile) variant: This male patient had disease onset at 16-year-old, when he went to hospital emergency room, after presenting with a three-week history of cough with white sputum, progressive dyspnea, and intermittent fever. Initial treatment with bronchodilators, corticosteroids, and antibiotics led to temporary improvement. However, he was reevaluated after a recurrence of symptoms, including hemoptoic expectoration and low-grade fever. Chest X-ray revealed marked cardiomegaly and pulmonary congestion without consolidation. Echocardiography showed

severe global hypokinesia of the left ventricle, most pronounced in the anterior wall, with a left ventricular ejection fraction (LVEF) of 20%, bilateral systolic dysfunction, and a mild pericardial effusion. Additional findings included severe PAH and mild mitral and tricuspid regurgitation. The patient was diagnosed with DCM and transferred to intensive care in stable condition. The patient was transplanted due to heart failure after spending weeks in the Intensive Care Unit. Genetic testing revealed the above mentioned rare missense variant in *RBM20* (NM_001134363: c.1910G>T; p.Ser637Ile), initially classified as a VUS based on PM2 (absent or rare in population databases) and PM1 (located in a mutational hotspot within exon 9 of *RBM20*, which is enriched for DCM-associated variants). Upon reanalysis, additional evidence emerged supporting reclassification. Notably, a different missense substitution affecting the same amino acid residue —p.Ser637Asn— had been reported in an unrelated individual with DCM and classified as LP (Sun et al., 2020), emphasising the critical functional role of this serine residue. Additionally, several P missense variants within exon 9, where this variant is located, have been associated with DCM (Brauch et al., 2009; D. Li et al., 2010), supporting a reclassification of the *RBM20* variant to LP.

2) *ACTC1* variant (NM_005159.5: c.28C>A; p.Leu10Met) was identified in a male patient diagnosed with HCM, based on a septal thickness of 15 mm. Patient's history included rheumatoid arthritis presented with resting chest pain and ventricular hypertrophy. CMR imaging in 2020 confirmed moderate to significant concentric left ventricular hypertrophy, particularly in the mid-basal septum, along with diffuse myocardial fibrosis and a subtle increase in extracellular volume in the apical segments, suggesting a possible early-stage infiltrative cardiomyopathy. Differential diagnoses such as amyloidosis (excluded by negative gallium-67 uptake) and Fabry disease (ruled out through normal alpha-galactosidase A activity) were dismissed. The clinical phenotype, combined with imaging and laboratory findings, strongly supported a diagnosis of non-obstructive HCM. The identified variant is a rare missense change affecting the cardiac α-actin protein, a critical component of the sarcomere. Initially, the variant was classified as a VUS, with limited evidence and conflicting interpretations across databases such as ClinVar, preventing application of ACMG/AMP criteria PP2 and PP5 according to the *ACTC1*-specific ClinGen guidelines (https://cspec.genome.network/cspec/ui/svi/doc/GN101). However, the consistent and specific phenotype observed in the patient allowed the application of PP4, supporting clinical relevance. Subsequent data from Health in Code (personal communications) enhanced the reclassification to LP, based on several factors: the variant was significantly

overrepresented in HCM cases [OR 5.86 (2.30–17.68), p<0.0001], and segregation data from multiple families (34 affected carriers across 3 meiosis) further supported pathogenicity. Additionally, the disease showed incomplete penetrance, with a mean septal thickness of 16.3 mm across carriers aged 10 to 70 years, though some individuals exhibited greater hypertrophy without alternative P variants. Considering the strength of phenotype correlation (PP4), segregation data (PP1), rarity in population databases (PM2), and the functional relevance of the affected domain (PM1), the variant was ultimately reclassified from VUS to LIP.

3) *MYH7* c.2312G>C (p.Gly771Ala) variant: The affected female patient had been diagnosed with HCM. Reevaluation of case revealed significant asymmetric septal hypertrophy with a basal septal thickness of 17 mm and left ventricular outflow tract obstruction (LVOTO) measured at 32 mmHg at rest, rising to 114 mmHg with Valsalva maneuver. Ejection fraction was preserved. Additional findings included systolic anterior motion (SAM) of the mitral valve, moderate mitral regurgitation, slight left atrial dilation, and mild to moderate pulmonary hypertension. Cardiac computed tomography confirmed maximal myocardial thickness of 22 mm in the basal anterior segment and severe left atrial enlargement, with no significant coronary artery disease. The identified rare missense variant *MYH7* c.2312G>C (p.Gly771Ala) is located in the motor domain of the β-myosin heavy chain, a known hotspot for P variants in HCM. Although initially classified as a VUS due to limited available data, reanalysis using updated ACMG/AMP guidelines and *MYH7*-specific expert recommendations allowed application of several key criteria: PM2 (absent from population databases), PM1 (located in a critical functional domain), PP3 (multiple in silico tools predicting deleterious impact), and PP4 (strong phenotypic match as assessed by clinical experts). The convergence of this molecular and clinical evidence supports reclassification of the variant as LP.

4) *ACTC1* c.644A>G (p.Gln215Arg) variant. The patient harboring this variant presented with severe DCM, ultimately undergoing heart transplantation. Pathological evaluation of the explanted heart confirmed the clinical diagnosis of DCM. Initially classified as a VUS based on ACMG/AMP criteria including PM2 (absent from population databases), PP2 (missense variant in a gene with low benign variation and known disease mechanism via missense mutations), and PP3 (multiple *in silico* tools predicting deleterious impact). No previous reports existed in ClinVar or the literature linking p.Gln215Arg to disease. However, subsequent clinical review highlighted a phenotype strongly consistent with *ACTC1*-related DCM and detailed correlation between genotype and pathological

phenotype, triggering the inclusion of PP4. *ACTC1* encodes cardiac α-actin, a highly conserved protein where several missense variants affecting the actin-myosin interaction domain —such as E101K, P166A, and A333P— have been firmly associated with both HCM and DCM. Although p.Gln215Arg itself remains novel, its location within a mutational hotspot critical for sarcomeric function, combined with the patient's severe phenotype and explant pathology, prompted the reclassification of the variant as LP.

5) *ACTC1* c.496C>T (p.Pro166Ser) variant. Identified in a male patient with a longstanding history of left ventricular non-compaction cardiomyopathy who was first diagnosed at the age of 49 following the identification of the same condition in his daughter. He had been told since adolescence that "he had an enlarged heart", and moderate systolic dysfunction had been documented for decades. A 2004 cardiac MRI showed a dilated left ventricle with pronounced apical trabeculation, consistent with left ventricular non-compaction cardiomyopathy. He was later diagnosed with brady-tachy syndrome and atypical atrial flutter in 2005, for which a dual-chamber pacemaker was implanted. In December 2007, a primary prevention implantable cardioverter-defibrillator was placed. Echocardiography in 2024 confirmed severe apical trabeculation, left ventricular dilation (LV diameter 62 mm), and reduced ejection fraction (EF 32%), with a mobile aneurysm of the interatrial septum protruding into the left atrium. Both mitral and tricuspid regurgitations were mild, and there was no evidence of pulmonary hypertension. The same variant was also identified in both of the patient's children, who are also clinically affected, supporting familial segregation. Initially classified as a VUS, the variant met PM2 (absent or extremely rare in population databases), PP2 (missense variant in a gene with low benign missense variation and known disease mechanism), PM5 (novel missense at a residue with other known P variants), and PP3 (computational evidence supporting a deleterious effect). Although it had not yet been curated in ClinVar, expert clinical review of the patient's imaging and disease course confirmed a phenotype highly consistent with *ACTC1*-associated LVNC, enabling application of PP4. With this additional phenotypic correlation and supporting segregation data, the variant was reclassified as LP under ACMG/AMP guidelines.

6) The *TTN* c.89812_89813insTGCC (p.Gln29938Leufs*7) frameshift variant, located in exon 335 within the A-band region of *TTN* (NM_001267550), was not reported during the initial analysis because the patient's referral phenotype was HCM, and the HCM gene panel—curated by ClinGen's expert panel—does not include *TTN* among its definitive or strong evidence HCM-associated genes. However, upon detailed clinical reassessment,

the phenotype was found to be non-specific cardiomyopathy rather than classical HCM. Given that truncating A-band *TTN* variants are a recognised cause of AD DCM, the variant was deemed relevant. This case underscores the importance of phenotype-driven reanalysis and flexible panel design: while *TTN* is excluded from HCM panels, reconsideration of clinical presentation allowed appropriate variant identification and diagnosis of DCM in this patient.

The reclassification of VUS and the identification of new variants in previously unexamined genes offer valuable clinical benefits. Molecular diagnoses enable more precise risk stratification, inform management decisions, and facilitate genetic counseling for patients and their families. For instance, a patient with a reclassified variant in the *RBM20* gene (p.Ser637Ile) could benefit from closer monitoring for arrhythmias, which are a known risk in *RBM20*-related DCM.

The presented results highlight the value of reanalysing existing exome sequencing data in a large cohort of ICCs patients, identifying 25 variants of interest in 742 cases (3.4%). This diagnostic yield aligns with the range reported in previous reanalysis-focused investigations, which generally report yields between approximately 4-23% depending on the study design, cohort characteristics, and analysis strategy (Chang et al., 2022; Josephs et al., 2024; Cuesta-Llavona et al., 2024). Notably, our findings emphasise that the majority of clinically relevant variants were located in genes originally sequenced but initially classified as VUS, underscoring the dynamic and evolving nature of variant classification over time. This observation parallels the reinterpretation studies cited in the introduction, such as those by Campuzano et al. (2020) and Novelli et al. (2022), which reported substantial rates of VUS reclassification to P/LP following updated evidence and expert clinical review.

Our study also identified a smaller subset of P variants in genes that were not included in the original diagnostic panels, exemplified by the *TTN* truncating variant detected in patient 7. This finding reinforces the importance of expanding gene panels and highlights how panel limitations can contribute to missed diagnoses in the initial analysis. This phenomenon echoes the findings from Austin et al. (2024), where a second-tier gene panel analysis shortly after genome sequencing revealed additional clinically relevant variants. Similarly, Mazzaccara et al., 2022 increased diagnostic sensitivity by up to 8% in a cohort of inherited cardiomyopathies and CHAN by using a panel of uncommon genes.

The clinical review was instrumental in correlating genotype with phenotype, resulting in six variants being classified as clinically concordant. Most of these were previously reported VUS that were upgraded to LP based on emerging evidence and phenotypic concordance, consistent with reclassification rates described in prior ICCs cohorts (Martin et al., 2024; Horgan et al., 2025). Conversely, some variants in genes such as *SCN5A*, *NEXN*, *ACTA2*, and *MIB1*, while initially flagged as candidates, were ultimately retained as VUS due to insufficient phenotype correlation, illustrating ongoing challenges in variant interpretation and the need for comprehensive genotype-phenotype databases. The patient's presentation with coronary artery aneurysms (CAAs) and the identification of a *NEXN* truncating variant (NM_144573: c.201G>A; p.Trp67)* present a unique and complex diagnostic challenge. The variant has been related to DCM and HCM (Al-Hassnan et al., 2020; Bruyndonckx et al., 2021; Rinaldi et al., 2020), and another LOF variant (NM_144573: c.298G>T; p.Gly100*) has been identified previously in a patient with a giant right coronary artery aneurysm among other conditions (Peng et al., 2022). *NEXN* (Nexilin) is a well-established gene implicated in various cardiomyopathies, particularly DCM, and to a lesser extent, HCM. It encodes a Z-disc protein crucial for sarcomere integrity and cardiac function, with truncating variants like the one identified typically considered P due to their impact on protein function. However, the existing literature does not generally associate *NEXN* variants directly with CAAs. CAAs are typically linked to distinct etiologies such as Kawasaki disease, atherosclerosis, or specific connective tissue disorders (e.g., MFS), and are not considered a direct manifestation of primary myocardial diseases like DCM or HCM. This discrepancy raises a critical question regarding the observed phenotype: is the CAA an independent co-morbidity, or does the *NEXN* variant, in this specific case, confer a broader vasculopathic predisposition not yet fully understood? While the primary role of *NEXN* in myocardial structure and function is clear, the presence of CAAs might suggest an uncharacterised pleiotropic effect of this particular truncating variant, possibly influencing vascular integrity in addition to its known cardiac involvement. Further investigation into potential mechanisms that could link *NEXN* dysfunction to vascular wall pathology is warranted, as this case highlights a potential novel phenotypic association that extends beyond the currently recognized spectrum of *NEXN*-related cardiomyopathies.

The patient with *SCN5A* finding was initially referred for aortic pathology study. While *SCN5A* is classically associated with various primary arrhythmia syndromes, notably BrS, LQTS, and CCDs, its direct link to aortic pathology is not established. The patient's ECG from 2019 was suggestive of BrS, though not diagnostic, but subsequent ECGs have not

shown Brugada patterns. Despite the clear pathogenicity of the identified *SCN5A* variant, the absence of overt and consistent BrS features, coupled with the lack of documented family history of arrhythmias, underscores the variable penetrance often observed with these genetic conditions. While *SCN5A* typically governs cardiac electrical activity through its role in encoding the alpha subunit of the cardiac sodium channel (Nav1.5), there is no known association between *SCN5A* and structural aortic pathologies such as aneurysms or dissections. Therefore, the *SCN5A* variant, though highly relevant to cardiac electrophysiology, is unlikely to explain any potential aortic concerns.

While *ACTA2* variants are well-established causes of various vascular pathologies, including familial TAAs and Moyamoya disease, and are associated with smooth muscle dysfunction syndrome, the index patient in this study presented with a clinical picture highly consistent with acute myocarditis. In 2023, the patient reported characteristic chest pain radiating to the left shoulder, along with vegetative symptoms and dyspnea on moderate exertion, with subsequent progressive pain improvement. Cardiac magnetic resonance imaging (MRI) definitively confirmed the diagnosis of myocarditis, and there is currently no suspicion of associated aortic pathology. A comprehensive review of echocardiographic measurements, including images from the initial assessment, revealed no signs of aortic dilation (aortic root z-score remained around +1.3), further supporting the absence of current aortic involvement. Nonetheless, given the patient's documented *ACTA2* variant (NM_001613.4: c.C289T; p.R97C), which is categorised as P and linked to severe vascular phenotypes, further genetic screening of the parents is strongly recommended to assess for familial inheritance and potential risk stratification. The patient, now 20 years old, remains under the diligent care of a local cardiologist, with close monitoring facilitated by the father, a family physician. The case of a patient with *MIB1* variant, who presented a dissecting aortic aneurysm at an age identical to his father's fatal aortic dissection, presents a compelling narrative for a strong underlying genetic predisposition to aortopathy. The presence of a P frameshift variant in *MIB1* (NM_020774: c.2596_2599del; p.C866Wfs*27) is a particularly intriguing finding in this context. While *MIB1* has recently been established as a novel gene associated with BAV through its crucial role in the Notch signaling pathway, its direct involvement in TAA and dissection, especially in the absence of a BAV, is less clearly defined. The conventional understanding posits BAV as a significant risk factor for aortopathy due to altered hemodynamics, suggesting an indirect link for *MIB1* to TAA via BAV. However, this patient's tricuspid aortic valve challenges this conventional pathway, compelling a deeper exploration into how *MIB1* dysfunction, specifically this frameshift variant, might directly

contribute to aortic wall fragility and dissection. The severity of the patient's aortic event, coupled with the absence of BAV, raises the hypothesis that the *MIB1* variant may induce a primary defect in aortic wall integrity, perhaps through more fundamental disruptions to vascular smooth muscle cell function or extracellular matrix homeostasis, independent of valvular morphology. Given the strong familial history of early-onset aortic dissection, further comprehensive phenotyping of affected and at-risk family members, including the siblings and children, is critical. This will not only aid in elucidating the penetrance and variable expressivity of this *MIB1* variant but also offer invaluable insights into potential novel mechanisms of aortopathy, warranting dedicated research into the pleiotropic effects of *MIB1* within the broader context of arterial wall development and maintenance.

Taken together, these results affirm that reanalysis, encompassing reinterpretation of known variants and panel expansion, constitutes a critical complementary approach to initial genetic testing. It leverages updated knowledge, refined bioinformatics pipelines, and multidisciplinary clinical input to improve diagnostic yield in ICCs. Future efforts should continue to integrate iterative data review with emerging functional data and long-term clinical follow-up to maximise the utility of genetic testing for patient care.

5.2. Reanalysis of the Suspected Fanconi anaemia cohort

Reanalysis of WES data of patients with suspicion of FA led to the identification of disease-causing variants in patients 1 and 2. In patient 1, a nonsense variant (NM_198253.3: c.337G>T; p.Glu113*) and a missense change (NM_198253.3: c.193C>A; p.Pro65Thr) were identified in the *TERT* gene. The nonsense variant shows an extremely low frequency in gnomAD, and LOF is a known mechanism of disease in the gene (Armanios et al., 2005). According to this evidence, the variant was classified as LP. The missense change also presents an extremely low frequency in gnomAD, and the gene shows a low rate of benign missense mutations, which are a common mechanism of disease. Although the variant was found in a patient with aplastic anaemia also carrying a second missense change (Arias-Salgado et al., 2019), it was classified as a VUS, as there was not enough available evidence to confirm its pathogenicity.

The *TERT* gene is associated with AD or AR DC, a telomere disease. While there is significant overlap in the clinical features of DC regardless of inheritance pattern, some nuances can exist. Severe forms of DC, like Hoyeraal-Hreidarsson syndrome—often involving cerebellar hypoplasia and developmental delay—can be associated with both AR and AD inheritance, depending on the specific gene involved (Marrone et al., 2007).

The age of onset and the rate of progression can vary between individuals and families, and this variation is not strictly tied to the inheritance pattern but more to the specific gene and mutation. The classic triad of nail dystrophy, lacy reticular pigmentation, and oral leukoplakia can be present in both AR and AD forms, though not all individuals with DC present with all three features. Both AR and AD forms of DC are characterised by shortened telomeres. The degree of telomere shortening may correlate somewhat with disease severity, though this is not strictly determined by inheritance. There is significant phenotypic variability in DC, even within families with the same mutation, and anticipation may occur in dominant forms, with successive generations exhibiting earlier onset and more severe symptoms (Armanios et al., 2005).

The studied patient presented severe telomere shortening (**Figure 4.5**) and developed bone marrow aplasia, which required an allogeneic transplant in 2003. In 2021, she was diagnosed with interstitial lung disease and received a lung transplant. The patient also experienced chronic diarrhea associated with irritable bowel syndrome and, in her most recent clinical report, presented with severe neutropenia. The variants were confirmed by Sanger sequencing, and the topoisomerase-based cloning analysis showed they were in trans. This provides ACMG/AMP PM3 evidence: a variant occurring in trans with a P variant for a recessive disorder, supporting classification as LP (Richards et al., 2015). DC caused by *TERT* has been associated with both dominant and recessive inheritance, with the latter form sometimes displaying additional features such as leukoplakia, failure to thrive, cerebellar hypoplasia, microcephaly, and developmental delay (Marrone et al., 2007), none of which were present in our patient. Her telomere length was 2,608 bp at 14.3 years of age, with a z score of -3.141, significantly below the 1st percentile (-2.326), consistent with telomere disease (Alter et al., 2015) and serving as a functional evidence of the variants pathogenicity.

In patient 2, the *RPL5* variant (NM_000969.5: c.175_176del; p.Asp59Tyrfs*53) was classified as P based on several criteria: it is a LOF variant, which is a known disease mechanism in *RPL5* (Gazda et al., 2008; Quarello et al., 2010); it is extremely rare (not found in gnomAD); and it has been reported in individuals with DBA (Gazda et al., 2008; Gerrard et al., 2013; Tsangaris et al., 2011). DBA is an inherited red blood cell aplasia that typically manifests in the first year of life, featuring normochromic macrocytic anaemia, reticulocytopenia, and almost absent erythroid progenitors in the bone marrow. Growth retardation is common, and 30-50% of patients have congenital malformations involving the face, limbs, heart, or urinary system (Gadhiya & Wills, 2025). Increased MCV,

elevated erythrocyte adenosine deaminase activity, and persistent fetal hemoglobin are also often observed, though some patients may not exhibit these traits. Even within the same family, phenotypic variation is common (Landowski et al., 2013).

The studied patient exhibited a mild phenotype, presenting in 2010 with anaemia (MCV at the normal-high limit for age) and mild leukopenia-neutropenia. In the most recent report, the anaemia had resolved, but mild leukopenia persisted. This aligns with the heterogeneity of DBA (Landowski et al., 2013), as even individuals with the same mutation can have differing clinical presentations (Errichiello et al., 2017; Gazda et al., 2008). Additionally, this patient had telomere shortening relative to controls: at 10.9 years of age, her telomere length was 7,116 bp (z score -1.557), which is below the 10th percentile but above the 1st, supporting a diagnosis of DBA as described by Alter et al. (2015).

5.3. Final discussion remarks

Our diagnostic yield (0.8% in ICCs patients and 33% in suspected FA patients) is lower than the reported range of 6.5% to 41% in previous NGS reanalysis studies of genetic diseases. However, the population selected for reanalysis significantly influences these outcomes (Van Der Geest et al., 2024). Hence, the comparison should be restricted to studies within the field of cardiology and bone marrow failure. The percentage of new diagnoses identified through a subsequent, broader analysis of WES data, which included a more comprehensive panel of genes implicated in IBMFSs, aligns with the diagnostic yields reported in larger cohorts (10-25%). This work underscores the importance of expanding the scope of genetic investigation beyond traditional FA genes in patients presenting SFA features but normal chromosome fragility. However, only one publication reporting a true diagnostic rate in the field of cardiology, for example, was found (Chang et al., 2022). While their rate of 4.8% exceeds ours, it is important to consider that our study analysed a larger cohort (742 cases versus 125). Research in other fields has shown that diagnostic yield tends to decrease with larger sample sizes (Van Der Geest et al., 2024). In addition, the higher yield reported may reflect their use of WGS and resequencing, methods that have been shown to significantly enhance diagnostic rates and the detection of variants (Chang et al., 2022; Cuesta-Llavona et al., 2024).

Another important factor is the recommended interval between the initial genetic testing and the reanalysis, which ranges from 6 to 36 months (Van Der Geest et al., 2024). For WES reanalysis in patients with Mendelian diseases or paediatric patients, a minimum of 12 to 18 months is suggested (Ewans et al., 2018; Stark et al., 2019) although there is no

established consensus in the literature regarding the optimal frequency of reanalysis for ICCs. Given that some of the cases in our study were initially analysed in 2022 and the reanalysis began in 2023, there is a possibility that with a longer interval could yield additional clinically relevant findings.

An essential aspect of the reanalysis strategy is the involvement of expert review by a multidisciplinary team of geneticists and cardiologists, as demonstrated in our study. This step is critical for verifying phenotypic concordance and resolving conflicting variant classifications. As databases and guidelines evolve, expert interpretation will remain indispensable for the precise diagnosis and management of inherited cardiac diseases.

Despite the clear benefits of reanalysis, several challenges remain. The diagnostic yield of this study (0.8% in cardiology patients and 33% in IBMFSs patients), though significant, is modest, especially in the ICCs cohort. Many of the variants identified could not be definitively linked to the clinical phenotypes, emphasising the complexity of inherited cardiac diseases. For instance, a variant in *TTN* was found in a patient with LQTS, a condition not traditionally associated with this gene. This example highlights the need for further research to better understand genotype-phenotype correlations and improve the interpretation of VUS.

Additionally, technical limitations, such as the inability to detect large SVs or deep intronic changes that affect splicing, may have contributed to the relatively low diagnostic yield. Short-read sequencing is known to have reduced sensitivity for SVs, due to the difficulty in assembling reads across complex genomic rearrangements, especially within repetitive regions (Kosugi et al., 2004). Furthermore, WES's exon-centric design hinders the detection of variants in critical non-coding regions (Burdick et al., 2020), such as deep intronic and regulatory sequences. Other limitations, including uneven coverage in GC-rich regions (Wang et al., 2017) and the difficulty in detecting small CNVs (Yao et al., 2017), also contribute to the potential for missed variants. Long-read sequencing could overcome these limitations by effectively resolving complex and repetitive regions, leading to improved detection of SVs and other variant types, including SNVs (Logsdon et al., 2020; Iyer et al., 2024; Warburton et al., 2023; Wohlers et al., 2022; Olivucci et al., 2024). Similarly, WGS provides a more comprehensive genomic view, allowing for the identification of SVs and CNVs frequently missed by WES and enabling the exploration of potentially P variants in non-coding regions (Bowman et al., 2025; Nomakuchi et al., 2024;

Brlek et al., 2024). Incorporating complementary approaches, including the ones just mentioned or RNAseq, could increase the diagnostic yield (Tan et al., 2020).

Another contributing factor may be the limited knowledge of many reviewed non-OMIM genes, which resulted in numerous rare VUS. In addition, there is also a possibility of a disease caused by the combined effects of multiple genes, each contributing a small risk. WES may identify some of these polygenic risk variants, but it is difficult to determine their overall contribution to the disease.

To improve this study and NGS reanalysis in general, automation plays an important role. While manual curation, as used in our study, is often associated with better outcomes (Van Der Geest et al., 2024), automated approaches facilitate the incorporation of up-to-date literature and databases, enabling the identification of novel gene-disease associations and variants, ultimately improving the diagnostic yield (Baker et al., 2019; Dai et al., 2022; James et al., 2020; Sarmady & Abou Tayoun, 2018). Artificial intelligence-based tools have shown promising results and seem to be valuable for clinical laboratories as they reduce the time and resources invested on reanalysis and benefiting a larger number of patients (O'Brien et al., 2022; Van Der Geest et al., 2024).

Lastly, the reanalysis of genomic data offers a significant advantage through the incorporation of newly discovered disease-causing genes. Many of these genes were simply not included in the original CES panels used at the time of initial analysis. This is a crucial factor, as these recently identified genes are now responsible for a substantial portion of new molecular diagnoses in rare diseases, as evidenced by studies across various fields (P. Liu et al., 2019; Mensah et al., 2022) and specifically within ICCs (Austin et al., 2024; Cuesta-Llavona et al., 2024).

The advent of NGS has undeniably revolutionised our understanding of genetic diseases and profoundly reshaped diagnostic approaches and shortened diagnostic odyssey. Despite its widespread integration into diagnostic workflows and the resulting explosion of genomic data, a notable proportion of patients still remain without a definitive molecular diagnosis. This persistent diagnostic gap highlights the critical, yet often underexplored, potential of iteratively reanalysing existing exome sequencing data. This ongoing reevaluation is key to unlocking previously missed diagnoses as new genetic knowledge and bioinformàtic and analytical tools emerge, ultimately offering hope and actionable insights for individuals with genetic disorders and their relatives.

6. Conclusions

1) Global Diagnostic Yield and Contributing Factors: Systematic reanalysis of inconclusive NGS data increased the molecular diagnostic yield across both cohorts, achieving a 0.8% increase in definitive diagnoses for ICCs and a notable 33.4% yield for IBMFSs). The primary drivers for these new diagnoses were the reclassification of previously ambiguous VUS and the successful identification of P variants through the inclusion of additional gene-disease associations not considered in initial analyses.

2a) Reanalysis Impact on ICCs:
For ICCs, reanalysis significantly improved diagnostic outcomes and clinical utility by successfully reclassifying five VUS to P/LP status, largely due to evolving scientific evidence and rigorous multidisciplinary clinical review. Furthermore, the identification of a diagnostic variant in a newly associated gene (*TTN*) in one patient underscored the value of expanding gene analysis beyond initial, phenotype-specific panels.

While reanalysis proved highly beneficial, the study also highlighted persistent challenges in achieving full diagnostic resolution. The difficulty in establishing clear genotype-phenotype correlations for some P/LP variants (e.g., *SCN5A, NEXN, ACTA2, MIB1*) underscores the complex expressivity and pleiotropy of genetic conditions, emphasizing the need for comprehensive patient phenotyping and continued research into gene-disease associations. Furthermore, technical limitations inherent to short-read sequencing, such as suboptimal detection of SVs and non-coding alterations, likely contributed to the remaining undiagnosed cases.

2b) Reanalysis Impact on SFA: In the SFA cohort, reanalysis effectively determined the underlying genetic causes in two out of six previously inconclusive cases, demonstrating a substantial diagnostic yield of 33.4%. This highlights the critical role of broader gene analysis within reanalysis for identifying disease-causing variants in patients where initial, more targeted genetic testing had proven insufficient.

As final closure, this work affirms that the iterative reanalysis of existing NGS data is an indispensable and cost-effective strategy in clinical genomics. It leverages the dynamic landscape of genetic knowledge and analytical advancements to provide actionable diagnosis, thereby enabling more precise risk stratification, informing tailored management strategies, and facilitating crucial genetic counseling for patients and their

families. Future efforts should focus on integrating longer reanalysis intervals, exploring complementary sequencing technologies like long-read sequencing and WGS, and incorporating advanced computational tools, including artificial intelligence, to further maximize the diagnostic yield and enhance patient care.

Publications derived from this thesis

This thesis led to the publication of the following article:

'Ending diagnostic odyssey by reanalysis of whole exome sequencing data: reclassification of suspected Fanconi anemia cases to dyskeratosis congenita and Diamond-Blackfan anemia' (https://doi.org/10.1186/s13023-025-03928-5)

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Abstract

Background: Initial Whole Exome Sequencing frequently fails to resolve rare disease cases. Bioinformatic reanalysis of existing genomic data utilizes advancing knowledge to enhance diagnosis without additional testing. This study investigated six patients with clinical features consistent with Fanconi Anemia but negative chromosomal breakage tests, whose initial genetic analyses were inconclusive.

Results: Whole Exome Sequencing data from these patients (collected 2005–2009) underwent comprehensive reanalysis, including single nucleotide variants, insertions/deletions, and copy number variants across genes beyond those typically associated with Fanconi Anemia. Telomere length was assessed via monochrome multiplex quantitative PCR. Reanalysis identified clinically significant variants in two patients (33.3% yield): one harboured a heterozygous pathogenic loss-of-function variant in the Diamond-Blackfan anemia gene *RPL5*, while the second exhibited compound heterozygous variants in the *TERT* gene, indicative of dyskeratosis congenita.

Conclusions: This study underscores the clinical value of reanalyzing existing genomic data in unresolved suspected genetic disorders, even when phenotype-specific assays are negative. The 33.3% diagnostic yield aligns with gains from larger reanalysis studies (10–25%). Systematic reassessment after sufficient time (24 + months) for genomic advancements offers a cost-effective diagnostic approach for long-undiagnosed cases, highlighting the dynamic nature of genomic interpretation as gene-disease understanding evolves.

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