Master Thesis

Overview of cardiogenetic cases at the Diagnostic and Research Institute of Human Genetics at the Medical University of Graz

submitted by

Dr. med. univ.

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Declaration

I declare on my honour that I have written this work independently and without outside help, that I have not used sources other than those indicated, and that I have marked as such the places taken literally or in terms of content from the sources used. Throughout this thesis, I followed the guidelines of "Good Scientific Practice".

Graz, 18.06.2020 *Dr. Lavinia Rech, MA eh*

Acknowledgement

First of all, I would like to thank Mr. Assoc.-Prof. Windpassinger, who took care of my work and guided me skilfully over small unevenness.

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Zusammenfassung / Abstract in German

Hintergrund. Die Genetik vererbter Krankheiten erstreckt sich mittlerweile in viele Bereiche der Medizin. Ebenso nimmt die Zahl der Testungen auf kardiogenetische Erkrankungen zu. Für viele Krankheiten gibt es bereits familiäre Varianten wie allen voran die vererbte hypertrophe Kardiomyopathie (HCM) oder das Long QT Syndrom (LQT). War man früher durch die Sanger Sequenzierung, und ihre Limitationen, noch eingeschränkt auf die Suche in sehr spezifischen Genen, so ermöglicht das Next-Generation Sequencing neue Möglichkeiten. Durch diese Technik ist es möglich, günstig und schnell, eine Vielzahl von relevanten Genen zu untersuchen und so die Wahrscheinlichkeit, eine Mutation zu finden, zu steigern.

Material & Methoden. Das Studienkollektiv umfasste von 2015 bis 2018 288 PatientInnen. Die Daten über Geschlecht, Alter, Arbeitsdiagnose, Mutationsart, Klassifikation und betroffene Gene wurden einem Excel Sheet entnommen und retrospektiv wiederum in Excel aufbereitet.

Ergebnisse. Die Geschlechterverteilung lag mit 62% vermehrt bei den Männern. Dies spiegelte sich auch in einigen Subgruppen (z.B. HCM) wieder. Andere bereits beschriebene Verteilungsmuster zeigten sich auch in unserer Kohorte schön; zum Beispiel die achtfach erhöhte Frequenz für Männern beim Brugada Syndrom oder die höhere Wahrscheinlichkeit für Frauen beim LQT. In unserer Kohorte konnte etwa die Hälfte der Fälle nicht gelöst werden. Das kann unter anderem darauf zurückgeführt werden, dass viele der 592 gefundenen Mutationen mit unclassified variant (UV) als Ursache für den Phänotyp nicht eindeutig sind.

Schlussfolgerung. Die Uneindeutigkeit der UVs bedeutet einen erhöhten Aufwand der Abklärung für die Symptome der PatientInnen, sowie eine weitere Klärung die Mutation selbst betreffend. Besonders bei den UVs zeigt sich der beschränkte Nutzen der genetischen Testung für diverse Erkrankungen. Die Folgen der Bekanntgabe eines UVs können für PatientInnen belastender sein als eine klare Diagnose. Dennoch bieten genetische Testungen auch Vorteile vor allem derzeit in der Forschung polygenetischer Formen kardiovaskulärer Erkrankungen.

Schlagwörter: Kardiologie, Genetik, Kardiogenetik, vererbte kardiologische Krankheiten

Abstract

Background. The genetics of inherited diseases now extends into many areas of medicine. The number of tests for cardiogenic diseases is also increasing. For many diseases, there are already familial variants such as inherited hypertrophic cardiomyopathy (HCM) or Long QT syndrome (LQT). Whereas Sanger sequencing, and its limitations, used to be restricted to the search in particular genes, next-generation sequencing opens up new possibilities. With this technique it is possible, cheap and fast, to examine a large number of relevant genes and thus increases the probability of finding a mutation.

Material & Methods. From 2015 to 2018, the study collective comprised of 288 patients. The data on gender, age, working diagnosis, type of mutation, classification and affected genes were taken from an Excel sheet and retrospectively processed in Excel again.

Results. The gender distribution was 62% in men. This was also reflected in some subgroups (e.g. HCM). Other distribution patterns already described were also found in our cohort; for example, the eightfold increased frequency for men in Brugada Syndrome or the higher prevalence of women in LQT. In our cohort, for about half the cases the underlying pathogenic mutation could not be detected. This can be explained, among other things, by the fact that many of the 592 identified variants are currently unclassified (UV) and therefore currently cannot be categorized as genetic cause for the phenotype respectively.

Conclusion. The ambiguity of the UVs means an increased effort of clarification of the patient's symptoms and further clarification of the mutation itself. Particularly in the case of UVs, the limited benefit of genetic testing for various diseases becomes apparent. The consequences of the announcement of an UV can be more stressful for patients than a clear diagnosis. Nevertheless, genetic testing also offers advantages, especially at present in research into polygenetic forms of cardiovascular disease.

Keywords: cardiology, genetics, cardiogenetic, inherited cardiological diseases

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Abbreviations and Definitions

2D	2 dimensional
ABCC9	ATP binding cassette subfamily C member 9
ACMG	American College of Medical Genetics and Genomics
ACAD9	acyl-CoA dehydrogenase family member 9
ACTC1	actin alpha cardiac muscle 1
ACTN2	actinin alpha 2
AKAP9	A-kinase anchoring protein 9
ANK2	ankyrin 2
ANKRD1	ankyrin repeat domain 1
ARVC	arrhythmogenic right ventricular cardiomyopathy
AV-block	atrioventricular block
B	benign
BAG3	BCL2 associated athanogene 3
bp	base pair
BrS	Brugada syndrome
CACNA1C	calcium voltage-gated cannel subunit alpha 1 C
CACNA2D1	calcium voltage-gated channel auxiliary subunit alpha 2 delta 1
CACNB2	calcium voltage-gated channel auxiliary subunit beta 2
CAD	coronary artery disease
CALM2	calmodulin 2
CALR3	calreticulin 3
CASQ2	calsequestrin 2
CAV3	caveolin 3
CHD	congenital heart disease
COX15	cytochrom c oxidase assembly homolog COX15
CPVT	catecholaminergic polymorphic ventricular tachycardia
CRT	cardiac resynchronization therapy
CRYAB	crystallin alpha B
CSRP3	cysteine and glycine rich protein 3
CTF1	cardiotrophin 1
CTNNA3	catenin alpha 3
CVD	cardiovascular disease

DCM	dilative cardiomyopathy
ddNTP	dideoxyribonucleoside triphosphate
DES	desmin
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
DMD	dystrophin
DSC2	desmocollin 2
DSG2	desmoglein 2
DSP	desmoplakin
DTNA	dystrobrevin alpha
ECG	electrocardiogram
EF	ejection fraction
ESC	European Society of Cardiology
EYA4	EYA transcriptional coactivator and phosphatase 4
FHL1	four and a half LIM domains 1
FLNC	filamin C
GAA	glycosidase alpha, acid
GPD1L	glycerol-3-phosphate dehydrogenase 1 like
HCM	hypertrophic cardiomyopathy
HCN4	hyperpolarization activated cyclic nucleotide gated potassium channel 4
HFrEF	heart failure with reduced ejection fraction
ICD	implantable cardioverter-defribrillator
ILK	integrin linked kinase
IVFA	idiopathic ventricular fibrillation arrhythmia
JPH2	junctophilin 2
JUP	junction plakoglobin
KCNE1	potassium voltage-gated channel subfamily E regulatory subunit 1
KCNE2	potassium voltage-gated channel subfamily E regulatory subunit 2
KCNE3	potassium voltage-gated channel subfamily E regulatory subunit 3
KCNH2	potassium voltage-gated channel subfamily H member 2
KCNJ2	potassium inwardly rectifying channel subfamily J member 2
KCNJ5	potassium inwardly rectifying channel subfamily J member 5
KCNQ1	potassium voltage-gated channel subfamily Q member 1

LAMA2	laminin subunit alpha 2
LAMA4	laminin subunit alpha 4
LAMP2	lysosomal associated membrane protein 2
LB	likely benign
LDB3	LIM domain binding 3
LMNA	lamin A/C
LP	likely pathogenic
LQT	long QT syndrome
LVAD	left ventricular assistant device
LVNC	left ventricular noncompaction cardiomyopathy
microRNA	micro ribonuclein acid
MRPL3	mitochondrial ribosomal protein L3
MYBPC3	myosin binding protein C, cardiac
MYH6	myosin heavy chain 6
MYH7	myosin heavy chain 7
MYH11	myosin heavy chain 11
MYLK2	myosin light chain kinase 2
MYO6	myosin VI
MYOM1	myomesin 1
MYPN	myopalladin
NEBL	nebulette
NEXN	nexilin F-actin binding protein
NGS	next-generation sequencing
NYHA	New York Heart Association
OBSCN	obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF
PDLIM3	PDZ and LIM domain 3
P	pathogenic
PCR	polymerase chain reaction
PKP2	plakophilin 2
PRKAG2	protein kinase AMP-activated non-catalytic subunit gamma 2
PSEN1	presenilin 1
PTSMA	percutaneous transluminal septal myocardial ablation
QTc	QT-interval corrected
RAF1	Raf-1 proto-oncogene, seine/threonine kinase

RANGRF	RAN guanine nucleotide release factor
RBBB	right bundle branch block
RBM20	RNA binding motif protein 20
RCM	restrictive cardiomyopathy
RNA	ribonuclein acid
RYR2	ryanodine receptor 2
SCD	sudden cardiac death
SCN10A	sodium voltage-gated channel alpha subunit 10
SCN1B	sodium voltage-gated channel beta subunit 1
SCN4B	sodium voltage-gated channel beta subunit 5
SCN5A	sodium voltage-gated channel alpha subunit 5
SGCD	sarcoglycan delta
SOS1	SOS Ras/Rac guanine nucleotide exchange factor 1
SUDS	sudden unexpected death syndrome
SQT	short QT syndrome
SYNE1	spectrin repeat containing nuclear envelope protein 1
SYNE2	spectrin repeat containing nuclear envelope protein 2
TASH	transcoronary ablation of septum hypertrophy
TCAP	titin-cap
TGA	transposition of the great arteries
TMEM43	transmembrane protein 43
TNNC1	trponin C1, slow skeletal and cardiac type
TNNI3	troponin I3, cardiac type
TNNT2	troponin T2, cardiac type
TPM1	tropomyosin 1
TRIM63	tripartite motif containing 63
TRPM4	transient receptor potential cation channel subfamily M member 4
TTN	titin
UV	unclassified variance/variant
VCL	vinculin
WES	whole exome sequencing
WGS	whole genome sequencing

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1 Introduction

Cardiovascular diseases (CVD) are one of the main problems in today's societies. The incidence of 2015 in the member countries of the European Society of Cardiology (ESC) was 11 million people and the prevalence of around 83,5 million. (1) Besides the significant causes for CVDs, the multifactorial composition of risk factors, the genetic background can lead to cardiac problems as well. These can appear in syndrome's like for example a Truncus arteriosus or a tetralogy of Fallot in Di-George-Syndrome, caused by a microdeletion on the long arm of chromosome 22. (2) For hypertrophic cardiomyopathies (HCM) or rhythmological problems like the long QT syndrome (LQT), many gene mutations are described likewise. Nevertheless, many symptoms which appear hereditary are still unknown. With establishing the new technology next-generation sequencing (NGS) with whole-genome/ exome sequencing (WGS/WES), many more mutations can be detected easier and faster. Thus, genetic testing increased in CVDs as nicely shown by Hofman et al. 2010 in the Netherlands. Their number of cardiogenetic testings increased from 25 patients in 1996 to more than 950 patients in 2008. (3) With this development, new phenotypes of already known CVD causing genes were found like Arndt et al. describe in their study from 2014. (4) They gave the example of SMAD3, which was already known for thoracic aortic aneurysm with premature osteoarthritis. (4, 5) Now, with NGS, it was possible to describe other forms of SMAD3 mutations with an aggressive form of vasculopathy with cerebrovascular problems. (4, 6–8) That genetic testing in CVDs is reasonable can nicely be shown by the Cardiac Inherited Diseases Registry New Zealand, discussed in the paper of Earle et al. 2018. This register includes 941 probands with suspected cardiac inherited diseases and their families. 68% underwent genetic testing and in total 23% a pathogenic (P) variant and in additional 6% a variant of uncertain significance (UV) could be found. (9) Therefore, genetic investigations in cardiology have their legitimacy.

At the Medical University of Graz, Austria, these patients are supported by the Diagnostic Research Institute of Human Genetics. This master thesis will generate an overview of the cardiogenetic cases analysed at this institute.

1.1 Clinics

This chapter will give an overview of the main CVDs with a genetic background already founded. To separate them, we use the typical terms of structural and rhythmological diseases. While structural diseases contain all non-coronary cardiac diseases with structural problems (10), rhythmological includes all types of changes in normal cardiac rhythm. The term ‘structural diseases’ includes i.a. hypertrophic cardiomyopathy (HCM), dilative cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), left ventricular noncompaction cardiomyopathy (LVNC) and arrhythmogenic right ventricular cardiomyopathy (ARVC). Under rhythmological diseases can be found i.a. the long QT-Syndrome (LQT), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), idiopathic ventricular fibrillation arrhythmia (IVFA) and the short QT-Syndrome (SQT). Other diseases not classified in one of these two terms can be, for example, the congenital heart disease (CHD) of transposition of the great arteries (TGA) or a situs inversus. (Fig. 1)

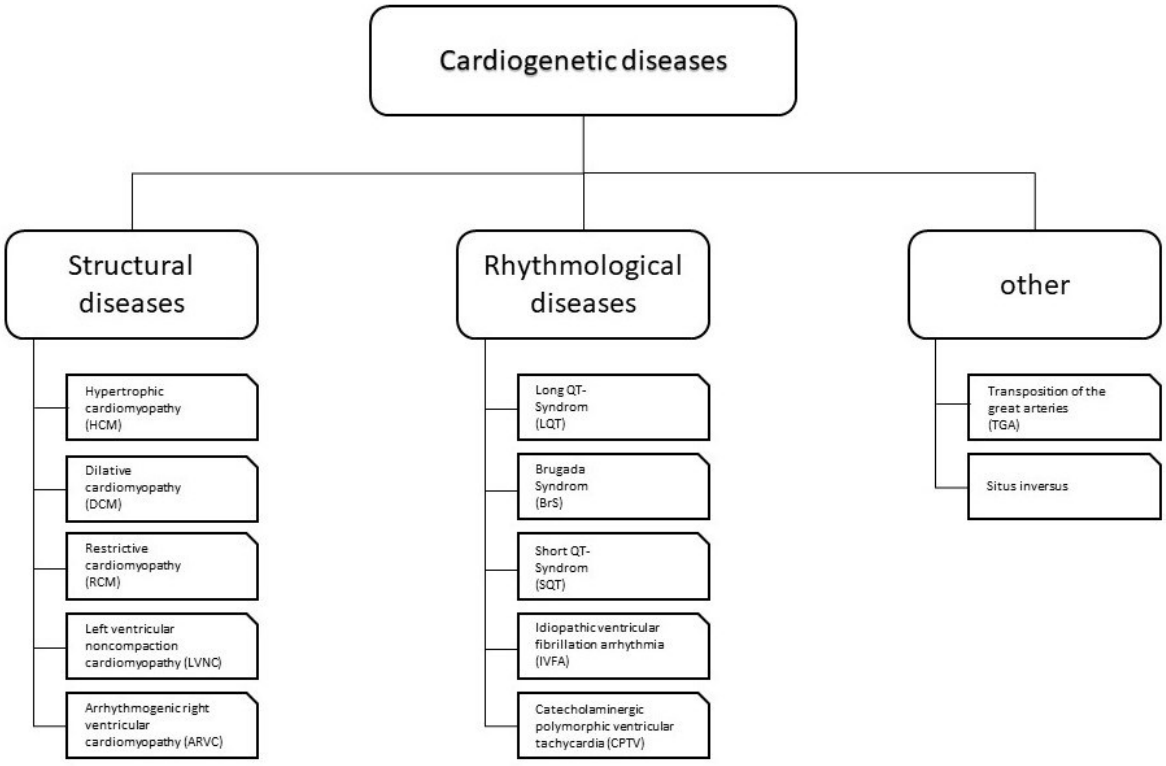


Figure 1: Differentiation of cardiogenetic diseases

1.1.1 Structural diseases

This chapter will give a clinical overview of the main structural diseases.

1.1.1.1 Hypertrophic cardiomyopathy

Primary hypertrophic cardiomyopathy (HCM) is defined by an increase of the ventricular wall thickness without dilation of the left ventricular. Important is that it is unexplainable by other reasons like amyloidosis, valve or coronary artery diseases (CAD). It is an isolated form not exclusively explained by abnormal loading conditions. The increase of all among left ventricular wall thickness results in diastolic dysfunction. (11–17) The incidence of HCM is 19/100,000 with a prevalence of around 200/100,000 people. (13, 17) Common symptoms are dyspnoea, angina pectoris and sometime swindle and syncope. (16, 17) HCM will be diagnosed by anamneses, ECG and especially 2-dimensional (2D) echocardiography. (16, 18, 19) The wall thickness can nicely be seen in the echocardiography, like in the picture from Jan et al. (Fig. 2). ESC Criteria for the diagnosis in adults is a wall thickness ≥ 15 mm in one or more segments of the left ventricular myocardium. For children, the criteria is a wall thickness with a z-score > 2. (15) HCM is the most common hereditary heart disease, occurring in over 50% of cases in a familiar setting. (13) Therefore, the ESC gives following IB recommendation for genetic testing: *"In the presence of symptoms and signs of disease suggestive of specific causes of HCM, genetic testing is recommended to confirm the diagnosis."* (15) In the beginning, HCM will be treated conservatively with medications for reducing the heart rate and the volume, for example, by beta-blocker or calcium antagonists. (13, 14) For reducing the wall thickness when necessary interventional therapy called percutaneous transluminal septal myocardial ablation PTSMA (=TASH) is possible or a surgical reduction of the myocardium can proceed. (13, 20, 21)

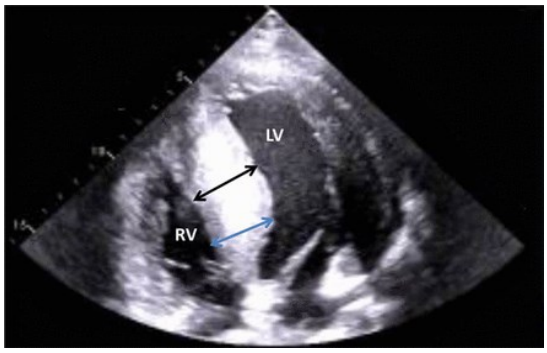


Figure 2: Echocardiography HCM, apical view
RV: right ventricle, LV: left ventricle; arrow marks the hypertrophy of the septal wall

1.1.1.2 Dilative cardiomyopathy

Dilative cardiomyopathy defines an enlargement of the ventricles, most the left, with a hemodynamic insufficiency, especially in the systole. It comes along with a reduction of the left ventricular ejection fraction (EF). Important is the absence of CAD and abnormal loading conditions as reasons for the dilatation. (11–14, 22) The incidence of DCM is 6/100,000 and the prevalence 36/100,000, with men two times more affected than women. (13, 17) Newer papers estimate a prevalence about >1 of 250 people respectively 1/500 individuals. (23, 24) Compared to the HCM, a DCM has normal wall thicknesses or following, a wall thinning. (17, 19) DCM accounts for 40% of cardiomyopathies. Symptoms are the same as for heart failure. Combined with a reduction of EF, the phenotype is the same as for the heart failure type called HFrEF (heart failure with reduced ejection fraction). (25) HFrEF has an ejection fraction < 40%. (26) The low EF is the reason for the common symptoms from DCM and heart failure like dyspnoea, oedema, especially in the lung and reduced exercise tolerance, categorised with the NYHA classification. (11, 16, 26) Dilation and ejection fraction are measured by 2D echocardiography are the easiest way to diagnose DCM (Fig.3). Normal values for the left ventricular are in females a diameter of 45.0 ± 3.6 mm or a volume of 45 ± 8 ml/m² body surface area (BSA) in diastole. In males, the values are 50.2 ± 4.1 mm or 54 ± 10 ml/m² BSA. (27) Genetic testing is recommended in idiopathic DCM, which counts 50% of cases. Ponikowski et al. claim in the ESC Guidelines for heart failure, that until this time, over 50 genes were identified with an association to DCM. (22, 26) First-line therapy is the same as for heart failure. It starts with reducing cardiovascular risk factors, like weight gain, alcohol and nicotine, (IC recommendation) and combines with medical treatment like a reduction of hypertension (IA recommendation) and rhythm control. (11, 26) Additionally, non-surgical devices, like implantable cardioverter-defibrillator (ICD) or cardiac resynchronization therapy (CRT), can be necessary to prevent cardiac arrest. (26) If necessary, surgical treatments like the reconstruction of the left ventricular or left ventricular assistant devices (LVAD) can be include. The last option for heart failure treatment is heart transplantation. (20, 21)

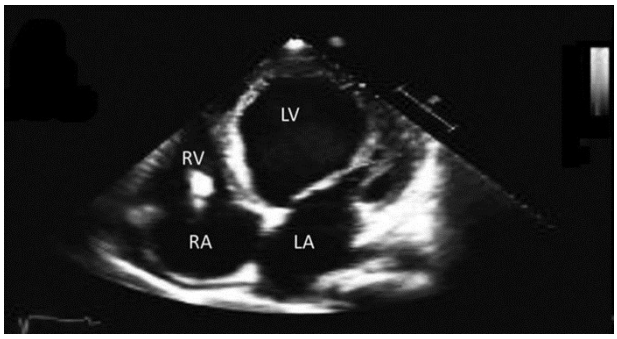


Figure 3: Echocardiography DCM, apical view
RV: right ventricle, RA: right atrium (enlarged),
LV: left ventricle (enlarged), LA: left atrium (enlarged)

1.1.1.3 Other cardiomyopathies

Restrictive cardiomyopathy

Restrictive cardiomyopathy is a diastolic dysfunction without a dilated ventricle or enlarged wall thickness. It is a rare disease with unknown prevalence. RCM is differentiated between non-infiltrative and infiltrative forms. The non-infiltrative form includes the idiopathic RCM and the endomyocardial fibrosis. Amyloidosis, Sarcoidosis or Morbus Fabry, for example, counts to the infiltrative form. Typical echocardiographical findings are the normal dimensions of the ventricles and the normal diameter of the wall, combined with enlarged atriums (Fig. 4). In Doppler sonography, the diastolic dysfunction can be seen in parallel with normal systolic function. Genetic causes for RCM are already recognised. Treatment is symptomatic and inspired by the once for heart failure, including heart transplantation as last option. (11–13, 16, 17, 19, 28–30)

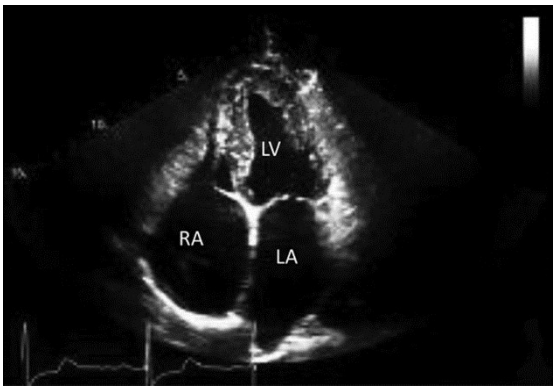


Figure 4: Echocardiography RCM, apical view
RA: right atrium (enlarged), LA: left atrium (enlarged)
LV: left ventricle

Left ventricular noncompaction cardiomyopathy

The left ventricular noncompaction cardiomyopathy (LVNC) is a rare disease with a prevalence of 0.0014 – 0.17 %. It accounts for 9% of infantile cardiomyopathies. It is characterised by in beginning normal dimensions of the ventricular. Its ventricular wall has prominent trabeculae, let it looks spongy (Fig. 5). In echocardiography, the left ventricular wall shows two layers, a compact one and a non-compact. One criterion for LVNC is a compact layer thickness of < 8mm. The non-compact layer is mostly found in the apex and the mid-ventricular lateral or inferior wall. Normally we find this kind of trabeculation in the early embryonic development, before the 8th week. Because of this, a genetic reason is still discussed. Symptoms and therapy are the same as for heart failure, described before. (13, 16, 19, 28, 31–35)

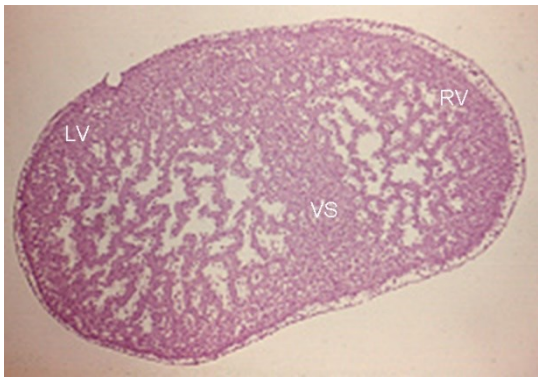


Figure 5: Histology slice LVNC
LV: left ventricle, VS: ventricular septum,
RV: right ventricle

Arrhythmogenic right ventricular cardiomyopathy

The arrhythmogenic right ventricular cardiomyopathy (ARVC) is also called arrhythmogenic right ventricular dysplasia. Its prevalence is estimated with 1:2000 to 1:5000 people. ARVC is known as a genetic disorder. 10-20% of all sudden cardiac death (SCD) is due to ARVC. It results in a fibrolipomatous degeneration of the right ventricle. This leads to a dilated right ventricle together with rhythmological changes. Main findings are young patients with palpitations, unexplained syncope or SCD. For diagnosis, a presence of right ventricular dysfunction is necessary together with histological findings or echocardiographic abnormalities like dilation of the right ventricle >32 mm (long axis) and akinesia, dyskinesia or aneurysm of the wall. In 20-30% of the patients, the abnormal electrical forwarding can be seen in the electrocardiogram (ECG) by an additionally epsilon wave between QRS-complex and T wave (Fig. 7). Therapy is based on the guidelines for right ventricular heart failure. (11–13, 16, 19, 28, 36, 37)

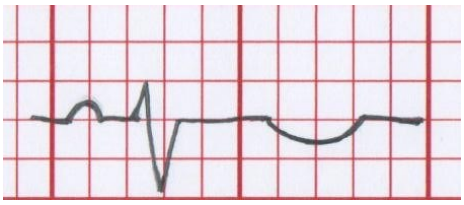


Figure 6: Normal ECG in ECG recording V1

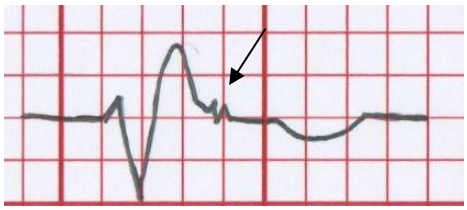


Figure 7: ECG V1 with epsilon wave (arrow)

1.1.2 Rhythmological diseases

This chapter will give a clinical overview of the central rhythmological diseases correlated with genetic mutations.

1.1.2.1 Long-QT Syndrome

The Long-QT syndrome (LQT) is an ion-channel disorder. This channelopathies lead to a different activation of the ion channels and therefore to a prolonged repolarisation. It can be seen by an extended QT-interval (Fig. 9). The QT-time is corrected to the frequency of the heart and is called corrected QT interval (QTc). Average values for the QTc are for females 440 ms ± 15% and for males

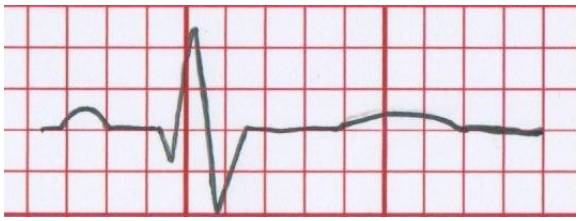


Figure 8: Normal ECG in ECG recording Einthoven I

390 ms ± 15%. In 90-95% of congenital LQT, a mutation in potassium channels for a cell inflow is the cause of the QT interval prolongation. Over 17 types of LQT are already known. Symptoms are tachycardia, palpitation and SCD. Reason of the SCD begins with tachycardia, which can result in a problematic rhythm called Torsade de Pointes, induced by a too early polarisation in the repolarisation period (Fig. 10). Due to this problem, pharmacological treatment will reduce heart rate and stabilise the repolarisation. Relevant is the carefully view on additional medications as an aside of a QT-time prolongation. Additionally, an ICD can be necessary (11, 13, 14, 37–40)

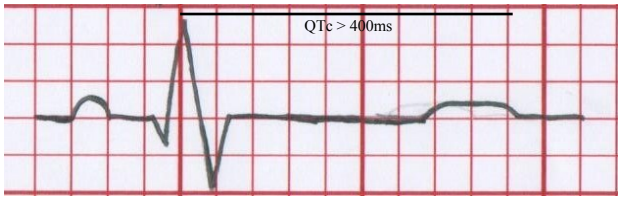


Figure 9: ECG Einthoven I with QT interval prolongation

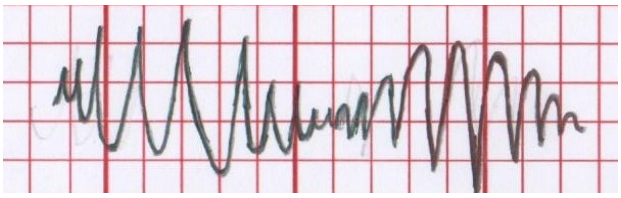


Figure 10: ECG Einthoven I: Torsade de Pointes

1.1.2.2 Brugada Syndrome

The Brugada Syndrome (BrS) is a channelopathy with an unknown incidence. One primary characteristic is the aborted SCD of heart-healthy patients. Interestingly, more men are affected than women (8:1). Although over 19 types are known, 25% of the cases are BrS type 1, which is a mutation in a sodium channel at the right ventricular outflow tract. In ECG, we can see an incomplete or complete right bundle branch block (RBBB) and an ST elevation in the recordings V1-V3 (Fig.11; normal ECG V1, see Fig. 6 page 6). No effective pharmacological therapy exists; therefore, an ICD is the only possible treatment. (11, 13, 14, 37, 39, 41)

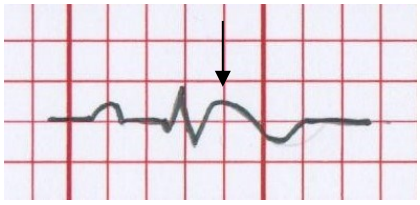


Figure 11: ECG Einthoven I: RBBB and ST elevation (arrow)

1.1.2.3 Other rhythmological diseases

Short-QT syndrome

An abnormally short QT interval characterises the short QT syndrome (SQT) by normally structured myocardium. Its primary symptoms are SCD or aborted SCD, family history of SCD and syncope. Diagnosis criteria is a short QT interval of QTc <340 ms (1C

recommendation ESC guidelines) and a large and pointed T wave (Fig. 12; normal ECG Einthoven I, see Fig. 8 page 6). At least six types are known, leading to a gain-of-function mutation in potassium channels or loss-of-function mutations in calcium channels. First-line therapy is an ICD. (11, 13, 14, 39, 42–45)

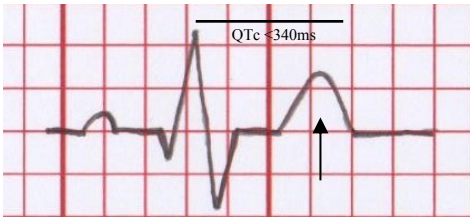


Figure 12: ECG Einthoven I with short QT interval and pointed T wave (arrow)

Idiopathic ventricular fibrillation arrhythmia

Idiopathic ventricular fibrillation arrhythmia (IVFA) is a rare disease with a nearly unknown genetic origin. It is defined as ventricular fibrillation without any reason like channelopathies or structural diseases. Therefore, it is considered a diagnosis of exclusion, which previously included some unknown channelopathies like SQT. IVFA accounts for 10% of the victims of SCD. There are some mutations associated with IVFA nowadays. In ECG, IVFA shows the same characteristics, like common ventricular fibrillation (Fig. 13; normal ECG Einthoven I, see Fig. 8 page 6). It is treated by antiarrhythmic medications or catheter ablation. (39, 46–48)

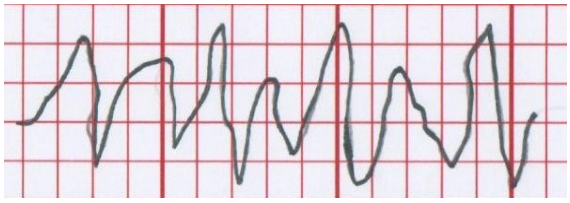


Figure 13: ECG Einthoven I: ventricular fibrillation

Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmia caused by physical activity or emotional stress. The prevalence is estimated to be around 1/10,000 people. It is characterized by syncope, dizziness and SCD. The genetical background is a mutation in calcium-related proteins in the sarcoplasmic reticulum, with 50-55% of the cases in the *RyR2* Gen. With a 1C recommendation of the ESC guidelines, CPVT is diagnosed by normal heart structure and regular ECG at rest and exercise- or emotional-induced bidirectional or polymorphic ventricular tachycardia (Fig. 14; normal ECG Einthoven I, see Fig. 8 page 6). Treatment is a prophylactic implementation of an ICD. (39, 45, 49, 50)

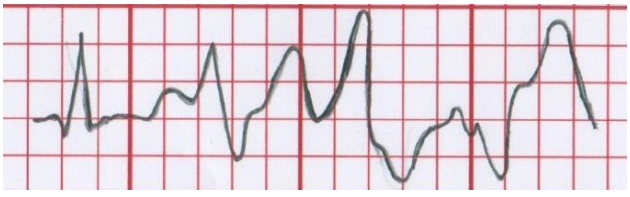


Figure 14: ECG Einthoven I: CPVT

1.1.3 Other cardiac diseases

This chapter will focus on two further hereditary cardiac diseases, even though there are a lot more. Most of them are characterized as congenital heart diseases (CHD).

Transposition of great arteries

The transposition of the great arteries (TGA) is a potentially lethal CHD. It accounts for around 5% of all CHDs. The pulmonary artery arises from the left ventricle, while the artery arises from the right ventricle (Fig. 15). According to this situation, the body circuit and the pulmonary circuit are separated, which results in less oxygenation of the whole body. The TGA is often associated with hereditary syndromes like heterotaxia, but non-syndromic types are existing. The TGA is corrected by an early arterial switch or Rastelli operation. (2, 21, 51–54)

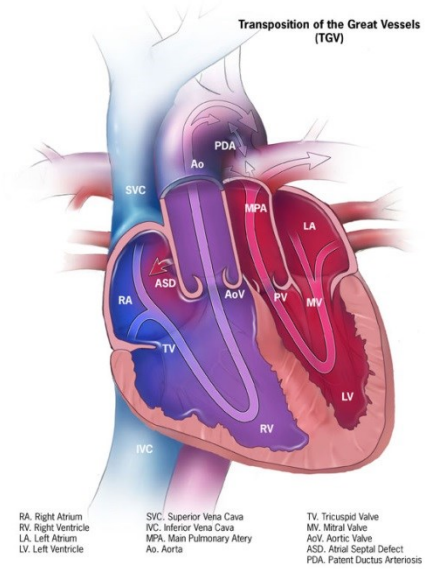


Figure 15: Scheme of TGA

Situs inversus

A situs inversus is a total left-right switch of all organs (Fig. 16). Usually, this has no pathogenic consequences. The situs inversus is developing during embryogenesis, for example by primary ciliary dyskinesia. The situs inversus can establish spontaneous in one person or as a family constitution. (55–57)

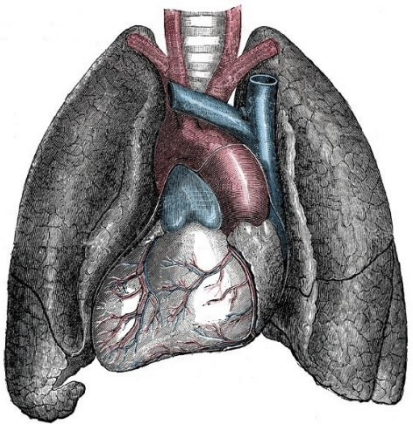


Figure 16: Situs inversus of lung, heart and great arteries

1.2 Molecular background

This chapter will give an overview of the types of gene mutation and their consequences, the methods of testing them and how mutations can be classified.

1.2.1 Types of mutations

At the genetic level in exons, we find point mutations like substitution, deletion and insertion. Substitutions can result in different types of mutation (Fig. 17). Even by changing a base, the result can be the implantation of the same amino acid. This type of mutation is called a silent mutation because the protein product does not change. A base exchange witch results in the implementation of a different amino acid is known as a missense mutation. Depending on the following protein structure, this can result in different consequences. Nevertheless, the protein may be still working like before. Otherwise, it can result in a loss-of-function or a gain-of-function action. A base exchange can also lead to an early stop of the protein sequence. It is called a nonsense mutation. Depending on the position of the new end, it is possible that the protein is still a bit functional or lost its function completely. A deletion or an insertion of bases are called in a frameshift mutation. It occurs to a base postponement which results in a different amino acid sequence. This can be elongated or stopped earlier as well. Is a triplicate of bases concerned in the sequence, this can lead to an in-frame mutation. In deletions one amino acid is missing, in an insertion, an

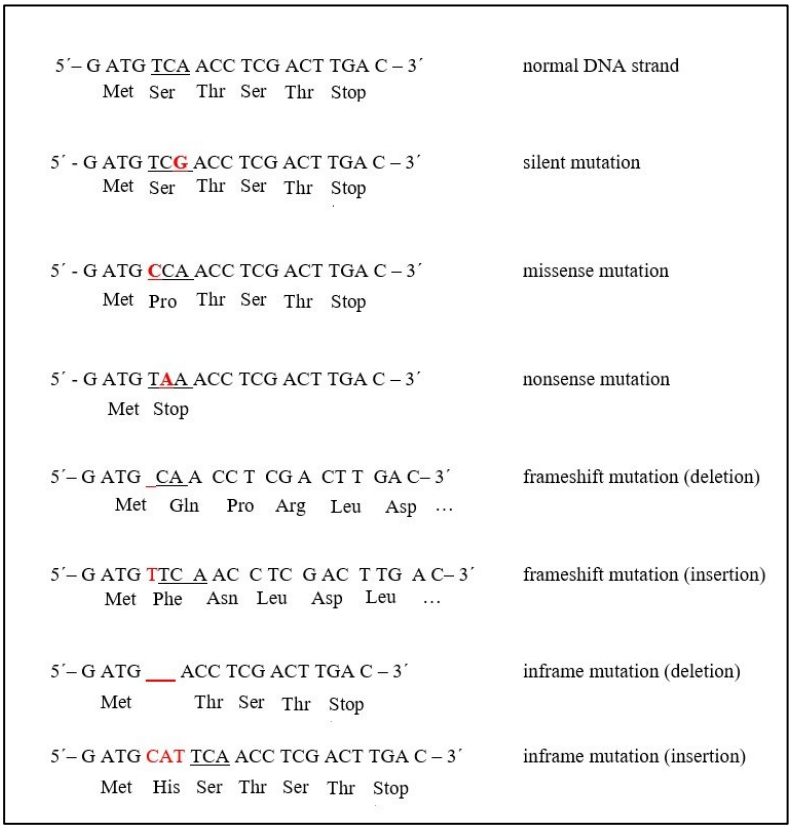


Figure 17: Examples of different types of mutations

additional amino acid can be found. The rest of the protein sequence is the same as before. Depending on the function of the amino acid within the protein and its place, it can result in complicate consequence for the protein function or does nothing. (58, 59)

Another possibility for modified functions of proteins can result from mutations in regulatory parts of the genes or at splice sides. Mutations in the promoter region, enhancers, silencers or like in the 3' UTR region can lead to loss-of-function or gain-of-function. Mutations at splice sides can ensue to wrong exon splicing, which will produce faulty proteins. Mutations in intron regions are still under research. Formerly known as junk, they are now partly known as regions for microRNA and thus for regulatory roles. (58, 59)

1.2.2 Molecular diagnostic methods

This chapter gives an overview of the two main sequencing methods for detecting single-base mutations.

1.2.2.1 Sanger sequencing

Sanger sequencing is a method to detect every base of a strand of max. 1000 base pairs (bp) based on a polymerase chain reaction (PCR). It starts with a multiplication of the deoxyribonucleic acids (DNA) part of interest by PCR. Then the actual sequencing can start. For every base exists its deoxyribonucleoside triphosphate (dNTP) as normal for synthases of the DNA strand. Additionally, the reagents contained dideoxyribonucleoside triphosphate (ddNTP). Every ddNTP is labelled with a different fluorescence colour. After a ddNTP is integrated into the DNA, this strand cannot be elongated anymore, and the sequence stops with this particular ddNTP. Statistically seen, each position on the DNA is stopped at least once by a ddNTP. Afterwards, capillary gel electrophoresis can sort them by length and detect the fluorescence of every strand. It results in the sequence of the DNA (Fig. 18). (58–61)

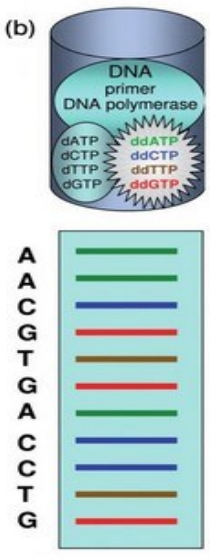


Figure 18: Scheme of reagents and detected sequence of Sanger

1.2.2.2 Next-generation sequencing

Next-generation sequencing (NGS) describes a group of new sequencing methods for massively and deep sequencing. With NGS, it is possible to sequence a human genome within one day and for around 1000 US$. (62) Several technics exists, nevertheless, the principle for all 2nd generation NGS systems are the same, library preparation, cluster amplification, sequencing and data analysis. Most commonly used is the technology from the company Illumina® and therefore, this technology is described further. During the library preparation, the DNA of interest (e.g. specific gene) or the whole genome will be prepared by fragmentation into smaller single-strand DNA, and adapters will be stick to their ends (Fig. 19A). After this step, the DNA fragments are rinsed over a flow cell. The flow cell consists of many adapters fitting to the once attached to the DNA in the library preparation. The DNA binds to the flow cell. By bridge amplification, the amount of DNA with the same sequencing is increasing in this spot, and they build clusters (Fig. 19B). The third step is the sequencing, base by base. Every nucleotide is labelled with a different fluorescence. The flow cell is flooded with all of them at once. The nucleotides are inserted at the right place, not more than one at the same time. Then the fluorescence is detected as pictures (Fig. 19C). Afterwards, the fluorescence of this turn is washed away, and the flow cell is flooded again with all nucleotides. This cycle is done several times. In the last step, the computer put all pictures together to create a sequence for every cluster. These short sequences are called reads. All reads are mapped to a reference genome (Fig. 19D). As a result, single mutations can be observed. (58, 59, 63–66) All founded mutations were filtered by allele frequencies, due to the expected inheritance and ClinVar classification to name some examples of this prioritization of variances.

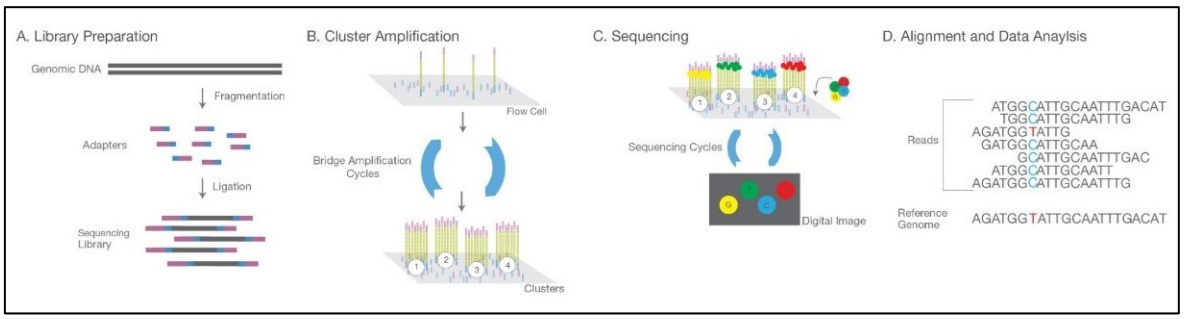


Figure 19: NGS steps Illumina

1.3 Classification of mutations

Mutations are classified by criteria published by the American College of Medical Genetics and Genomics (ACMG) together with the Association for Molecular Pathology. These criteria characterise the mutation by its place in the gene, allele frequencies, recent reports and many other aspects. These characteristics can be arranged into a list of 30 severities from very pathogenic variances to absolute benign once (Tab. 2). To make it easier to classify a specific mutation, the 30 severities are organized into five groups; pathogenic (P), likely pathogenic (LP), likely benign (LB), benign (B) and unclassified variation (UV) (Tab. 1). (67, 68)

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

Table 1: ACMG criteria combined for classification

_ PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

_ PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
_ PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
_ PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
_ PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls
_ PP1 (Strong evidence) Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

_ PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
_ PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
_ PM3 For recessive disorders, detected in trans with a pathogenic variant
_ PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants
_ PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
_ PM6 Assumed de novo, but without confirmation of paternity and maternity
_ PP1 (Moderate evidence) Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

_ PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease
_ PP2 Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
_ PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
_ PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology
_ PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

_ BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease
_ BP2 Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern
_ BP3 In-frame deletions/insertions in a repetitive region without a known function
_ BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)
_ BP5 Variant found in a case with an alternate molecular basis for disease
_ BP6 Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation
_ BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

_ BS1 Allele frequency is greater than expected for disorder
_ BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age
_ BS3 Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing
_ BS4 Lack of segregation in affected members of a family

_ BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Table 2: ACMG criteria

2 Material and Methods

2.1 Study design

Aim of this study is to generate an overview of cardiogenetic diseases, analysed at the Diagnostic and Research Institute for Human Genetics at Medical University of Graz. This work is a reconditioning of analysis in patients with inherited heart diseases. Data were initially organised in a Microsoft© Excel sheet. Only numerical and fully anonymised data, not including any personal information nor including detailed information of particular genetic variants, were used for this thesis, and therefore, no ethics committee vote was necessary.

2.2 Study collective

Included were 288 patients with genetic analysis in order of a cardiovascular disorder recruited from 2015 to 2018. The genetic analysis was performed in the Diagnostic and Research Institute for Human Genetics at Medical University of Graz.

2.3 Methods

2.3.1 Methods of original analyses

At the beginning, the library preparation was performed using the HaloPlex Target Enrichment System by Agilent Technologies®. The investigators must select the genes of interest, and the right sequences for the gene amplification must be announced. The hybridization of the DNA fragments is done as circulated DNA. (69–71)
Afterwards, library preparation was done with the Illumina® kit TruSight™ One. This kit includes amplification reagents for > 4800 genes associated with specific clinical phenotypes. With many genes tested, we only have a look at genes of interest while the rest is still blinded. Our genes of interest are listed in Table 3. (72)

With both library preparation steps, the following steps of NGS were the same. The analysis was performed on an Illumina® MiSeq™. The analysis was done with the software VariantStudio™ by Illumina®.

Cardiac Phenotypes	Cardiac Genes
Long-QT-Syndrome (LQT)	KCNH2, KCNQ1, SCN5A, AKAP9, ANK2, CACNA1C, CALM1, CALM3, CAV3, KCNE1, KCNE2, KCNE3, KCNJ2, KCNJ5, SCN4B, SNTA1
Short-QT-Syndrome (SQT)	KCNH2, KCNJ2, KCNQ1, CACNA2D1, CACNB2
Brugada-Syndrome (BrS)	SCN5A, SCN10A, ABCC9, CACNA1C, CACNA2D1, CACNB2, GPD1L, HCN4, KCND3, KCNE1L, KCNE3, KCNH2, KCNJ8, PKP2, RANGRF, SCN1B, SCN2B, SCN3B, SCNN1A, TRPM4
Idiopathic ventricular and atrial fibrillation (IVFA)	KCNA5, KCNE2, ABCC9, DSC2, GATA4, GATA6, GJA5, JPH2, KCND3, KCNE1, KCNE1L, KCNE3, KCNJ2, KCNJ8, KCNQ1, LMNA, NKX2-5, NKX2-6, NPPA, NUP155, SCN10A, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, TNNI3
Atrioventricular blockage	SCN5A, CALM1, DES, GJA5, HCN4, KCNA5, KCNE2, KCNJ2, KCNQ1, LMNA, NKX2-5, PRKAG2, SCN1B, TBX5, TRPM4
Catecholaminergic polymorphic ventricular tachycardia (CPVT)	CASQ2, KCNJ2, RYR2, CALM1, CALM3, LMNA, TRDN
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	DES, DSC2, DSG2, DSP, JUP, PKP2, TMEM43, CTNNA3, LMNA, RYR2, TGFB3
Hypertrophic cardiomyopathy (HCM)	MYBPC3, MYH7, TNNI3, TNNT2, TPM1, ACAD9, ACTC1, ACTN2, ANKRD1, CALR3, CAV3, COX15, CRYAB, CSRP3, CTF1, DES, FHL1, FHL2, FLNC, GAA, GLA, ILK, JPH2, KLF10, LAMA4, LAMP2, LDB3, MRPL3, MYH6, MYL2, MYL3, MYLK2, MYO6, MYOM1, MYOZ2, MYPN, NDUFV2, NEBL, NEXN, PDLIM3, PLN, PRKAG2, RAF1, RYR2, SCN5A, TCAP, TNNC1, TTN, TTR, VCL
Dilatative cardiomyopathy (DCM)	LDB3, LMNA, MYBPC3, MYH7, PLN, TNNT2, ABCC9, ACTC1, ACTN2, ANKRD1, ATP2A2, BAG3, CHRM2, CRYAB, CSRP3, CTF1, DES, DMD, DNAJC19, DSC2, DSG2, DSP, DTNA, EMD, EYA4, FKTN, FLNC, ILK, LAMA2, LAMA4, LAMP2, MYH6, MYL2, MYL3, MYOM1, MYPN, NEBL, NEXN, OBSCN, PDLIM3, PSEN1, PSEN2, RAF1, RBM20, SCN5A, SDHA, SGCB, SGCD, SYNE1, SYNE2, TAZ, TBX20, TCAP, TMPO, TNNC1, TNNI3, TPM1, TTN, TTR, VCL
Left ventricular non-compaction cardiomyopathy (LVNC)	LDB3, ACAD9, ACTC1, CASQ2, DTNA, LMNA, MYBPC3, MYH7, NEBL, PRKAG2, RYR2, TAZ, TNNT2, TPM1
Restrictive cardiomyopathy	MYH7, TNNI3, ACTC1, CSRP3, DES, FLNC, GLA, LDB3, LMNA, MYBPC3, MYL2, MYOZ2, MYPN, PLN, TAZ, TCAP, TNNC1, TNNT2, TPM1, TTN
Sudden Infant Death Syndrome (SIDS), Sudden Unexplained Death Syndrome (SUDS)	KCND3, KCNH2, KCNQ1, RYR2, SCN5A, AKAP9, ANK2

Table 3: Genes of interest

2.3.2 Methods for this thesis

This study used Microsoft® Excel 2019 (Microsoft Corporations) for the statistical line-up of anonymised data, as well as for graphic illustrations.

3 Results

3.1 Collective

This study includes 288 patients at the timepoint 18.05.2018. Herein there were 179 males and 109 females (Fig. 20). The age was from 4 weeks up to 78 years, but not known in 8 patients (Fig. 21).

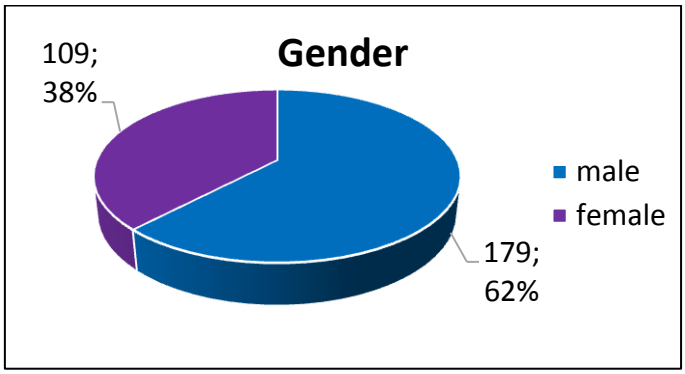


Figure 20: Gender distribution

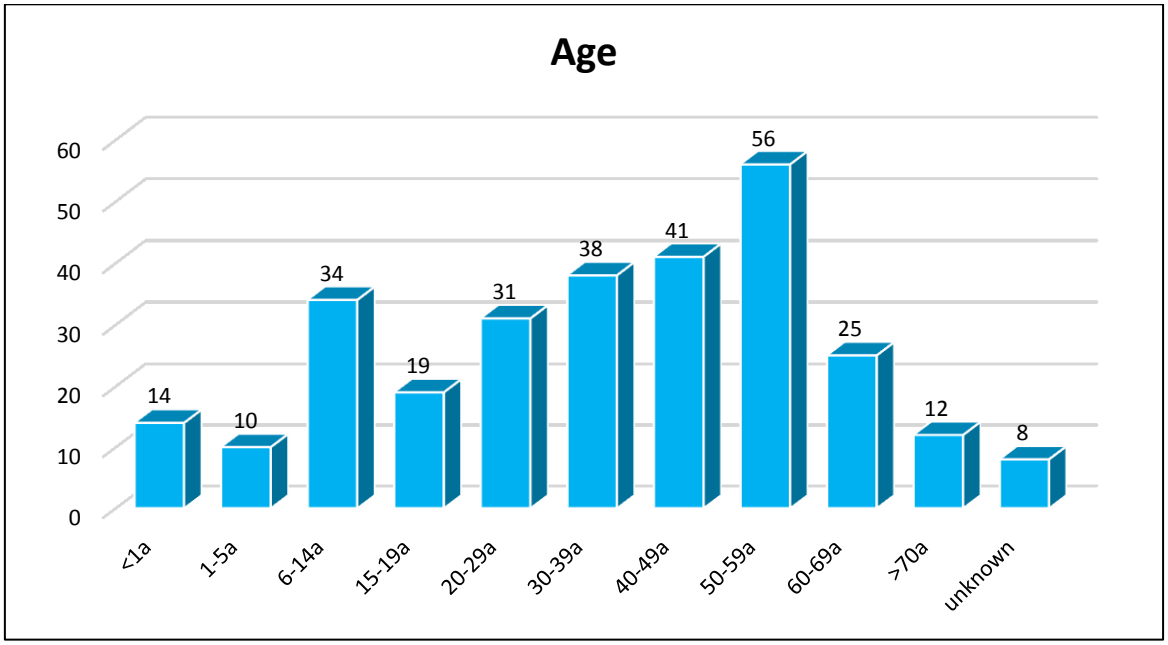


Figure 21: Age distribution

Indications were separated between structural heart diseases, rhythmological heart diseases or both. In 81 patients, no indication was available, 105 patients had structural diseases as an indication, 88 rhythmological and 14 both (Fig. 22)

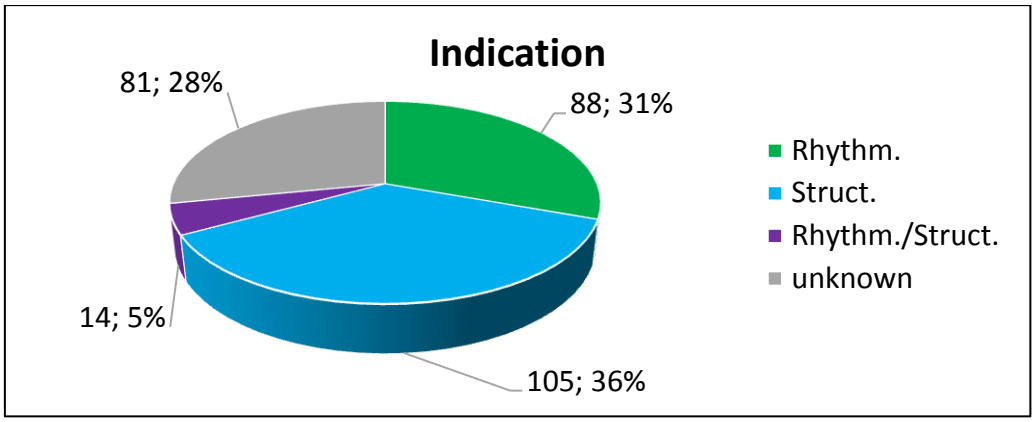


Figure 22: Indication

The most common allocation diagnosis was hypertrophic cardiomyopathy (HCM) with 90 patients, followed by long QT-syndrome (LQT) with 63 patients. Other diagnoses were dilative cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), short QT-syndrome (SQT), Brugada Syndrome (BrS), sudden unknown death syndrome (SUDS), arrhythmogenic right ventricular cardiomyopathy (ARVC), idiopathic ventricular fibrillation arrhythmia (IVFA), catecholaminergic polymorphic ventricular tachycardia (CPVT) and left ventricular noncompaction cardiomyopathy (LVNC). In 49 patients, more than one working diagnosis was given, or other diagnoses like, e.g. transposition of the great arteries (TGA), situs inversus or aneurysm was indicated (Fig. 23).

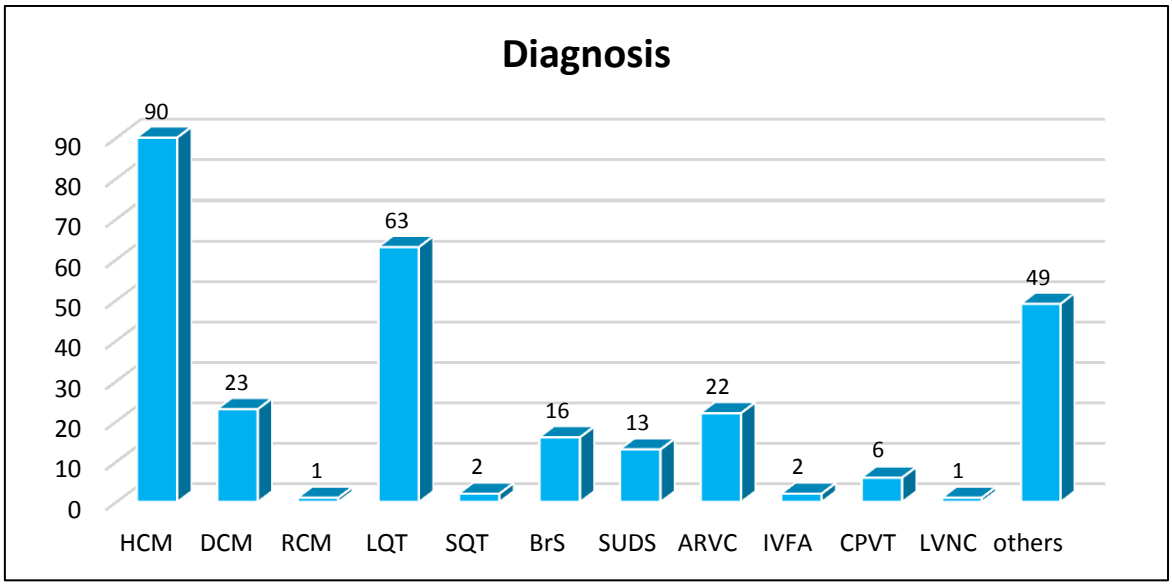
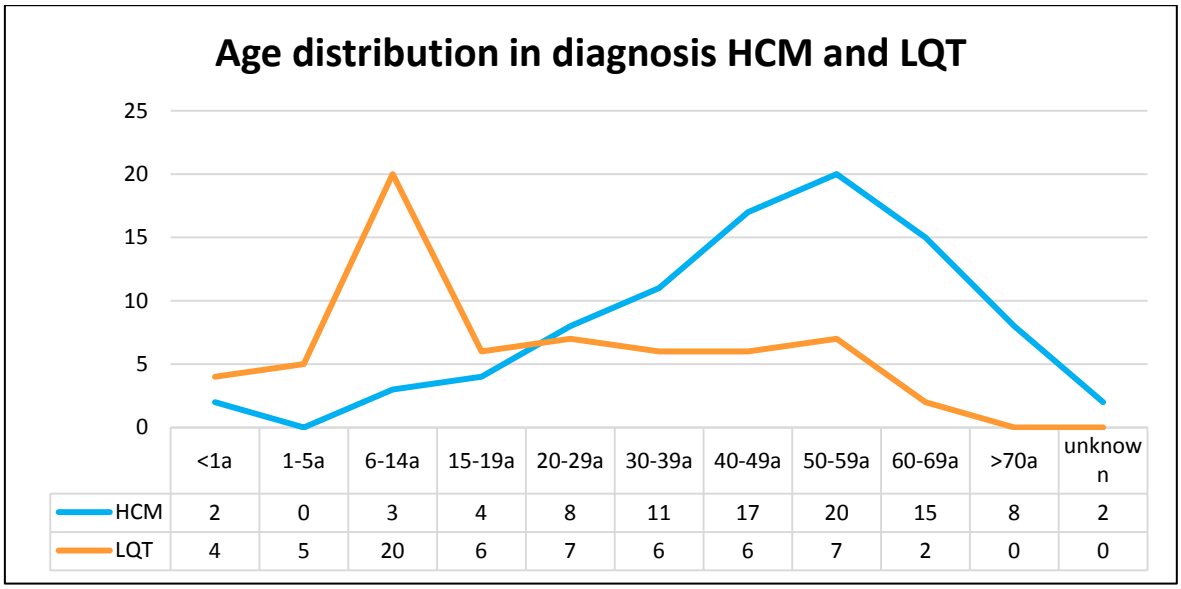


Figure 23: Allocation diagnosis

Interestingly, in the most common diagnosis HCM and LQT, the differentiation between the groups of ages were different. While in the group of HCM, the mean 46.30 ± 18.75 years with a peak in a group of the 50s, the mean of the LQT group was 23.97 ± 18.15 years with a peak in a group of the children between 6 to14 years (Fig. 24).



	<1a	1-5a	6-14a	15-19a	20-29a	30-39a	40-49a	50-59a	60-69a	>70a	unknown
HCM	2	0	3	4	8	11	17	20	15	8	2
LQT	4	5	20	6	7	6	6	7	2	0	0

Figure 24: Age distribution in diagnosis HCM and LQT

In these 288 patients, we found 582 mutations. No mutations were found in some cases, while we found in others up to 9 different mutations (Fig. 25).

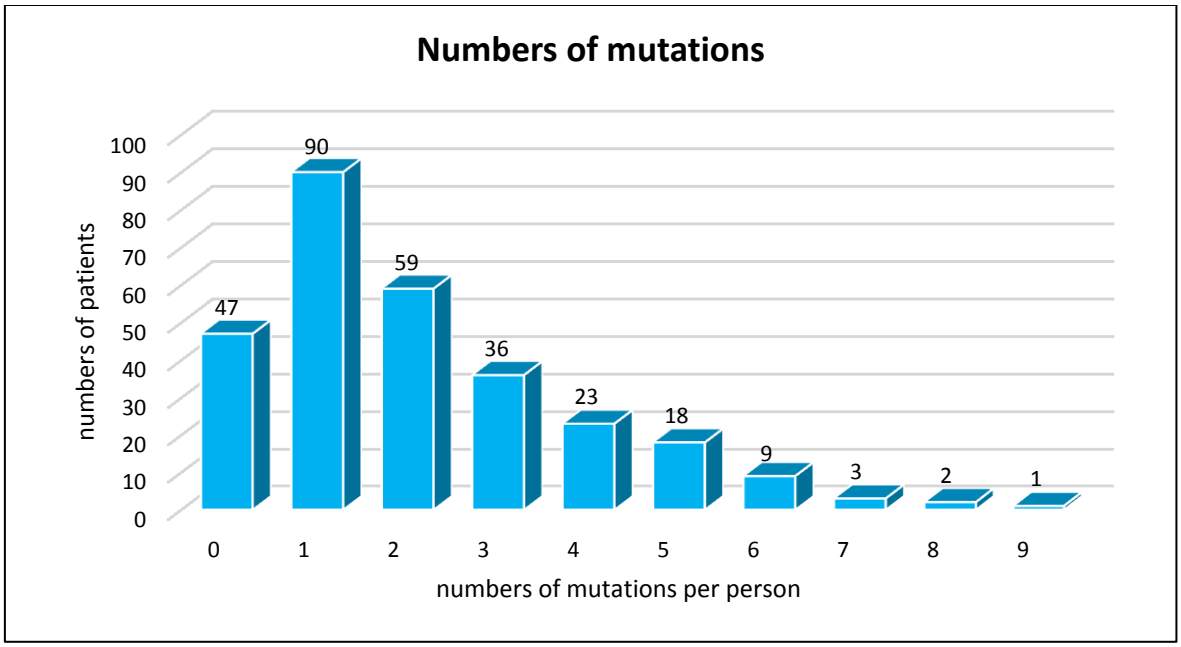


Figure 25: Numbers of mutation per person

In 76 cases, mutations declared the diagnosis, in another 74 cases, the investigator thought that these cases are likely solved, because of the mutation, the area of the change and the type of mutation. The remaining 138 cases are unsolved (Fig. 26).

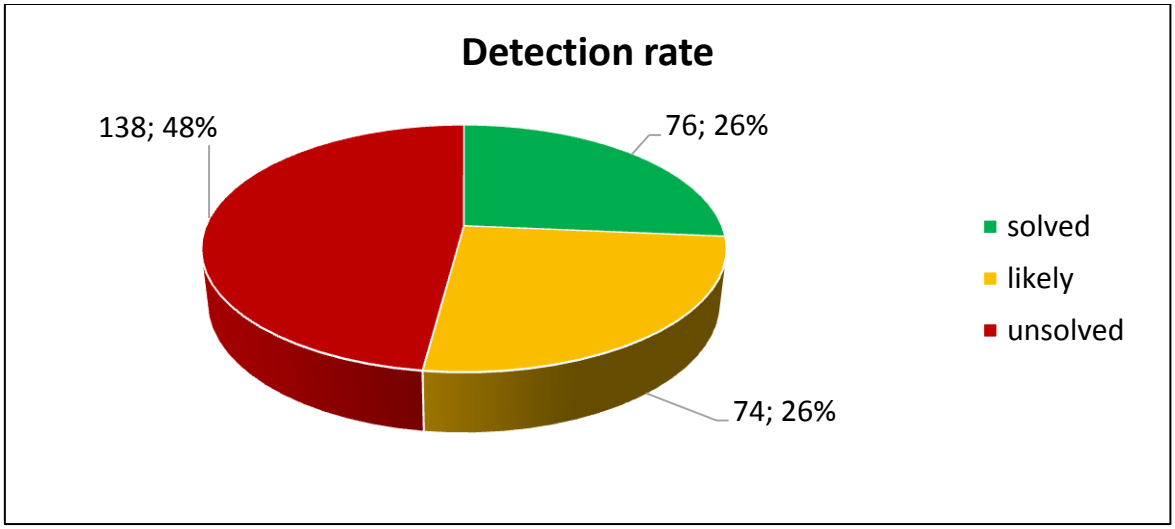


Figure 26: Detection rate overall

Compared to their allocation diagnosis, except for the case with just one or two patients, the group with HCM has the best detection rate.

In total, 179 cases of the 288 were male patients (62 %) compared to 109 female ones (38 %). In the subgroup of patients with HCM, this ratio is almost the same. Interestingly in the second most frequent subgroup, the ones for LQT, we found more female patients (54 % vs 46 %). The rest of the subgroups showed different distributions, but because of low numbers, no conclusions can be established (Tab 4).

	number	solved	likely	unsolved	male	female
overall	**288**	**76**	**74**	**138**	**179**	**109**
		26,39 %	25,69 %	47,92 %	62,15 %	37,85 %
HCM	90	40	24	26	60	30
		44,44 %	26,67 %	28,89 %	66,67 %	33,33 %
DCM	23	4	15	4	17	6
		17,39 %	65,22 %	17,39 %	73,91 %	26,09 %
RCM	1	1	0	0	0	1
		100 %	0 %	0 %	0 %	100 %
LQT	63	17	6	40	29	34
		26,98 %	9,52 %	63,49 %	46,03 %	53,97 %
SQT	2	0	0	2	2	0
		0 %	0 %	100 %	100 %	0 %
BrS	16	1	3	12	13	3
		6,25 %	18,75 %	75 %	81,25 %	18,75 %
SUDS	13	0	3	10	7	6
		0 %	23,08 %	76,92 %	53,85 %	46,15 %
ARVC	22	5	6	11	12	10
		22,73 %	27,27 %	50 %	54,55 %	45,45 %
IVFA	2	0	0	2	2	0
		0 %	0 %	100 %	100 %	0 %
CPVT	6	1	1	4	3	3
		16,67 %	16,67 %	66,67 %	50 %	50 %
LNVC	1	0	0	1	0	1
		0 %	0 %	100 %	0 %	100 %
others	49	7	16	26	34	15
		14,29 %	32,65 %	53,06 %	69,39 %	30,61 %

Table 4: Overview of detection rate and gender in a different diagnosis

3.2 Mutations

We found mutations in 85 genes within total 592 mutations, most prevalent in *TTN* with 160 events, followed by *MYBPC3* with 51 events (Fig. 29). Comparing the types of mutations, missense mutations are the most frequent with 385 events, followed by silent mutations with splice mutation component in question. Further types were frameshift mutation, splice mutations, a mutation which initialised stop in translation, deletions in-frame, insertion in-frame, intronic mutations, deletions, nonsense mutations as well as mutations in regulatory parts in question (Fig. 27). Compared to the classification (see chapter 1.3 Classification, pages 13-14) we found 76 pathogenic (P) mutations, 109 likely pathogenic (LP), 147 likely benign (LB) mutations and 250 unclassified variances (UV) as the highest amount. Ten mutations were not classified until the end of this study (Fig. 28).

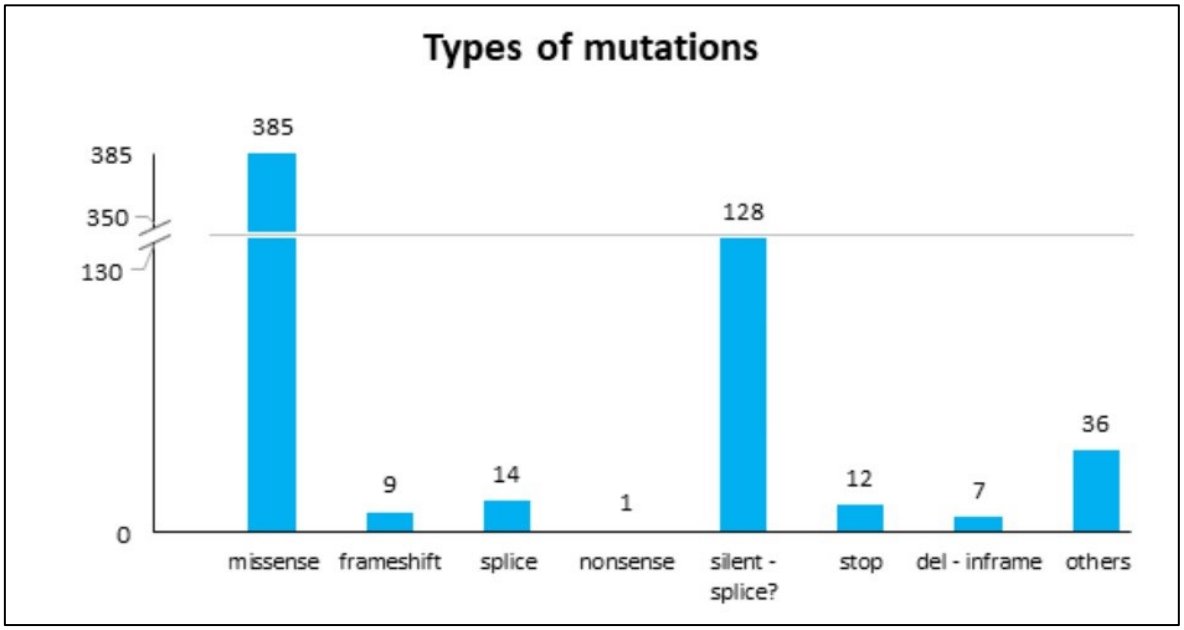


Figure 27: Types of mutations

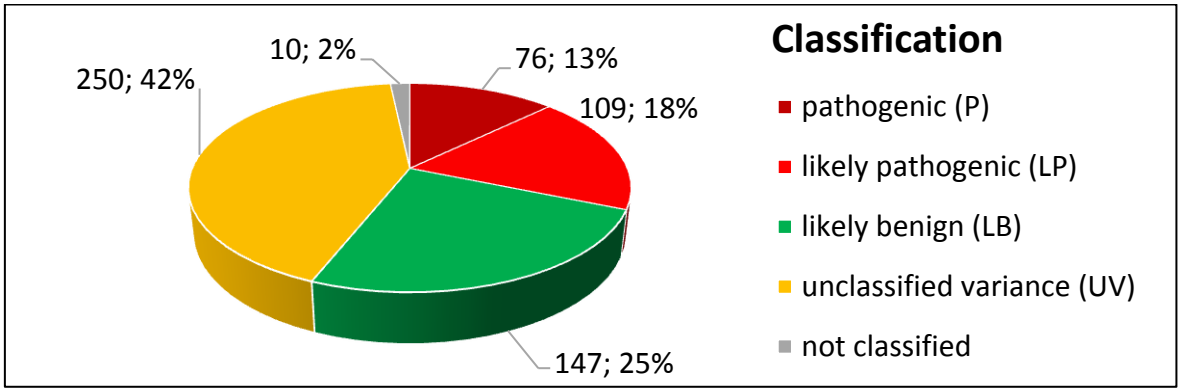


Figure 28: Classification

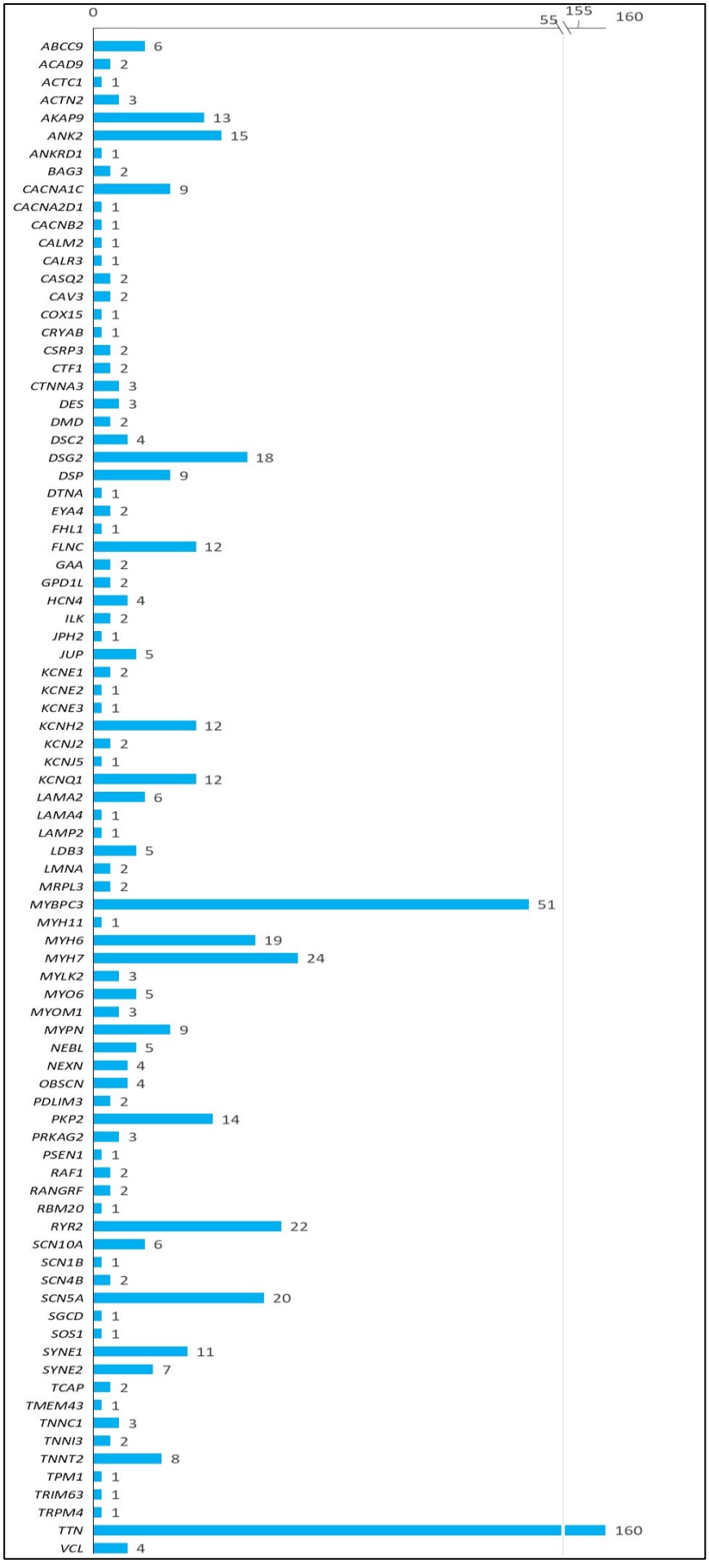


Figure 29: Numbers of mutations in different genes

Combining the two main types of mutations, missense and silent-splice? (silent-splice? : in first line as silent mutations declared, but eventually a splice mutation), with the classification, we can nicely observe, that while in both around 46% are UV, in the missense mutations group 37% are P or LP, in the silent mutations group 51 % are LB (Fig 30 + 31)

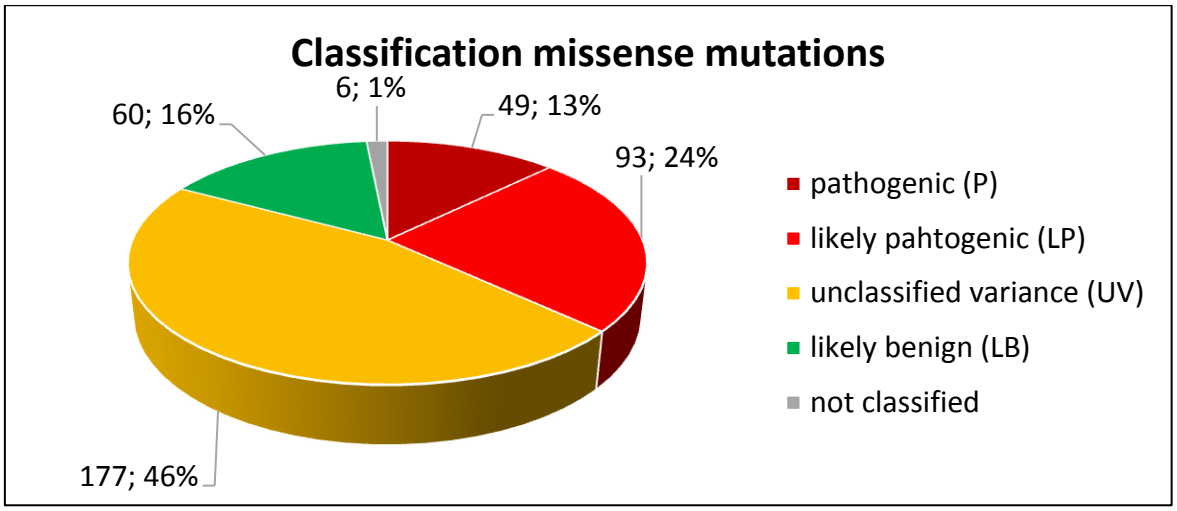


Figure 30: Classification "missense mutation"

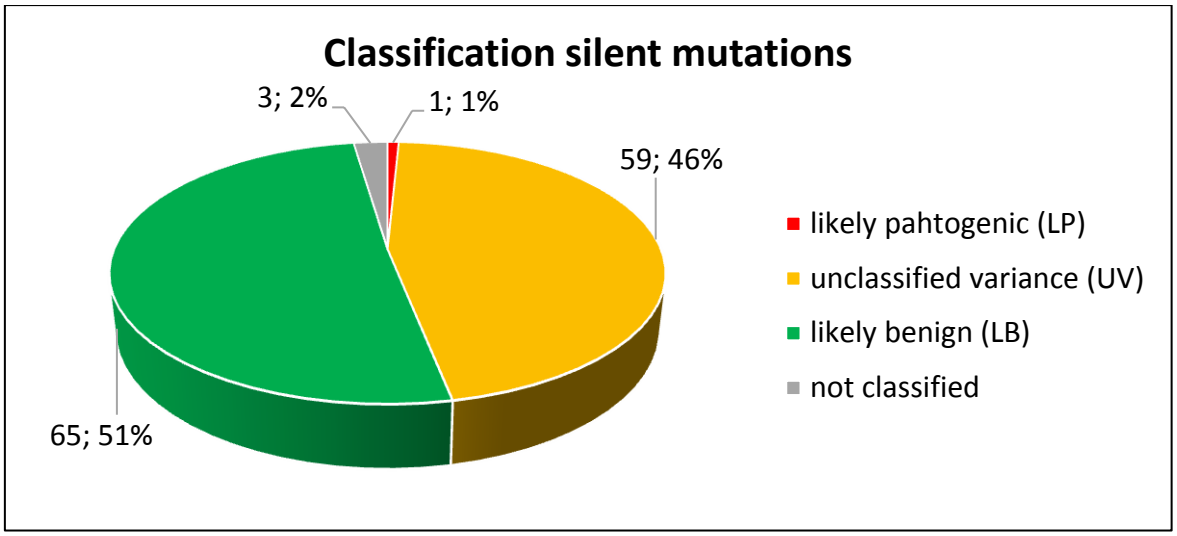


Figure 31: Classification "silent-splice? mutation"

Fourteen genes, with more than 10 cases, were selected for the differentiation of sex. The overall distribution was 412 male patients (70 %) and 180 female patients (30 %).

This is comparable seen in the genes *AKAP9, ANK2, MYBPC3, MYH6, PKP2, RYR2, SCN5A, SYNE1* and *TTN*. Other genes like *DSG2, KCNH2, KCNQ1* and *MYH7* shown different gender distribution, but that could be due to the small numbers of cases. Interestingly for the gene FLNC, mutations were only found in male patients (Tab. 5; Fig 32).

	number	male	female
overall	**592**	**412**	**180**
		69,59 %	30,41 %
AKAP9	**13**	**8**	**5**
		61,54 %	38,46 %
ANK2	15	9	6
		60 %	40 %
DSG2	18	14	4
		77,78 %	22,22 %
FLNC	12	12	0
		100 %	0 %
KCNH2	12	7	5
		58,33 %	41,67 %
KCNQ1	12	7	5
		58,33 %	41,67 %
MYBPC3	51	32	19
		62,75 %	37,25 %
MYH6	19	13	6
		68,42 %	31,58 %
MYH7	24	19	5
		79,17 %	20,83 %
PKP2	14	9	5
		64,29 %	35,71 %
RYR2	22	15	7
		68,18 %	31,82 %
SCN5A	20	13	7
		65 %	35 %
SYNE1	11	7	4
		63,64 %	36,36 %
TTN	160	111	49
		69,38 %	30,63 %

Table 5: Gender distribution in number and percentage in selected genes

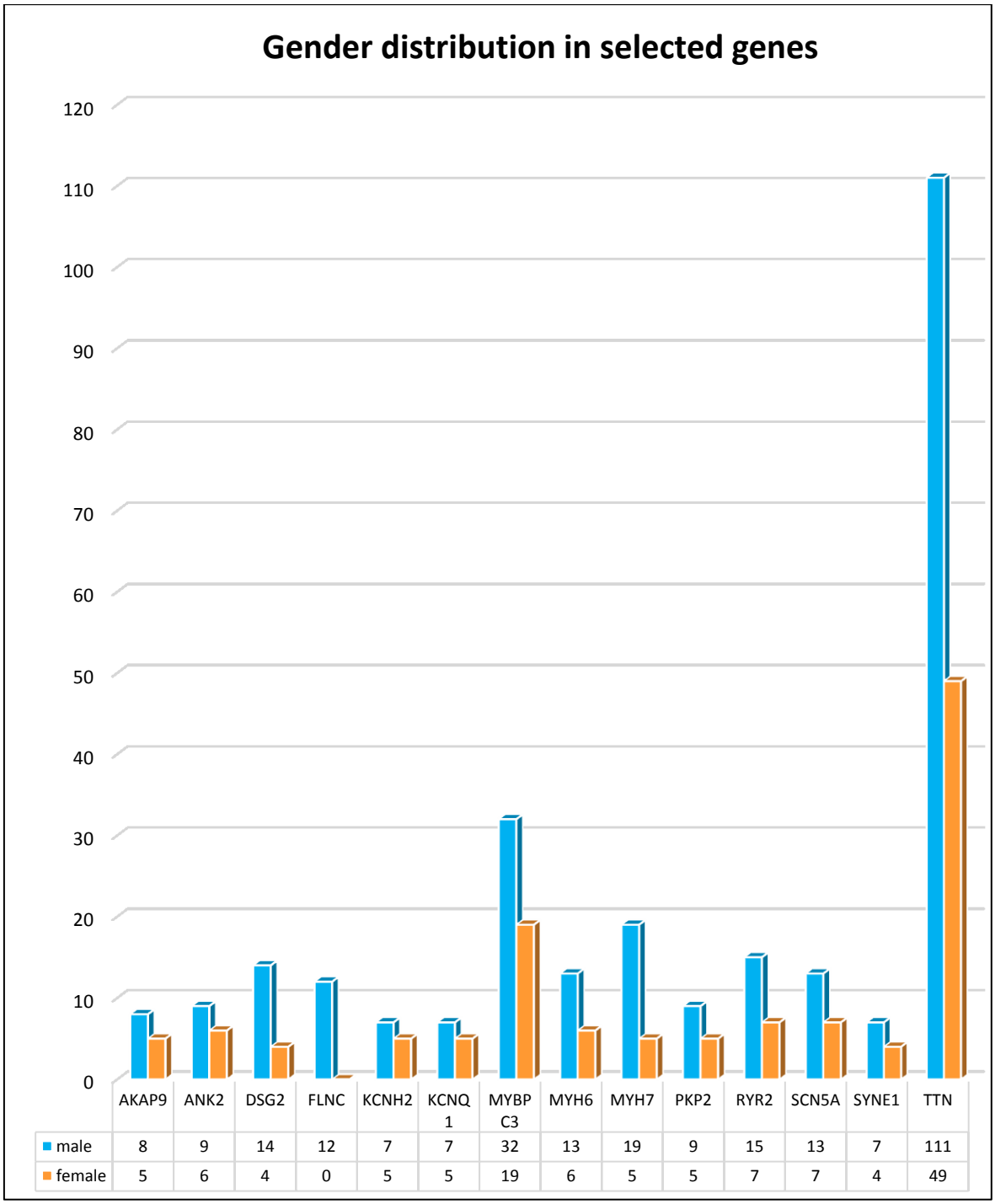


Figure 32: Gender distribution in selected genes

When taken the two mutations with the highest number of cases, TTN and MYBPC3, we see, that the distribution of diagnosis (Fig. 33) and types of mutations (Fig. 34) is relatively comparable. This is not seen anymore, in the classification. In the *MYBPC3* gene, 76% of the mutations are pathogenic (P) or likely pathogenic (LP) while in *TTN* gene just 11% are LP. Instead, there are 57% unclassified variances (UV) and 31% likely benign (LB) in *TTN* compared to 14% UV and 10 % LB in *MYBPC3* (Fig. 35 + 36).

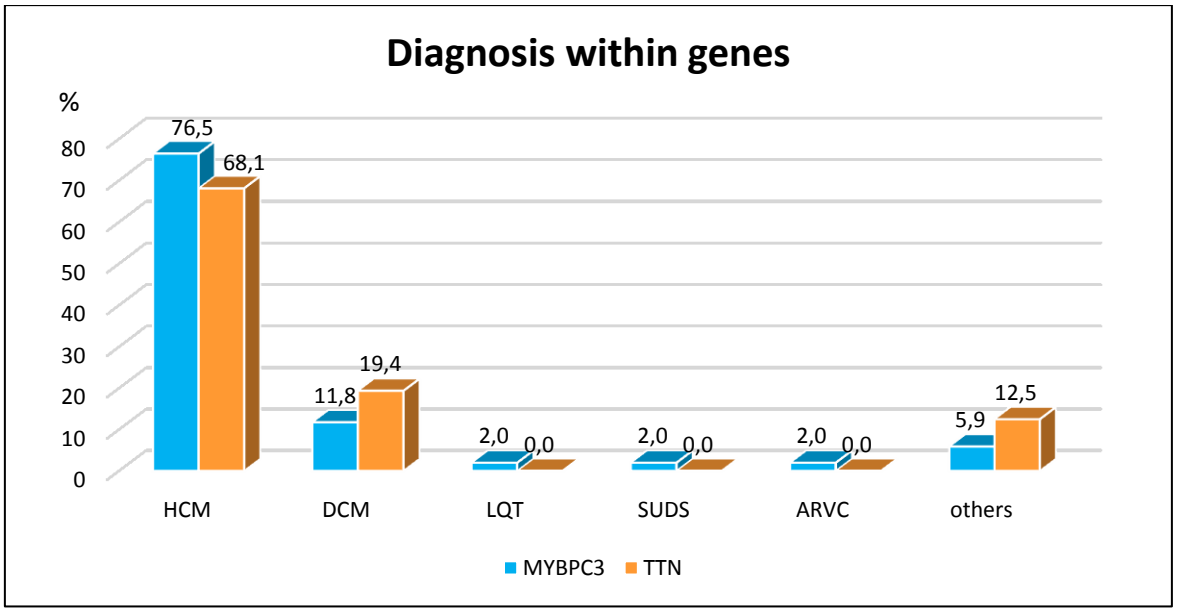


Figure 33: Diagnosis within the genes *MYBPC3* and *TTN*

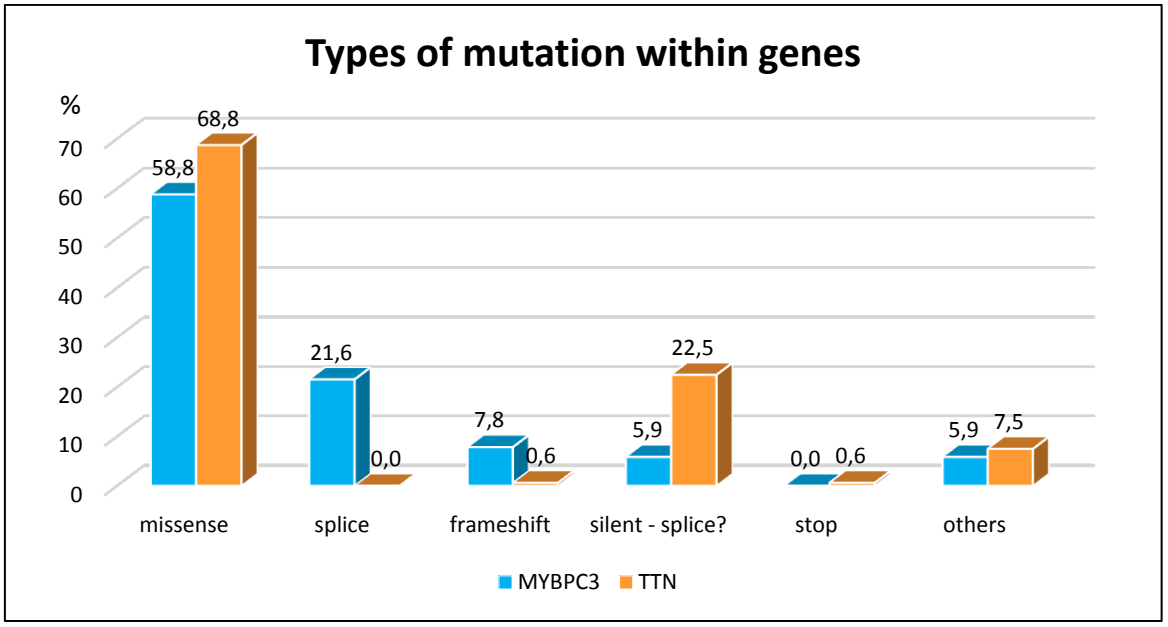


Figure 34: Types of mutations within the genes *MYBPC3* and *TTN*

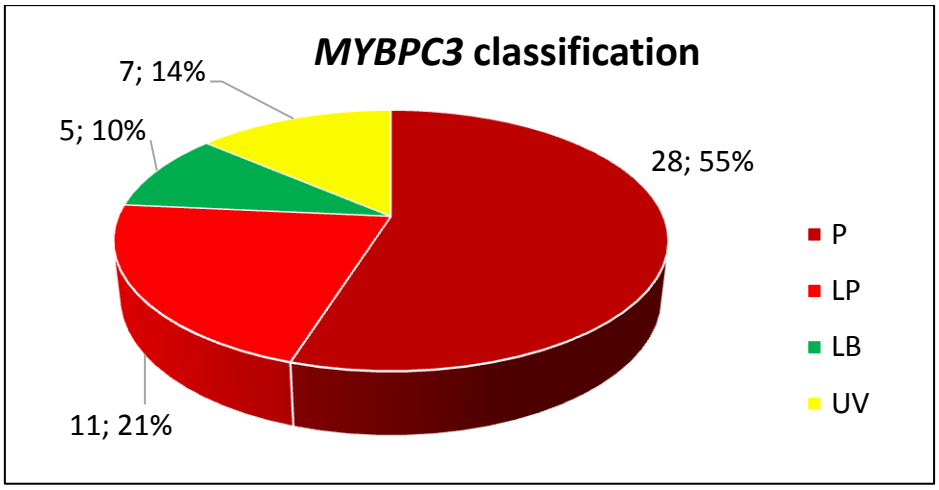


Figure 35: *MYBPC3* classification

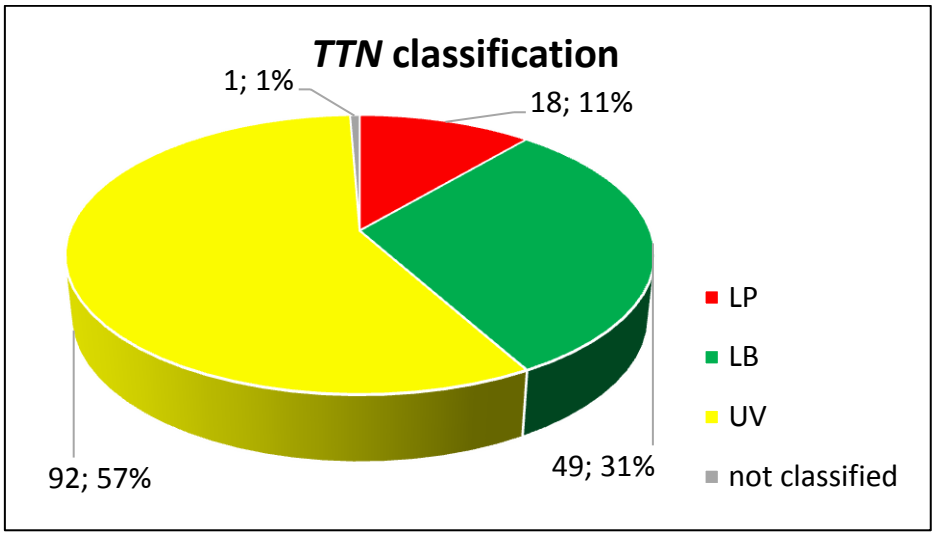


Figure 36: *TTN* classification

4 Discussion

4.1 Results

In this study, more men were diagnosed than women. However CVDs are nearly equal in women and men with a prevalence of 35.0% and 37.4%, shown by Mosca et al. (73) Nevertheless, a variation in gender is known for some inherited cardiac diseases. (74) However, it can be suggested that this gender distribution in our study results of physician-dependent transmissions to the Diagnostic and Research Institute for Human Genetics at Medical University of Graz. It is commonly known that there is a difference in diagnostic of CVDs in women and men, towards men. (75) Thus can result in a higher number of tested men.

The age distribution increases with age, according to the total population of CVD patients. (76, 77) A peak can be seen at the 50s. In my opinion, the fact of decreasing numbers of genetic testing in patients with age above 60 years, is due to the increase of non-genetic issues in diseases, especially cardiomyopathies. Therefore, no genetic testing is requested. The other peak in the age group of children between 6 and 14 years exists probably because of a strong relationship between the Institute of Human Genetics and the Division of Paediatric Cardiology of the Medical University of Graz. (69)

HCM and LQT are the most frequent inherited cardiovascular diseases. (13, 78) This is seen clearly in our cohort. If combining the age distribution with the two diseases HCM and LQT, we can see the two peaks nicely distributed, the lower peak for LQT and the higher peak for HCM. HCM is a disease that develops. The existing mutations can still be compensated in younger years. In the case of LQT, the peak in childhood seemed logical, since children in Styria need a cardiological examination before taking up sports competitions in a club to protect them from sudden cardiac death. (79)

In 49 patients, no mutation was found. This can account of the possibility that there is no mutation existing in the sequenced area or even when the technic of genetic testing quite well, errors exists. Moreover, owing to the tremendous amount of data mutations can get lost while the filtering steps for the prioritization of variants. The rest of the curve presents an obviously curve, with a decreasing number of patients with increasing numbers of mutation. Since humans have 100 to 200 novel private mutations on the whole genome and the coding regions only account 1% of the genome, there should exist statistically 1-2 mutations in whole-exome. (80) In this study, just a few exons/genes were investigated and

analysed, so the numbers should be even less. Caused by the Gaussian normal distribution, also exist patients with more mutations founded, but in a decreasing number.
Nearly half of all cases could not be solved. These can occur on the one hand by the 47 patients with no found mutations and on the other hand by mutations with no evidence for a pathologic expression, but mutations like benign (B, LB) or mutations of uncertain variance (UV), which cannot explain the pathological phenotype. One possibility of unidentified mutations can be attributed to the technic of library preparation. Notably, the HaloPlex System needs a well-known background of the genes by the investigator, respectively, the person who orders the kit because of its customer dependent production. (69) Additionally, the HaloPlex library preparation needs a restrictive enzyme, which has the potential to cleave a target site of interest. (81)
The overall gender distribution in our study describes around 2/3 men affected and 1/3 women. The same distribution can be seen in the group of patients with HCM. Compared to CDVs, patients with HCM (genetic and non-genetic) are predominantly male (2/3), as can be seen in the study from Olivotto et al. (82) Within the groups of less than ten patients, differentiation is not appropriate. The same applies to the mixed group of all other cases. In the group of DCM patients, more men were affected as Schultheiss et al. described in their paper as well. (24) Of interest is the gender distribution in LQT. In our group, we found just a few more women. This differs from the paper from Linde et al. where they showed, that interestingly women were more affected by LQT, from their puberty. (74, 83) Especially visible is the disparity of the gender distribution in BrS with 80% affected men and 20% women. This in line with the fact of an 8 times higher incidence of men compared to women. (13) Furthermore, the equal dissemination of the ARVC is the same as Choudhary et al. claimed in der study. (84)

The overall classification, according to the ACMG criteria, of the mutations in our study shows 42% uncertain variances (UV), 25% likely benign (LB), 18% likely pathogenic (LP) and 13% pathogenic (P) variances. Therefore, it is nearly consistent with the findings from van Lint et al., who investigated 46% of UVs. However, their number of LP and P mutations were lower, 7% (LP) and 9% (P). (85) This can lead to different used panels with a various set of genes.
The dominant type of mutation was the ‘missense mutation’ in our cohort. That seems logical, due to the fact, those missense mutations have a high possibility to lead to unfunctional proteins since patients, which come to a genetic test performed, have a

pathological phenotype. Group of the second largest is the ‘silent-splice?’ typed one. These mutations seem like silent mutations, but an adverse effect on splicing remains possible. Comparing the two groups to their dedicated classification distribution, it is nicely seen, that the group of missense mutation account just 16% LB mutations compared to the silent mutation group with 51%. This reflects the background kind of mutation, that a silent mutation doesn’t change the resulting amino acid and is therefore in all probability benign. In contrast, LP or P mutations are much higher in the missense group, with 37% opposed to 1% in the silent group. The 1% in the silent group represent the speculation of an additional splice mutation capability.

Fourteen genes with more than ten mutations can be represented. Six are likely associated with HCM (*FLNC, MYBPC3, MYH6, MYH7, RYR2, TTN*), five with LQT (*AKAP9, ANK2, KCNH2, KCNQ1, SCN5A*), two with ARVC (*DSG2, PKP2*) and one with DCM (*SYNE1*) as working diagnose. The gender distribution was 70% male and 30% female in these 14 genes. That is mostly seen in the genes themselves as well. Just *FLNC* shows just men affected. Since it is located on chromosome 7, the only possible answer to this situation is by random. (86) Interestingly, also the genes associated with LQT figures more men with mutations than women. According to Linde et al., more women would be expected to carry mutations in these genes. (74) That can result from the fact, that all numbers of mutations were counted, or just the count of patients and a patient can have several numbers of mutations, even in one gene or genes associated with a particular disease. While the genes *KNCH2* and *KCNQ1* are responsible for 70-95% of all hereditary LQT, in our shortened group just 33% belongs to these genes. Compared to this, the rare genes for LQT, *AKAP9* and *ANK2*, with a describes frequency of less than 1%, present 39%. (39) Due to no other explanation, it has to be count as a random finding. Within the HCM group of the selected genes, mutations in *MYBPC3* and *MYH7* are described as the reason for 50-70% in all cases. (87) In our cohort, these are found in 60% of all HCM cases. Nevertheless, the two most commonly affected genes in this study group are *MYBPC3* and *TTN*. With *TTN*, known as *titin*, we have the greatest resulting human protein, based on 365 exons. (88) Due to this fact, it is logical, that more mutation could be found, in our study 160, according to the second most common gene with 51 identified mutations by a size of 51 exons. (89) With a closer look at the distribution of types of mutation in these two genes, *MYBPC3* shows 89% in the types of missense, splice and frameshift mutations, whereas *TTN* has 70%. In contrast, *TTN* demonstrates more silent mutations than *MYBPC3*. Furthermore, *MYBPC3* shows clearly more LP and P classified mutations with 76%, unlike

TTN with 11% LP mutations. That presents impressively the correlation between basically pathogenic mutations types and the pathologic phenotypic findings in patients. On the contrary, *TTN* shows 57% UVs, unlike *MYBPC3* with 14%, and 31% LB according to the high number of silent mutations.

The high number of UVs found in our study cohort may be problematic. Patients with a phenotype for a particular disease come to genetic consultations, and we find a variant in a potentially pathogenetic gene and cannot say, this is causing their symptoms or it is a benign variant with no effect on them. We just do not know. We can just offer these patients to come back in several years, and we can re-evaluate this variant, caused to the fact, that with more genetic testing in the world, such UVs can more accurately be sorted into a specific group. (90) That will possibly increase their anger, sadness or anxiety. In a study from 2019, Makhnoon et al. investigated the experience of 26 patients to participated with a UV diagnosis. *"Most expressed negative effect on learning of their VUS result and uncertainty about its impact on clinical management."*(91)

However, also the group with confirmed genetic mutations causing a disease fight with these emotions. (92) Aatre et al. gave, in their paper from 2010, an excellent overview of psychological problems patients with especially HCM and LQT were faced. They discovered that a positive result, can motivate the patients for risk modification and behavioural changes and decrease their doubt about the diseases. In contrast, a negative result can increase anxiety and depression and cause scepticism about the clinical diagnosis. (90) The study team also noticed, that studies existing, which supported the potential benefit of gain of knowledge about a variance. (90, 93) Friederike Damme, a woman with diagnosed hereditary HCM, wrote a short article about her experience with this disease. She mentioned the problems as a child, like the impossibility of sports and be like the other children with the same power. Due to the symptoms of her disease, she was slower than the others, both in movements and thinking. After feeling much better, after a surgical operation, she felt freed and suppressed possible problems until an incident occurs. She got an ICD, which influence several parts of her life. Now she also thinks more about her life continuing; for example, about having children. (94) These are some psychological problems caused by cardiovascular genetic disease.

4.2 Limitations

Limitations arise out of the fact that in this study only summarised and cumulative data were accessible and included in the analysis, in order to avoid any usage of personalised data. However, this limits the possibility of analysing the mutations in more detail.
Furthermore, it is unfortunately no longer possible to ask the principal investigator of the data about this list, so that some of the data were subject to my interpretation.

4.3 Conclusion

This study shows that there is already some success in the diagnosis of cardiogenetic diseases. Nevertheless, some phenotypes remain genotypically unrecognized. On the one hand, they may simply not be genotypic, but on the other hand, they could also be polygenic.

Here, in particular, NGS opens up a large field of research. Hadji et al. were able to find a locus for syncopations and collapses with a genome-wide association study. (95) Furthermore, Zeng et al. were also able to show that coronary heart disease also has genetic components. (96) The William Harvey Lecture on Basis Science, sponsored by the ESC, will be awarded to Professor Connie Bezzina in 2019 for the identification of polygenic factors for the development of a BrS. (97)

This shows that genetic testing makes sense, both for individuals and for the population. However, we cannot ignore the psychological factors that still affect individuals.

5 Literature

1. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE et al. European Society of Cardiology: Cardiovascular Disease Statistics 2019. Eur Heart J 2018; 39(7):508–77.

2. Libby P, Braunwald E, editors. Braunwald's heart disease: A textbook of cardiovascular medicine. 8. ed. Philadelphia, Pa.: Elsevier Saunders; 2008.

3. Hofman N, van Langen I, Wilde AAM. Genetic testing in cardiovascular diseases. Curr Opin Cardiol 2010; 25(3):243–8.

4. Arndt A-K, MacRae CA. Genetic testing in cardiovascular diseases. Curr Opin Cardiol 2014; 29(3):235–40.

5. van de Laar IMBH, Oldenburg RA, Pals G, Roos-Hesselink JW, Graaf BM de, Verhagen JMA et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. Nat Genet 2011; 43(2):121–6.

6. van de Laar IMBH, van der Linde D, Oei EHG, Bos PK, Bessems JH, Bierma-Zeinstra SM et al. Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome. J Med Genet 2012; 49(1):47–57.

7. van der Linde D, van de Laar IMBH, Bertoli-Avella AM, Oldenburg RA, Bekkers JA, Mattace-Raso FUS et al. Aggressive cardiovascular phenotype of aneurysms-osteoarthritis syndrome caused by pathogenic SMAD3 variants. J Am Coll Cardiol 2012; 60(5):397–403.

8. Wischmeijer A, van Laer L, Tortora G, Bolar NA, van Camp G, Fransen E et al. Thoracic aortic aneurysm in infancy in aneurysms-osteoarthritis syndrome due to a novel SMAD3 mutation: further delineation of the phenotype. Am J Med Genet A 2013; 161A(5):1028–35.

9. Earle NJ, Crawford J, Hayes I, Rees MI, French J, Stiles MK et al. Development of a cardiac inherited disease service and clinical registry: A 15-year perspective. Am Heart J 2019; 209:126–30.

10. Steinberg DH, Staubach S, Franke J, Sievert H. Defining structural heart disease in the adult patient: current scope, inherent challenges and future directions. European Heart Journal Supplements 2010; 12(Suppl E):E2-E9.

11. Baenkler H-W. Kurzlehrbuch Innere Medizin. 2., aktualisierte Aufl. Stuttgart: Thieme; 2010.

12. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J 2008; 29(2):270–6.

13. Herold G. Innere Medizin 2012. Köln: Selbstverl.; 2012.

14. Longo DL, Dietel M, Harrison TR. Harrisons Innere Medizin. 18. Aufl., dt. Ausg., Sonderausg. Berlin, London, Berlin: Lehmanns Media; McGraw-Hill; ABW Wissenschaftsverl.; 2013.

15. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. Eur Heart J 2014; 35(39):2733–79.

16. Stierle U, Probst-Wiemuth B, editors. Klinikleitfaden Kardiologie: Mit dem Plus im Web ; Zugangscode im Buch. 5. Aufl. München: Elsevier Urban & Fischer; 2014.

17. Levine GN, editor. Cardiology secrets. Fifth Edition. Philadelphia, PA: Elsevier; 2018.

18. Böhmeke T, Schmidt A. Checkliste Echokardiographie. 4., vollst. überarb. und erw. Aufl. Stuttgart: Thieme; 2008. (Checklisten der aktuellen Medizin).

19. Jan MF, Tajik AJ. Modern Imaging Techniques in Cardiomyopathies. Circ Res 2017; 121(7):874–91.

20. Schmid C. Leitfaden Erwachsenenherzchirurgie. 3. Aufl. Berlin: Springer; 2014. (SpringerLink).

21. Ziemer G, Haverich A. Herzchirurgie: Die Eingriffe am Herzen und den herznahen Gefäßen. 3., völlig neu bearbeitete Auflage. Berlin, Heidelberg: Springer-Verlag Berlin Heidelberg; 2010.

22. McNally EM, Golbus JR, Puckelwartz MJ. Genetic mutations and mechanisms in dilated cardiomyopathy. J Clin Invest 2013; 123(1):19–26.

23. Hershberger RE, Hedges DJ, Morales A. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat Rev Cardiol 2013; 10(9):531–47.

24. Schultheiss H-P, Fairweather D, Caforio ALP, Escher F, Hershberger RE, Lipshultz SE et al. Dilated cardiomyopathy. Nat Rev Dis Primers 2019; 5(1):32.

25. Seferović PM, Polovina MM, Coats AJS. Heart failure in dilated non-ischaemic cardiomyopathy. Eur Heart J Suppl 2019; 21(Suppl M):M40-M43.

26. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2016; 37(27):2129–200.

27. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015; 28(1):1-39.e14.

28. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation 2006; 113(14):1807–16.

29. Muchtar E, Blauwet LA, Gertz MA. Restrictive Cardiomyopathy: Genetics, Pathogenesis, Clinical Manifestations, Diagnosis, and Therapy. Circ Res 2017; 121(7):819–37.

30. Familial restrictive cardiomyopathy; 2020 [cited 2020 Mar 24]. Available from: URL: https://ghr.nlm.nih.gov/condition/familial-restrictive-cardiomyopathy#statistics.

31. Loria V, Colizzi C, Vaccarella M, Franceschi F, Aspromonte N. Left Ventricular Noncompaction: Cause or Consequence of Myocardial Disease? A Case Report and Literature Review. Cardiology 2019; 143(3-4):100–4.

32. Ritter M, Oechslin E, Sütsch G, Attenhofer C, Schneider J, Jenni R. Isolated noncompaction of the myocardium in adults. Mayo Clin Proc 1997; 72(1):26–31.

33. Oechslin EN, Attenhofer Jost CH, Rojas JR, Kaufmann PA, Jenni R. Long-term follow-up of 34 adults with isolated left ventricular noncompaction: a distinct cardiomyopathy with poor prognosis. J Am Coll Cardiol 2000; 36(2):493–500.

34. Weiford BC, Subbarao VD, Mulhern KM. Noncompaction of the ventricular myocardium. Circulation 2004; 109(24):2965–71.

35. Samsa LA, Yang B, Liu J. Embryonic cardiac chamber maturation: Trabeculation, conduction, and cardiomyocyte proliferation. Am J Med Genet C Semin Med Genet 2013; 163C(3):157–68.

36. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. Br Heart J 1994; 71(3):215–8.

37. Schuster H-P, Trappe H-J. EKG-Kurs für Isabel: Lernprogramm zur EKG-Befundung. 5., überarb. und erw. Aufl. Stuttgart: Thieme; 2009.

38. Priori SG, Barhanin J, Hauer RN, Haverkamp W, Jongsma HJ, Kleber AG et al. Genetic and molecular basis of cardiac arrhythmias: impact on clinical management parts I and II. Circulation 1999; 99(4):518–28.

39. Issa ZF, Zipes DP, Miller JM. Clinical arrhythmology and electrophysiology: A companion to Braunwald's heart disease. Third edition. Philadelphia, PA: Elsevier; 2019.

40. Giudicessi JR, Wilde AAM, Ackerman MJ. The genetic architecture of long QT syndrome: A critical reappraisal. Trends Cardiovasc Med 2018; 28(7):453–64.

41. Brugada P. Brugada syndrome: More than 20 years of scientific excitement. J Cardiol 2016; 67(3):215–20.

42. Bjerregaard P. Diagnosis and management of short QT syndrome. Heart Rhythm 2018; 15(8):1261–7.

43. Perike S, McCAULEY MD. Molecular Insights into the Short QT Syndrome. J Innov Card Rhythm Manag 2018; 2018:3065–70.

44. Rudic B, Schimpf R, Borggrefe M. Short QT Syndrome - Review of Diagnosis and Treatment. Arrhythm Electrophysiol Rev 2014; 3(2):76–9.

45. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J 2015; 36(41):2793–867.

46. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 1998; 392(6673):293–6.

47. Marcus FI. Idiopathic ventricular fibrillation. J Cardiovasc Electrophysiol 1997; 8(9):1075–83.

48. Visser M, van der Heijden JF, Doevendans PA, Loh P, Wilde AA, Hassink RJ. Idiopathic Ventricular Fibrillation: The Struggle for Definition, Diagnosis, and Follow-Up. Circ Arrhythm Electrophysiol 2016; 9(5).

49. U.S. National Library of Medicine. Catecholaminergic polymorphic ventricular tachycardia; 2020 [cited 2020 Mar 23]. Available from: URL: https://ghr.nlm.nih.gov/condition/catecholaminergic-polymorphic-ventricular-tachycardia#statistics.

50. Leenhardt A, Denjoy I, Guicheney P. Catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol 2012; 5(5):1044–52.

51. Baumgartner H, Bonhoeffer P, Groot NMS de, Haan F de, Deanfield JE, Galie N et al. 2010 ESC Guidelines for the management of grown-up congenital heart disease. Eur Heart J 2010; 31(23):2915–57.

52. Hraška V, Murín P. Surgical Management of Congenital Heart Disease I. 1st ed. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012.

53. Schmid C, Asfour B. Leitfaden Kinderherzchirurgie (German Edition). Dordrecht: Springer; 2009.

54. Unolt M, Putotto C, Silvestri LM, Marino D, Scarabotti A, Valerio M et al. Transposition of great arteries: new insights into the pathogenesis. Front Pediatr 2013; 1:11.

55. Casey B. Genetics of human situs abnormalities. Am J Med Genet 2001; 101(4):356–8.

56. Kosaki K, Casey B. Genetics of human left-right axis malformations. Semin Cell Dev Biol 1998; 9(1):89–99.

57. Sutherland MJ, Ware SM. Disorders of left-right asymmetry: heterotaxy and situs inversus. Am J Med Genet C Semin Med Genet 2009; 151C(4):307–17.

58. Murken J, Grimm T, Holinski-Feder E, Zerres K, editors. Taschenlehrbuch Humangenetik. 9., teilaktualisierte Auflage. Stuttgart: Thieme; 2017.

59. Nordheim A, Knippers R, editors. Molekulare Genetik. 11., unveränderte Auflage. Stuttgart, New York: Thieme; 2018.

60. Men AE, Wilson P, Siemering K, Forrest S. Sanger DNA Sequencing. In: Janitz M, editor. Next Generation Genome Sequencing: Towards Personalized Medicine. 1., Auflage, neue Ausg. Weinheim: Wiley-VCH; 2011. p. 3–10.

61. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 1977; 74(12):5463–7.

62. National Human Genome Research Institute, editor. The Cost of Sequencing a Human Genome; 2019 2019 Oct 30. Available from: URL: https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost [cited 2020 Mar 30].

63. Illumina Sequencing by Synthesis; 2016.

64. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed 2013; 98(6):236–8.

65. Illumina, editor. An introduction to Next-Generation Sequencing Technology. Available from: URL: https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf [cited 2020 Mar 30].

66. Sikkema-Raddatz B, Johansson LF, Boer EN de, Almomani R, Boven LG, van den Berg MP et al. Targeted next-generation sequencing can replace Sanger sequencing in clinical diagnostics. Hum Mutat 2013; 34(7):1035–42.

67. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al. Standards and guidelines for the interpretation of sequence variants:: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17(5):405–24.

68. Kleinberger J, Maloney KA, Pollin TI, Jeng LJB. An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. Genet Med 2016; 18(11):1165.

69. Lafer I. Kardiogenetik: Im Hochdurchsatz zur Verbesserung der Partientenversorgung und Abklärung von bisher nicht diagnostizierbaren genetischen Ursachen bei Patienten mit schwer abgrenzbaren Erkrankungen [Dissertation]. Graz: Medical University of Graz; 2015.

70. HaloPlex Target Enirchment System; 2018 [cited 2020 Mar 31]. Available from: URL: https://www.agilent.com/cs/library/usermanuals/public/G9900-90001_HaloPlex_Target_Enrichment%20_Manual.pdf.

71. Agilent Technologies. HaloPlexHS: Target Enrichment-Molecular barcodes testimonial from Dr. Ioannis Ragoussis; 2016.

72. TruSight One Series of Sequencing Panels [cited 2020 Mar 31]. Available from: URL: https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_trusight_one_panel.pdf.

73. Mosca L, Barrett-Connor E, Wenger NK. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. Circulation 2011; 124(19):2145–54.

74. Linde C, Bongiorni MG, Birgersdotter-Green U, Curtis AB, Deisenhofer I, Furokawa T et al. Sex differences in cardiac arrhythmia: a consensus document of the European Heart Rhythm Association, endorsed by the Heart Rhythm Society and Asia Pacific Heart Rhythm Society. Europace 2018; 20(10):1565-1565ao.

75. Keteepe-Arachi T, Sharma S. Cardiovascular Disease in Women: Understanding Symptoms and Risk Factors. Eur Cardiol 2017; 12(1):10.

76. Bhatnagar P, Wickramasinghe K, Wilkins E, Townsend N. Trends in the epidemiology of cardiovascular disease in the UK. Heart 2016; 102(24):1945–52.

77. Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. Heart Fail Clin 2012; 8(1):143–64.

78. Firth HV, Hurst JA. Clinical genetics and genomics. 2 ed. Oxford: Oxford University Press; 2017. (Oxford desk reference).

79. Bauer S. Pädiatrische Sportmedizin in Österreich [Diplomarbeit]. Graz: Medical University of Graz; 2016.

80. Wellcome Sanger Institute. We are all mutants: Measurement of mutation rate in humans by direct sequencing; 2009.

81. Choffnes ER, Olsen LA, Wizemann T, Forum on Microbial Threats, Board on Global Health, Institute of Medicine (U.S.). The science and applications of microbial genomics: Workshop summary. Washington, District of Columbia: The National Academies Press; 2013.

82. Olivotto I, Maron MS, Adabag AS, Casey SA, Vargiu D, Link MS et al. Gender-related differences in the clinical presentation and outcome of hypertrophic cardiomyopathy. J Am Coll Cardiol 2005; 46(3):480–7.

83. Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovascular Disease. Physiol Rev 2017; 97(1):1–37.

84. Choudhary N, Tompkins C, Polonsky B, McNitt S, Calkins H, Mark Estes NA et al. Clinical Presentation and Outcomes by Sex in Arrhythmogenic Right Ventricular Cardiomyopathy: Findings from the North American ARVC Registry. J Cardiovasc Electrophysiol 2016; 27(5):555–62.

85. van Lint FHM, Mook ORF, Alders M, Bikker H, Lekanne Dit Deprez RH, Christiaans I. Large next-generation sequencing gene panels in genetic heart disease: yield of pathogenic variants and variants of unknown significance. Neth Heart J 2019; 27(6):304–9.

86. National Library of Medicine. FLNC filamin C: [Homo sapiens (human)]; 2020 [cited 2020 Apr 4]. Available from: URL: https://www.ncbi.nlm.nih.gov/gene/2318.

87. Girolami F, Frisso G, Benelli M, Crotti L, Iascone M, Mango R et al. Contemporary genetic testing in inherited cardiac disease: tools, ethical issues, and clinical applications. J Cardiovasc Med (Hagerstown) 2018; 19(1):1–11.

88. National Library of Medicine. TTN titin: [Homo sapiens (human)]; 2020 [cited 2020 Apr 4]. Available from: URL: https://www.ncbi.nlm.nih.gov/gene/7273.

89. National Library of Medicine. MYBPC3 myosin binding protein C3: [Homo sapiens (human)]; 2020 [cited 2020 Apr 4]. Available from: URL: https://www.ncbi.nlm.nih.gov/gene/4607.

90. Aatre RD, Day SM. Psychological issues in genetic testing for inherited cardiovascular diseases. Circ Cardiovasc Genet 2011; 4(1):81–90.

91. Makhnoon S, Garrett LT, Burke W, Bowen DJ, Shirts BH. Experiences of patients seeking to participate in variant of uncertain significance reclassification research. J Community Genet 2019; 10(2):189–96.

92. Cirino AL, Ho CY. Genetic testing for inherited heart disease. Circulation 2013; 128(1):e4-8.

93. Reinhard W, Trenkwalder T, Schunkert H. Praktische Durchführung der genetischen Diagnostik in der Kardiologie. Herz 2017; 42(5):485–91.

94. Damme F. Hypertrophe Kardiomyopathie: Wie es ist, mit einem Herzfehler zu leben. ze.tt 2019 Oct 6 [cited 2020 Apr 4]. Available from: URL: https://ze.tt/hypertrophe-kardiomyopathie-wie-es-ist-mit-einem-herzfehler-zu-leben/.

95. Hadji-Turdeghal K, Andreasen L, Hagen CM, Ahlberg G, Ghouse J, Bækvad-Hansen M et al. Genome-wide association study identifies locus at chromosome 2q32.1 associated with syncope and collapse. Cardiovasc Res 2020; 116(1):138–48.

96. Zeng L, Talukdar HA, Koplev S, Giannarelli C, Ivert T, Gan L-M et al. Contribution of Gene Regulatory Networks to Heritability of Coronary Artery Disease. J Am Coll Cardiol 2019; 73(23):2946–57.

97. Nicholls M. The William Harvey Lecture on Basic Science. Eur Heart J 2019; 40(43):3511–2.

6 References to figures

Figure 1: Created by the author in Microsoft® PowerPoint 2019

Figure 2: Jan MF, Tajik AJ. Modern Imaging Techniques in Cardiomyopathies. Circ Res 2017; 121(7):874–91.

Figure 3: Jan MF, Tajik AJ. Modern Imaging Techniques in Cardiomyopathies. Circ Res 2017; 121(7):874–91.

Figure 4: Jan MF, Tajik AJ. Modern Imaging Techniques in Cardiomyopathies. Circ Res 2017; 121(7):874–91.

Figure 5: Espinola-Zavaleta N, Soto ME, Castellanos LM, Játiva-Chávez S, Keirns C. Non-compacted cardiomyopathy: clinical-echocardiographic study. Cardiovasc Ultrasound 2006; 4:35.

Figure 6 – 14: Drawing of the author

Figure 15: https://commons.wikimedia.org/wiki/File:D-tga-575px.jpg

Figure 16: Gray H, Lewis WH. Gray's Anatomy. 20 th Edition: Lea & Febiger; 1918.

Figure 17: Created by the author in Microsoft® Word 2019

Figure 18: Men AE, Wilson P, Siemering K, Forrest S. Sanger DNA Sequencing. In: Janitz M, editor. Next Generation Genome Sequencing: Towards Personalized Medicine. 1., Auflage, neue Ausg. Weinheim: Wiley-VCH; 2011. p. 3–10.

Figure 19: Illumina, editor. An introduction to Next-Generation Sequencing Technology. Available from: URL: https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf [cited 2020 Mar 30].

Figure 20-36: Created by the author in Microsoft® Excel 2019

7 References to tables

Table 1: Kleinberger J, Maloney KA, Pollin TI, Jeng LJB. An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. Genet Med 2016; 18(11):1165.

Table 2: Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17(5):405–24.

Table 3: Muin D. Targetedsequencing for identification of cardiac variants in unexplained intrauterine death - a retrospective pilot study [Masterthesis]. Graz: Medical University of Graz; 2018.

Table 4-5: Created by the author in Microsoft® Excel 2019 based on the data