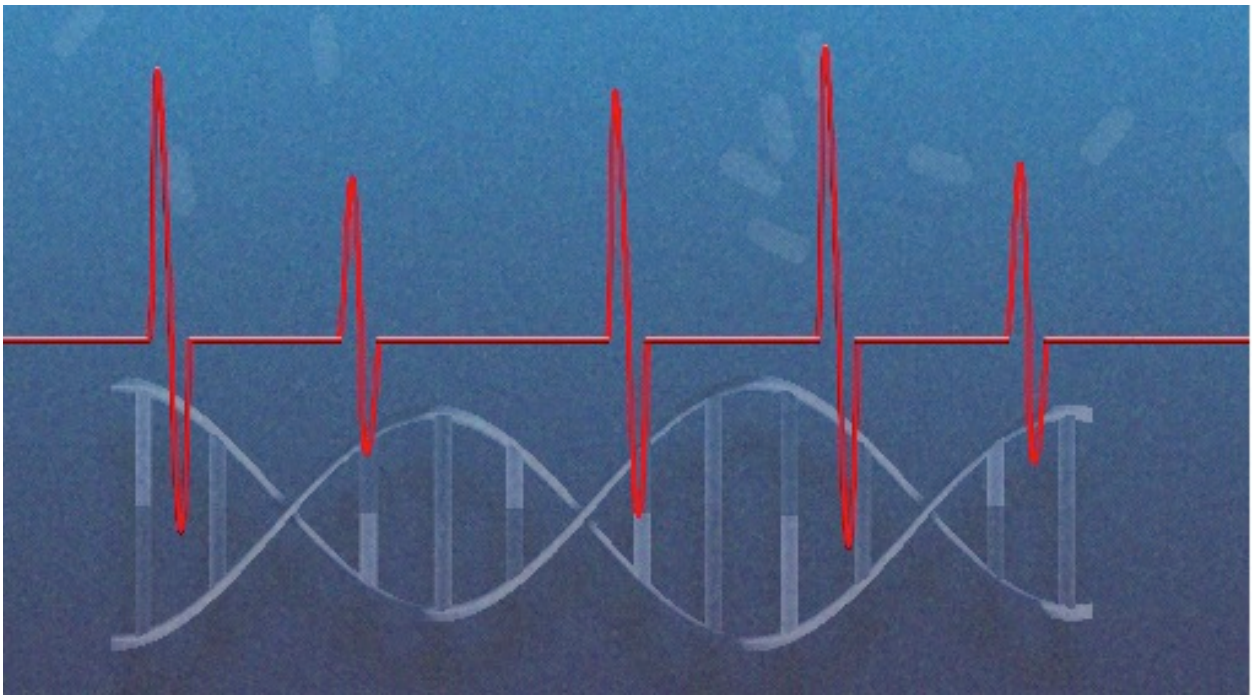
Implementation of routine predictive genetic testing for cardiovascular disease.



Implementation of routine predictive genetic testing for cardiovascular disease.

Clinical application and ethical aspects.

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Abstract

Cardiovascular diseases are the leading cause of death worldwide and are caused by a combination of genetic and environmental influences. Hence they have a great social and economic impact on our society. The onset and treatment of these diseases can be greatly influenced and controlled if it is known beforehand if a patient has a hightened risk to develop a CVD. In such cases it would be benifitial to reliably determine an individual's risk to develop these diseases based on their genetic data using preventive genetic screening.
Several published studies are discussed in this thesis that investigate the impact of adding genetic data to commonly used CVD risk prediction models. The addition of a SNP located in the Chr9p21.3 region that is strongly associated with a significantly higher risk for CVD surprisingly does not lead to an iprovement in predicting individual risk for developing CVD. This shows that based on genetic data alone it is difficult to predict risk for disease development. Because of its strong genetic association with CVD, addition of this SNP did help improve classification of persons into risk profiles, which could be beneficial for treatment.

Due to the development of next generation sequencing technologies and the possibility to sequence complete human genomes, it becomes more and more interesting to start using NGS based tests in the cinics for such purposes. However the complicated amounts of genomic data that are being generated in these tests are difficult to interpret and lead to a whole new series of complicated new technical and ethical issues.
Due to the fear of genetic discrimination it is important that current rules and regulations are updated specifically for the purpose of genetic testing.

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1. Introduction

This thesis focuses on the feasibility of routine predictive genetic testing for cardiovascular diseases and the accompanying ethical issues. Cardiovascular diseases are the leading cause of death worldwide and are caused by a combination of genetic and environmental influences. Hence they have a great social and economic impact on our society. The onset and treatment of these diseases can be greatly influenced and controlled if it is known beforehand if a patient has a hightened risk to develop a CVD. In such cases it would be benifitial to reliably determine an individual's risk to develop these diseases based on their genetic data using preventive genetic screening.

Several research questions will be addressed to answer the main research question of this thesis:

How does the general public health benefit from the implementation of routine predictive genetic testing for cardiovascular disease?

With the recent development of next generation sequencing (NGS) technologies and the resulting decrease in DNA sequencing costs it is now possible to analyze our complete genomic sequence and gather information about our risk to develop certain diseases. Genomic sequence information can be used to predict and prevent the onset of specific diseases in individuals and their families. We will discuss the following questions in chapter 2: What is next generation sequencing? What are the technological possibilities and the possible clinical applications of NGS? What future developments are required for the implication of NGS in the clinic? What ethical issues are arising as a consequence of this?
Due to this technological development a whole new medical field termed personalized, predictive, preventive and participatory medicine (P4 medicine) is evolving [1]. In general three application fields for P4 medicine can be defined: pharmacogenomics, genetic risk assessment for common diseases, and identification of rare disease-causing genetic variants.

Pharmacogenomics studies the genetic variation in genes involved in drug response pathways that influence the action of a drug by their role in regulating drug absorbtion, distribution, metabolisis, and excretion. This genetic information can be used to predict how a drug wil interact with its target and determine its mechanism of action. Hence it is possible to predict both efficacy and toxicity of a drug independently for every patient. This would obviously greatly increase the treatment potential and makes pharmacogenomics a very interesting and promising field. A clinical example is the use of the drug Trastuzumab in the treatment of breast cancer. When it is determined that a patient has an amplified *ERBB2(HER2)* gene they can be treated with the drug Trastuzumab. The drug binds to the HER2 receptor and so inhibits cell growth of these breast cancers. However only 30% of the patients respond to this treatment showing there is still considerable optimization required [2].

The identification and characterization of genetic variants to identify the molecular basis of rare and common genetic diseases is another application that generates a lot of interest. Rare genetic diseases affecting less then 1% of the population are still having an influence on more than 25 million people worldwide. If individuals who are carriers for such diseases could be identified early in life the risk of passing on their genetic mutations to future generations could be limited and controlled. Additionally the identification of rare genetic variants using P4 medicine can also be applied to predict a person's risk in developing common diseases like rheumatoid arthritis, Alzheimer's disease and cardiovascular diseases. The focus of this thesis is on cardiovascular diseases and in chapter 4 we discuss the following research questions: Why are cardiovascular diseases interesting for predictive genetic testing? Which cardiovascular diseases exist and what is their genetic factor? What cardiovascular diseases are suitable for predictive genetic screening?

The risk for a person to develop a certain disease is difficult to predict exactly because of the combination of genetic and environmental influences. In chapter 5 we discuss the following research questions: Which cardiovascular diseases can we currently predict and how is this applied in the clinic? With what certainty can we predict (cardiovascular) diseases? Which factors are important and play a role in the development of cardiovascular disease? How do these factors influence the disease outcome? How does disease prediction improve treatment? What do we already know from previous studies about predicting cardiovascular disease related to genomic variance?

The personal advantages of P4 medicine are clear; it can be very beneficial for the general public health if diseases are prevented and treated in time. This will reduce our health care costs and it will have a strong positive psychological effect. But if someone is diagnosed with a disease that cannot be cured the effect can be completely adverse. The strong phsycological and social implications are leading to a whole new series of disease specific ethical issues influenced by the severity and the type of disease.
Currently there is a lot of debate on how to prevent discrimination by employers or insurance companies based on genomic information. There is also much debate on how to protect our privacy. It is important that our genomic data is safely stored and not just everyone has access to a person's genomic information. In chapter 6 we will discuss the following research questions: Which ethical aspects are currently considered in the clinic and applied to predictive medicine? What are the current regulations and how should they be adjusted, especially regarding the informed consent and right not-to-know? What are the specific ethical issues regarding cardiovascular disease? How do we deal with informed consent when someone is tested for a genetic disorder and other unwanted information is gained? Specialized genetic counseling is required and probably new laws have to be made.

In the past medicine was more diagnosis based and treatment started only after symptoms of disease arise. The evolution of P4 medicine will lead to a change of medicine to a more pro-reactive field focussing more on prediction of the onset of disease and maintaining a healthy life style with for example dietary restrictions. It is being speculated that in the future every person's medical file wil contain gigabytes of data from not only their own and their families genomes, but also their enviroment and social lives[2]. Therefore it is very interesting to investigate what factors play a role in the onset of a disease and how these factors can help with disease prediction.

2. Next Generation Sequencing

2.1 Sequencing platforms

Before we discuss the possibilities of predicting cardiovascular diseases using next generation sequencing (NGS) data we first have to take a look at the technological possibilities that are currently available. How does NGS work, which platforms are available and what are the current technical possibilities and limitations?

The principle of NGS is fundamentally different from the Sanger sequencing method, which has been the dominant sequencing technology since it was first published in 1975 [3]. Sanger sequencing was the method used to sequence the entire genome in the human genome project [4,5]. The sequencing machines used for these projects could read around 2 million bases of DNA sequence per day. Since the size of the human genome is around 3 billion base pairs this was a very costly and time inefficient method.

Next generation sequencing technologies were introduced on the market around 2005, they parallelize the process (hence the term massive parallel sequencing) and increase the throughput of a sequencing machine up to 50 billion bases a day. This technical development resulted in a dramatic decrease in the cost of sequencing per base and an increase in throughput.

However the costs of sequencing an entire human genome still remain high and the ultimate goal is to be able to sequence the entire human genome for $100 [6].

There are multiple NGS platforms available from different companies that are all continuously improving their methods to make sequencing cheaper, faster and more reliable. The three leading sequencing systems in the market are the 454 from Roche, the ABI/SOLiD and Illumina's Hi-seq. Every company developed its own method of sequencing. An overview of the characteristics of each platform is given in table 1 [7].

Platform	**Roche 454**	**ABI/SOLID**	**Illumina**
PCR	Emulsion PCR	Emulsion PCR	Solid Phase amplification
Cluster formation	One DNA fragment per bead	One DNA fragment per bead	One DNA fragment per cluster
Sequencing method	Pyro-sequencing	Sequencing by ligation	Sequencing by synthesis
Read Length	400-500 bp	50 bp	100 bp

Table 1. Technological features of three leading next generations sequencing platforms
For every NGS platform the characteristics for library generation, sequencing technology and read length are summarized [7].

2.2 Technological principle

The sequencing strategies of the different NGS platforms are largely based on the same technological principles (Figure 1). Genomic DNA is isolated from for example blood, and fragmented. Sequence adapters are ligated to the fragments and then hybridised to a slide in the case of Solexa sequencing or attached to a bead (SOLiD, 454). At that location the DNA is amplified to form clusters containing DNA molecules with identical sequences. These DNA sequences are then sequenced by the platforms own methods. The sequences are read by registration of the incorporated fluorescent signals for each cluster at the same time. The obtained DNA sequences are then aligned in silico and compared to a reference sequence and further analyzed. Each NGS platform has its own advantages and disadvantages and they are usually chosen depending on the experimental purpose [6,7].

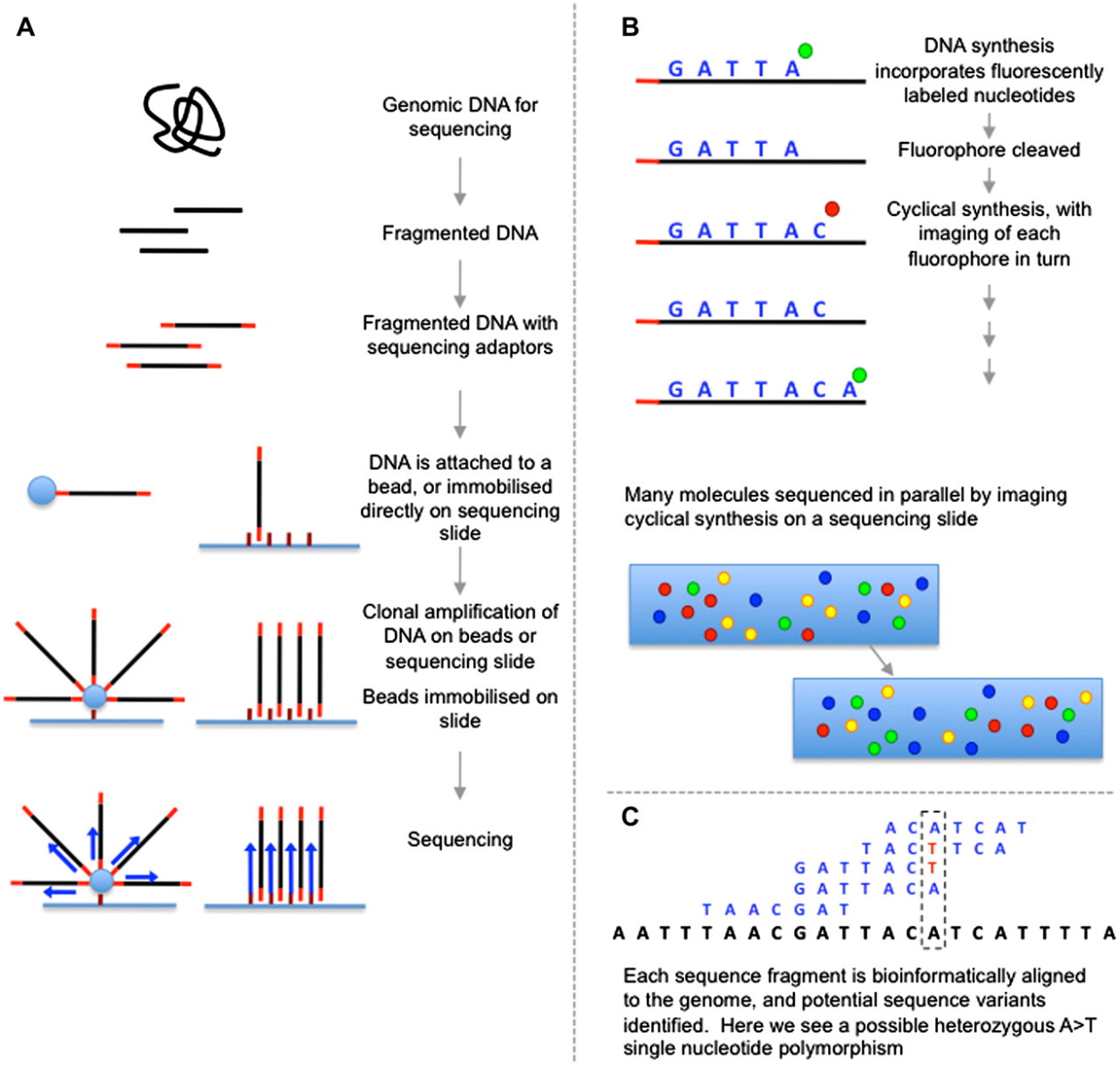

Figure 1. Schematic overview of next generations sequencing technology
A Genomic DNA is fragmented and platform specific 'adaptors' are attached. The DNA is immobilized and then clonally amplified to provide a cluster of molecules with identical sequences and sequenced. **B** DNA is sequenced by incorporatin of fluorescet nucleotides and these are imaged to read the sequence. **C** The sequenced DNA fragments are aliged in silico to a reference genome and can be further analyzed [6].

2.3 Clinical application

The very high resolution of NGS (every base pair is read and identified) makes it very suitable for detecting mutations. Most Mendialien disorders are caused by genomic variations that are small variations of 1 bp up to a few kb and therefore making them difficult to detect [8]. The methods that are currently used like chromosome karyotyping, FISH and micro-array analysis all have a MB resolution and therefore sometimes miss mutations. Additionally with NGS it is possible to multiplex samples allowing the sequencing of small regions of multiple samples in a single lane/well and thereby significantly lowering the costs.

The clinical application of NGS is still in its infancy, but there are several clear advantages over traditional diagnostic technologies. First the resolution of the generated data is very high. Every single nucleotide in a genomic region can be sequenced at a very high depth. This greatly improves the chances of locating mutations and identifying rare variants [9].
Secondly NGS provides an unbiased view of the genome. There is no prior knowledge of the patient of disease required before analysis. This greatly improves discovery of novel genetic variations [10].
A third major advantage is the limited amount of DNA that is required. Some patients' sample material can be limited or hard to collect. For Sanger sequencing usually a few micrograms of DNA is required [4,5] but for NGS as little as 50 ng of DNA can be enough [11]. And a recently improved sample preparation technique using Illumina paired-end sequencing only requires picogram quantities of DNA [12].
A final advantage are the many different research applications in which NGS can be applied, of which some examples are: whole-genome re-sequencing, exome sequencing, targeted re-sequencing and RNA-sequencing. All these methods have their own possibilities and limitations for clinical application.

2.3.1 Targeted re-sequencing

Targeted re-sequencing is the specific sequencing of only part of the genome, for example a gene locus that is involved in a certain type of disease. The gene is first specifically enriched for by PCR or micro-array and subsequently the enriched fragments are amplified and sequenced. [13]
Several advantages of this technique are the low costs, because less reads are required to generate enough coverage, and the simplified data analysis. A disadvantage is ofcourse that prior knowledge of the disease locus is required.
Targeted re-sequencing can for instance be used in traditional cytogenetic and Mendelian disorder diagnosis, the diagnosis and prognosis of cancer, and genetic testing for common diseases.
A recently published study shows the application of targeted re-sequencing in the clinic for diagnosis of X-Linked intellectual disability genes and testing of up to 84 human genes implicated in hearing loss. [14]

2.3.2 Exome sequencing

Exome sequencing is essentially also a form of targeted re-sequencing, but here the sample is first enriched for all exons, the coding regions of the genome. Exons only make up approximately 1% of the complete human genome, but carry 85% of the mutations with large effects on disease [15]. This makes whole exome sequencing very effective in finding disease-causing mutations, much cheaper than whole-genome sequencing and is therefore probably the most promising clinical application of NGS. Exome sequencing could for instance be applied in prenatal diagnostics, the screening for genetic disorders, cancer diagnostics and personalized medicine. Exome sequencing has already revealed many novel disease genes [16, 17].
In a recent paper exome sequencing has been used to identify de nove mutations in patients with intellectual disability. DNA from 100 patients was sequenced to analyze more than 21,000 genes and they identified 79 de novo mutations in 53 of 100 patients. [18] The identification of some mutations in known intellectual-disability genes provided clinically useful information for clinicians and for patients and their families, since much is known about the prognoses associated with these mutations. For some patients this reulsted in effective treatments based on the findings in this study, showing the power of whole exome sequencing as a diagnostic tool.

2.3.3 Whole genome sequencing

Whole genome sequencing (WGS) could have many applications in the determining the genetics of common complex diseases, in GWAS studies and in personalized medicine. Currently it has already been used a lot in cancer studies [19]. In these studies they usually compare the genome of healthy and tumor tissue of a patient providing us with important data of pathways involved in the origin of cancer and could help in providing targeted treatments. [20]
However despite all its possible applications, WGS is at present too costly for routine application. Another major drawback is our current lack of knowledge about genomic variants and their direct relation to disease. WGS generates a lot of data besides the genomic date about the locus of interest and these are not always wanted. Therefore targeted re-sequencing and exome sequencing will probably be the methods of choice for clinical application and WGS will probably be used in a more research setting but not for standard routine application.

2.4 Future developments

Besides the mentioned advantages for the application of NGS technologies in the clinic, there are ofcourse also some disadvantages, with the major disadvantage being the high costs. Currently it costs around $1000 to sequence a sample. The costs can be diminished by for example using exome sequencing instead of WGS, although they are still too high for most clinical labs.
A second disadvantage is the complexity and the storage of the data. NGS generates a high amount of data that requires a sophisticated computing infrastructure and skilled bio-informaticians. This makes it very impractical for small diagnostic labs and clinics. The cost of managing, storing and analyzing NGS data runs into hundreds of thousands of dollars and is currently a major bottleneck for the routine application of NGS in the clinic. [21]
To overcome some of these problems new machines are being developed of which the first already have been released (table 2). These machines are all based on the same principle as their predecessors described in the previous section and are a great step towards the routine implication of NGS in the clinic.

Name	Read length	Throughput	Advantages	Disadvantages
Illumina MiSeq	35-150	Upto 1GB/ run	Small sized, low cost instrument ($125.000) Low error rate Short run time Acceptable read length High level of multiplexing	Complex data analysis High costs of data generation
4545 GS Junior	400	35 MB / run	Small sized, low cost instrument Short run time Long read length	Low throughput Complex data analysis
Life Technologies SOLiD	35-75	Upto 15 GB / run	Low error rate Flexability and scalability Short run time Acceptable read length	Low throughput

Table 2. Ooverview of NGS platforms developed for the clinic.
For every platform the characteristics for read length, throughput and the advantages and disadvantages are summarized [7].

Besides these smaller and cheaper NGS systems, completely new third generation sequencers are also being developed. Whereas the platforms in the previous discussed sequencing techniques are all based on the amplification of DNA fragments, these new tecnhiques are based on sequencing a DNA molecule directly. This eliminates the need for PCR amplification, which can introuce a bias in your data [22]. Examples of these so called single molecule sequencers that are currently developed are the Heliscope single molecule sequencer, Single molecule real time (SMRT) sequencer, Single molecule real time (RNAP) sequencer, Nanopore DNA sequencer and The Ion Torrent sequencing technology [7].

It is clear that NGS will lead to a revolutionary improvement in clinical diagnosis and might increase the efficiency and efficacy of treating patients with all sorts of disorders, common diseases and cancer. However for the routine application of NGS in the clinic a lot of technical developments are still required and costs have to be diminished. These technical improvements will definitely occur in the coming years. A final very important consequence that needs to be considered is the fact that implementation of NGS in the clinic asks for the generation of new laws and rules and an ethical guideline for physicians. It will be difficult to interpret all the data generated with especially WGS and the availability of the data will have a strong impact on individual's life and pshcycoligal health.

3. Cardiovascular disease

3.1 Cardiovascular disease

Cardiovascular disease (CVD) is a general term for a large group of diseases that are related to the heart and the vasculatory system (Appendix I). The most common types are cardiomyopathies (HCM, DCM) myocardial infarction, stroke, and sudden cardiac death (SCD).
Cardiovascular disease (CVD) is the leading cause of death in Europe and the USA [23, 24] and responsible for 30 percent of all deaths worldwide [25]. In The Netherlands 29 percent of all deaths in women and 28 percent of deaths in men were caused by a CVD, in 2011 [26]. From these numbers it is clear that CVD have a great impact on our health and our social economic systems.
To reduce the number of deaths caused by CVD several public health campaigns have been initiated world wide to raise awareness of the risk factors like smoking, a high blood pressure and maintaining a healthy diet. These campaigns have been successful and have even lead to a decrease in prevalence of CVD in the western world. In addition the treatment has improved due to a more personalized drug treatment by analyzing specific symptoms individually thereby preventing or delaying the onset in individuals with a high risk of developing a CVD.
Nevertheless worldwide the CVD mortality rate has been increasing due to an aging population, life style changes (the third world is becoming more westernized), an increase in obesity and people suffering from type 2 diabetes [27]. As a result more than 80 percent of CVD-related deaths worldwide now occur in low and middle-income countries [28]. It is thought that CVD will in the future probably still be a major cause of death and that percentage might even increase [29]. Therefore to improve treatment and prevention it is crucial to generate a better understanding of the genetic background and molecular mechanisms contributing to CVD.

3.2 Risk factors

There are different factors that can lead to an increased risk of CVD, some of these factors can be modified, but some are beyond our control, like genetics. If a first-degree blood relative has had coronary heart disease or stroke before the age of 55 years (in the case of a male) or 65 years for a female, the risk of CVD increases significantly. Besides genetics there are some risk factors that cannot be controlled and are based on for instance gender, age and ethnic origin. People with African or Asian ancestry are at higher risks of developing cardiovascular disease than other racial groups. As a man you are at greater risk of heart disease than a pre-menopausal woman. But once past the menopause, a woman's risk is similar to a man's. The risk for stroke doubles every decade after age 55 and is similar for men and women. [24]

3.2.1 Modifiable risk factors

Besides genetics, CVD can be caused by modifiable risk factors that are affected by a persons life style, such as high blood pressure, obesity, use of tobacco, diabetes, cholesterol levels and lack of physical activity[26].
A raised blood pressure is currently the leading CVD risk factor but if diagnosed in time this can be prevented and treated successfully. The use of tobacco especially increases risk if you started smoking when at a young age, you smoke heavily or are a woman. Unfortunately passive smoking is also a risk factor. Stopping tobacco use can reduce your risk of cardiovascular disease significantly, no matter how long you have smoked [26,92].
Blood lipid levels are another risk factor for CVD. Raised blood glucose, high total cholesterol, high levels of triglycerides, high levels of low-density lipoprotein or low levels of high-density lipoprotein (HDL) cholesterol all increase the risk of heart disease and stroke. These emphasize the importance of a healthy diet, enough exercise and medication. These factors can all modify your blood lipid profile [26,92].
Physical inactivity increases the risk of heart disease and stroke by 50%. In addition obesity is a major risk for cardiovascular disease and predisposes you to diabetes, which is also a risk factor for cardiovascular disease. Having diabetes makes you twice as likely as someone who does not to develop

cardiovascular disease. A diet high in saturated fat increases the risk of heart disease and stroke. It is estimated to cause about 31% of coronary heart disease and 11% of stroke worldwide [26, 92].
A chronically stressful life, social isolation, anxiety and depression increase the risk of heart disease and stroke. Having one to two alcohol drinks a day may lead to a 30% reduction in heart disease, but above this level alcohol consumption will damage the heart muscle [26,92].
The risk of heart disease also increases by the use of certain medicines like the contraceptive pill and hormone replacement therapy (HRT) [30].

The influence and combined effects of all these different risk factors makes CVD a very difficult disease to predict and prevent. Currently there is only limited information available on whether combining genetic information with traditional risk factors improves prediction of heart disease.

3.3 Genetics of cardiovascular disease

CVD is a complex trait with a heterogeneous phenotype. Multiple diseases can for instance lead to the outcome of CVD but can have completely different genetic backgrounds. This makes it very difficult to identify the genetics underlying the molecular disease mechanisms in part due to the classification of the disease. [31] It is known from literature that CVD have a strong genetic background but there is no Mendelian inheritance for most types [32]. This is probably caused by the fact that multiple genes have an effect on the development and severity of the disease in combination with environmental risk factors.
CVD can roughly be divided into two groups based on their genetics; Inherited cardiac conditions and the common cardiovascular conditions.

3.3.1 Inherited cardiovascular conditions

Inherited cardiac conditions (ICC) are some of the most common human genetic disorders and can be roughly divided into four groups; cardiomyopathies, aortopathies, arrhythmias and cardiovascular malformations. These conditions usually have Mendelian forms of inheritance and are mainly caused by mutations in single genes and mostly affect children and young adults. Additionally congenital heart malformations are the most common cause of birth defect and the leading cause of death in infants [33]. The ICC will not be further discussed in this thesis.

3.3.2. Common cardiovascular conditions

Myocardial infarction, stroke, and sudden cardiac death (SCD) are some of the most common types of CVD. Beause these disorders are polygenic and are influenced by genetic and non-genetic factors it is difficult to identify the genes involved. However using GWAS 33 loci have now been identified and replicated by several studies [27,34,35]. (Table 2 and 3) Interestingly most of the discovered loci were found in genomic regions that have not previously been implicated in coronary artery disease [35]. Several genetic variants have been identified for arrhythmias, ventricular fibrillation, SCD and the sick sinus syndrome (Table 2). In addition loci associated with ischemic stroke, intracranial aneurysm, peripheral arterial disease, aortic aneurysm, venous thromboembolism, and erythrocyte phenotypes where identified (Table 3). Interestingly some of these loci are shared between coronary artery disease and myocardial infarction, suggesting a common genetic background.
It has proven difficult to identify and verify loci associated with heart failure and death from heart failure. Mainly caused by the fact that GWAS for heart failure had a limited number of cases to study have been limited by modest numbers of cases of heart failure (relative to the number of such studies and the heterogeneous nature (and thus heterogeneic sets of cases) of heart failure.

These summarized GWAS results indicate that a lot of loci related to common CVD have been identified in the past years. However the effect size of the identified variants is small and they probably have to be combined with non-genetic risk factors to use them efficiently in risk prediction [27].

During the rest of this thesis the focus wil be on coronary artery disease and myocardial infarction. These two forms of CVD are the leading cause of death in the world and although there is no clear mendelian

form of inherentce for these 2 diseases there is strong evidence that genetics play an important role in the development of these diseases [36].

Gene	Disease	Gene	Disease
ACTC1	HCM	GPD1L	BrS2
CSRP3	HCM, DCM	CACNB2	BrS4
MYBPC3	HCM, DCM	SCN1B	BrS5
MYH6, MYH7	HCM, DCM. SS	KCNE3	BrS6
MYL2, MYL3	HCM	SCN3B	BrS7
PRKAG2	HCM	CASQ2	AR-CPVT
TNNC1	HCM, DCM	FBN1	MS
TNNI3	HCM, DCM	GATA-4	AVSD
TNNT2	HCM, DCM	TBX5	AVSD
TPM1	HCM, DCM	NKX2	AVSD
TTN	HCM	APOB	FHP, SHC
VCL	HCM, DCM	**PCSK9**	**FHP, SHC, MI**
ABCC9	DCM	ANGPTL3	FHP
ACTC	DCM	**LDLR**	**SHC, MI**
ACTN2	DCM	ABCG5, ABCG8	SHC
DES	DCM	ARH	SHC
DSG2	DCM, ARVD	NOTCH1	BAV, CAV
DSP	DCM	**MRAS**	**CAD, MI**
EYA4	DCM	**SLC22A3**	**CAD, MI**
FCMD	DCM	**LPAL2**	**CAD, MI**
LAMP2	DCM	**LPA**	**CAD, MI**
LDB3	DCM	**CELSR2, PSRC1, SORT1**	**MI**
LMNA	DCM	**MIA3**	**MI**
NEXN	DCM	**WDR12**	**MI**
PLN	DCM	**PHACTR1**	**MI**
PSEN1, PSEN2	DCM	**CDKN2A, CDKN2B**	**MI, IA**
RBM20	DCM	**CXCL12**	**MI**
SCN5A	DCM, LQT3, BrS1	**BRAP**	**MI**
SGCD	DCM	**SLC5A3, MRPS6, KCNE2**	**MI**
TCAP	DCM	NINJ2	Stroke
TMPO	DCM	CELSR1	Stroke
TNN	DCM	BOLL, PLCL1	IA
DSC2	ARVD	SOX17	IA
DSP	ARVD	PITX2	IS
JUP	ARVD	SORT1	MI
PKP2	ARVD	ABO	CAD
RYR2	ARVD, AD-CPVT	ADAMTS7	CAD
TGFB3	ARVD	LIPA	CAD
TMEM43	ARVD	BAZ2B	SCD
KCNQ1	LQT1	GPC5	SCA
KCNH2	LQT2	ACTA2	TAA
ANK2	LQT4	MYH11	TAA & PDA
KCNE1, KCNE2	LQT5	TGFBR1, TGFBR2	TAA
KCNJ2	LQT7	DTNA	LVNC
CACNA1C	LQT8, BRrS3	TAZ	LVNC
CAV3	LQT9	AKAP9	LQT11
SCN4B	LQT10	SNTAI	LQT12

Table 2. Genes associated with CVD
HCM: hypertrophic cardiomyopathy, DCM: dilated cardiomyopathy, LVNC: left ventricular noncompaction, ARVD: arrhythmogenic right ventricular dysplasia, TAA: Thoracic Aortic Aneurysms, LQT: Long QT syndrome, BrS: Brugada syndrome , CPVT: catecholaminergic polymorphic ventricular tachycardia AR: Autosomal recessive, AD: Autosomal Dominant, AVSD: Atrial or ventricular septum defects, FHP: Familial hypobetalipoproteinemia, SHC: Severe hypercholesterolemia, BAV: Bicuspid aortic valve, CAV Calcific aortic valve disease, MS: Marfan's syndrome, CAD: coronary artery disease, MI: myocardial infarction, IA: Intracranial aneurysm, IS: Ischemic Stroke, SS: Sick sinus syndrome, SCD: Sudden Cardiac Death, SCA: Sudden Cardiac Arrest. [27,34,35]

3.4 Coronary Artery Disease and Myocardial Infarction

Coronary artery disease (CAD) is caused by an accumulation of so called atheromatous plaques within the coronary arteries. These plaques are made up of fat and cholesterol and form on the lumen inside the arteries. The disease takes years to progress and is usually not noticible in the beginning. After some time the formed plaques can rupture and are released from the lumen. In combination with blood cloth formation they obstruct the vessel and limit the blood flow, at one point completely obstructing the vessel, limiting blood flow to the heart. This usually results in a myocardial infarction, or heart attack, resulting in an irreversible death of heart cells [37].

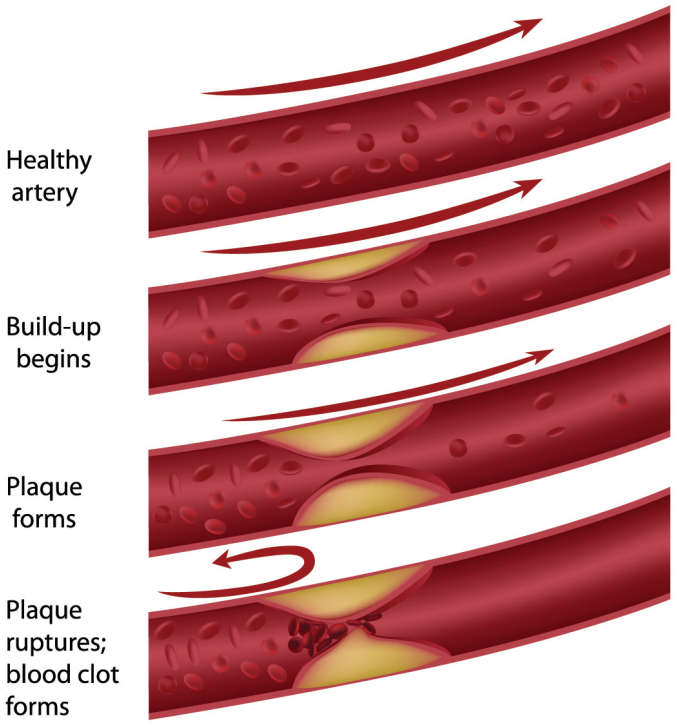

Figure 2. Stages of artherosclerosis
Artherosclerosis occurs when plaques are forming within the arteries. The plaques are formed by accumulation of fat and cholesterol. The plaques can eventually completely block the artery leading to a myocardial infarction.

3.4.1 Symptoms and diagnosis

Chest pain is the most common symptom, which can irradiate to for instance the left arm or the neck. Other symptoms are shortness of breath, heavy sweating, light-headedness, nausea, vomiting and palpitations. In the worst case scenario there is a loss of consciousness and eventually death. There are differences in the symptoms between men and women. At least one-fourth of all myocardial infarctions are silent, without chest pain or other symptoms [38].

Diagnosis for myocardial infarction is based on chest pain persisiting for longer then 20 minutes in combination with a rise in Troponin levels. Usually an ECG (Echocardiography) is performed to make an echo from the heart. From this the physician can determine the size and shape of the heart (internal chamber size quantification), pumping capacity, and the location and extent of any tissue damage. A physical exam can be performed and if required a coronary angiogram can be made.

Diagnosis for CAD is made based on imaging techniques like ECG, Exercise ECG, Coronary angiography, Intravascular sound and MRI [39].

3.4.2 treatment and prevention

For CAD several treatment options are possible depending on the state of the disease. Drugs that can be used are cholesterol lowering medications, beta-blockers, nitroglycerin and calcium antagonists. Surgery where they widen the artery using a balloon catheter or by implanting a coronary stent is also possible. In severe cases of obstruction coronary artery bypass grafting surgery is applied.

Treatment of a MI requires immediate medical attention where they usually try to rescue as much of the healthy myocardium as possible. Oxygen, aspirin and nitroglycerin are drugs that are commonly used in the prevention of further complications [40].

3.5 Genetics in treatment and prevention of CVD

As discussed in the previous sections there are many different risk factors that can affect the prevalence of CVD in combination with genetics. Consequently genetics can play an important role in the treatment and prevention of cardiovascular disease. In the past years a lot of research in the field of pharmacogenomics and effects of environmental factors and the genetics underlying CVD has been performed.

3.5.1 Pharmacogenomics

One of the best-studied CVD related genes are those involved in the cytochrome P450 pathway. Genetic variants in these genes affect the metabolism of a number of commonly used CVD drugs [41]. Copy number variants in the *CYP2D6* gene for instance affect the metabolism of *β-blockers and of* t*he drugs flecainide, propafenone.* The copy number variants affect the metabolosis of the drugs by affecting the metabolic rate of the enzyme or they produce an enzyme that is unstable or contains reduced to no enzymatic activity. Other studies have shown that the metabolism of the drugs warfarin and losartan is reduced in persons with genetic variants of the *CYP2C9* gene [42].
There are also genetic variants that can affect the drug target itself. For example 2 SNPs have been identified in the *NADPH* gene that are associated with smaller reductions in cholesterol in subjects treated with pravastatin. Additionaly several common variants in the *ESR1* (Estrogen receptor 1*)* gene further increase HDL-C levels after using estrogen therapy [43].

3.5.2 Genetic and environmental interactions

Another important and complicated factor in CVD treatment is the interaction between environment and genes. The great number of different risk factor have as a result that the same genotype can produce a different phenotype. A well-known example is blood pressure response to a low-sodium diet influenced by a SNP in the *AGT*-6 gene. The *AGT*-6 AA genotype is associated with a significant decrease in blood pressure for individuals on a low salt diet. Knowledge of the effect of these kinds of SNPs can improve treatment. Patients with the *AGT* -6 AA genotype could be placed on a low-sodium diet before exhibiting elevated blood pressure, thereby avoiding hypertension and consequent end-organ damage. [44]

3.5.3 Clinical application

Before the available genetic data can be translated to the clinic and used for the treatment of CVD some barriers remain. There are currently only a few genetic testing services available for relevant pharmacogenetic CVD variants, and the services that are available are costly and not standardized well. There is still a lack of rigorously performed clinical research that provides the evidence necessary to justify incorporation of pharmacogenetic testing into routine clinical practice. Many physicians are not familiar with the principles of genetics and the mechanisms by which gene variants might influence clinical decisionmaking. A considerable amount of work also remains to be done to elucidate both the genes contributing to CVD and the optimal interventions to address the genetic risk. The total costs of CVD and its treatment in our society are so great that even modest gains achieved through application of pharmacogenetics to CVD care could have a substantial impact on attributable risk and cost [45].

4. Predictive genetic testing for CVD

4.1 Genetic testing vs genetic screening

Studying genetic variation can be done in the context of genetic testing or genetic screening. It is important to clarify the difference between these two forms of genetic studies because the applications and laws are different. Genetic testing is a targeted analyis, which searches for specific genetic variants that are known to predispose individuals to a disease. These test are usually performed based upon prior knowledge by symptoms or familial reasons and is only performed in a diagnostic, predictive, or reproductive context. The genetic testing of embryos and fetuses is sometimes considered reproductive screening because no prior knowledge is required to perform these tests [46].
Genetic screening is usually performed among large groups of people without any specific prior reason. A well-known example of genetic screening is the Guthrie test, being performed on all new borns since 1975, to test for PKU. In 2005 the program was adjusted and the screening was broadened to test for in total 17 diseases, which are all rare disorders that can be treated when identified early [47].

4.1.1 Current applications of predictive genetic testing for CVD in the clinic

Predictive genetic testing for CVD in The Netherlands is only done on a diagnostic basis and is available for a number of monogenic forms of CVD. This is always based on familial history and is performed at dedicated cardiogenetics outpatient clinics at any university medical centre in the Netherlands. Disorders that can be tested for are listed in table 3. The request for testing is usually done by a clinical geneticist or general practioner. Before predictive DNA testing in asymptomatic relatives is possible, a disease causing mutation has to be identified in the index patient or proband. This so called proband is the first clearly affected person from his or her family to undergo DNA testing. Patients first receive a counselling session with a genetic counsellor and a cardiologist to discuss the clinical and psychosocial consequences of DNA diagnostics for the patient and his or her relatives. During this counselling session a pedigree is made and based on available data of the patient and his or her family the counsellor decides which genes can be tested and in what order. When the results of the DNA diagnostics are known, the patient and the referring physician are informed about the results and screening of relatives can be started. DNA testing can also be performed on preserved tissue of a deceased proband. In families with a disease causing mutation relatives are invited for a counselling session by means of a family letter to discuss the pros and cons of (predictive) DNA diagnostics. Blood withdrawal is possible after this counselling session [48]. Besides the disorders mentioned in table 4 it is also possible to test for familial hypercholesterolaemia en VSD en ASD at other clinics [49, 50]

Long QT Syndrome
Jervell-Lange Nielsen Syndrome
Andersen syndrome,
Timothy Syndrome
Short QT syndrome
Atrial Standstill
Sick Sinus Syndrome
Brugada Syndrome
Exercise induced polymorphic ventricular tachycardia
Wolff-Parkinson-White Syndrome
Hypertrophic cardiomyopathy
Dilated cardiomyopathy
Non compaction cardiomyopathy
Restrictive cardiomyopathy
Arrhythmogenic right ventricular dysplasia/cardiomyopathy
Carney complex, type 1
Danon Disease
Congenital heart defects

Table 3. CVD syndromes for which genetic tests are available at the AMC
Overview of genetic tests available at the AMC in Amsterdam. These tests are only performed based on familial history [50].

4.2 Genetic discrimination

Because of the enormous various implicative effects of genetic testing it is very important there are laws that regulate what can and what can not be done with the data obtained from genetic tests, based on all the ethical implications. Most of these laws are focusing on genetic discrimination.
With the application of NGS in the clinic one of the most heavily debated subjects has been the use of genetic information by insurance companies and/or employers. The fear is that this will lead to a "genetic underclass' with people that are uninsurable, unable to get a mortgage and will have trouble finding a job. These fears will also have an effect on the healthcare system. To keep genetic information out of their medical records, and thus out of the hands of insurers and/or employers, patients sometimes refuse genetic testing or screening [51].

In a recent study by Geelen et al.[52] the fears of dicrimination and impact of genetic testing were investigated by following six Dutch families involved in genetic testing for HCM. They conducted interviews with 57 members of these families and based on their anwers the fear of discrimination was surpisingly found to be based in the social and life-planning of these families more than the fear of discirimination by employers or insurance companies. An interesting finding was that this fear was mostly based on earlier experiences of discrimination of diseased family members more than of their own experiences. Therefore it is important for counselers to focus less on the information provision of genetic non-discrimination legislation and more on the role of family dynamics and individual strategies to cope with the social consequences of living with HCM as possible barriers for uptake of genetic testing [52].

4.2.1 Laws and regulations in The Netherlands

In The Netherlands we have several laws and regulations concerning genetic testing and screening.
The Dutch Medical Examination Act (MEA) restricts private insurers and employers in requesting a genetic test and using genetic test results from individuals who want to obtain a civil employment contract, a pension, or a life or disability insurance. The act states that – for a life insurance below a predefined ceiling of 160 000 Euro – no questions may be asked about untreatable hereditary disease or about the results of genetic tests for such diseases in the applicant and his/her relatives, except in case of an already manifest disease. Nevertheless the fear of genetic discrimination persists [53].
Additionally there are different laws and regulations concerning genetic testing and screening. There is the WGBO (Wet op de Geneeskundige BehandelingsOvereenkomst) that was accepted in 1995 and is about patient rights and responsibilities of the medical care person [54]. In addition there is the WBP (Wet Bescherming Persoonsgegevens) about gathering, storing and application of sensitive data like medical and genetic data [55].
The WMO (Wet Medisch-wetenschappelijk Onderzoek met mensen) states that NGS can only be used when the protocol gets permission from a METC (Medisch Ethische toetsingscomissie) and the test subject has inclined with the genetic analysis and oher aspects of the test [57].
With respect to screening, the Netherlands' government accepted the act "Wet op het bevolkingsonderzoek" in July 1996, in which the acceptance criteria are described for population-based research. It states that for some types of genetic research permition is needed from the Minister of Health, Welfare and Sports. Permission is needed for tests that use ionizing radiation, cancer research, research for severe diseases and traits for which no cure or prevention is available. This system was introduced to establish and guarantee a fair balance between the right of self-determination of individuals and the need to protect them against (potentially) harmful screenings techniques [57].
In the Act, population screening is defined as: "a medical examination which is carried out in response to an offer made to the entire population or to a section thereof and to detect diseases of a certain kind or certain risk indicators, either wholly or partly for the benefit of the persons to be examined." Offering and performing tests for detecting (risk indicators of) cancer and incurable diseases without a licence is unlawful. Moreover, performing these screening methods without permission is a punishable offence.
Unfortunately the Act does not set quality norms for the information to be provided to the (potential) test subjects, consent, the use of samples, and counselling to be provided. Nevertheless, health care workers and companies wishing to perform a population screening programme have to comply with the professional medical practice standards that entail the main rights of the patient as laid down in the Dutch Civil Code [53, 58].

4.3 Informed Consent

Besides the importance of having laws and regulations concerning genetic testing, it is also important to inform every person undergoing a genetic test or screening with the implications, limitations and possibilities [58, 59]. Before a genetic test is performed a patient receives a pre-test counselling session where they need to give their consent on what will happen with the test results and how much information they require. The counseller has to make sure the patient has a clear understanding of the facts, implications, and future consequences of the test performed. This is particularly important to protect the patient against unwanted test procedures and from receiving undesired information. Furthermore the patient needs to be capable of deciding for him or herself whether or not to receive information with regard to their health status or to undergo a physical examination or intervention. If a patient is incapable of deciding for him or herself another person is generally authorized to make this decision. For a child this is usually their parents [58, 59].
Important aspects that are generally discussed during the counselling session are the chances of finding additional information and how to report any additional findings. Genetic information acquired from the test can also have implications for family members of the patient [58].
With the new era of NGS based techniques and the possibility for genetic screening a lot more (undesired) information becomes available. This make the pre-test counselling session important. The right not to know will become a very important aspect of the counselling session.

Before consenting in a genetic test a patient can decide not to be informed with the result of the test or any of the additional information gained. This right not to know is also part of the WBO and will become particularly important with NGS based testing [58]. There are several reasons one can think of why a patient wouldn't want to know the outcome. First there is a huge psychological impact; It has been described that for many people, the discovery that they have a genetic condition that places them at a high risk of suffering certain untreatable diseases could so depress them that the quality, joy, and purpose of their lives would be affected. Secondly there is the dilemma of how to deal with informing their family members and again the psychological pressure this yields. Lastly there is a more practical disadvantage in the application for health insurance or getting a mortgage. Every patient therefore has the right not to know the outcome of a test.

It can seem that the right not to know is contrary to the doctor's "duty to disclose" risks to patients. Therefore it should be noted that the right not to know can be overruled by the physician if he thinks that the information is required for the health of the patient and their family members. This is related to another important aspect. The individual who chooses not to know his or her genetic status, thereby putting him or herself in a position of being unable to disclose that vital information to family members, could be said to be acting against solidarity [58].

For future application of NGS in the clinic it is of crucial importance to consider the type and of information that is being generated by NGS-based diagnostic approaches. There are new rules and laws required to prevent genetic discrimination and to make sure the generated data is stored and protected in an adequate manner. Furthermore it is of crucial importance that patients are correctly informed before performing tests and after test date has been acquired. These points are essential for a correct ethical implementation of NGS in the clinic.

5. CVD risk prediction

An accurate risk prediction for CVD is important for a reduction in the number of people affected by this group of diseases and could greatly improve treatment. If an individual has been diagnosed with a high risk for developing CVD a specialized treatment through lifestyle modification and/or drug therapy aimed at risk factor modification can be applied. As previously described most cardiovascular diseases like CAD are polygenic and are caused by a combination of genetic variants combined with non-genetic risk factors. Because all these risk factors have to be considered simultaneously it is very complicated to predict an individual's risk for CVD. However several risk prediction models have been developed in the past years.

5.1 CVD risk prediction models

The first developed CVD risk prediction model was the Framingham Risk Score, which can be used to assess the 10-year risk for CHD [60,61]. The Framingham Risk Score (FRS) is based on data obtained from the Framingham Heart Study and determines the risk separately for men and women. The risk factors taken into account by this model are dyslipidemia, age range, hypertension treatment, smoking, and total cholesterol [62].
The FHS started in 1948 with 5209 individuals. Original cohort participants were examined approximately every 2 years. Subsequently, in 1971, the Framingham Offspring Study enrolled 5124 children and spouses of the children of the original cohort. In 2002, the Framingham Third Generation study enrolled 4095 children of the Offspring cohort. Participants of the Framingham Offspring Study were evaluated approximately every 4 years.
Other models that have been developed to predict CVD risk are: the assessing cardiovascular risk to Scottish Intercollegiate Guidelines Network to assign preventative treatment (ASSIGN) score, systematic coronary risk evaluation (SCORE) score, Prospective Cardiovascular Münster (PROCAM) score, QRESEARCH cardiovascular risk (QRISK1 and QRISK2) algorithms and the Reynolds risk score.
All these studies have a similar efficiency in estimating disease risk, and they do not take genetics into account [63].

GWAS studies have revealed around 33 loci involved in CAD and it would be interesting to know what effect the genetic knowledge of a person would have on the prediction of disease. As an example I will describe the 9p21.3 locus below along with its effect on risk prediction for coronary artery disease.
This will be done by discussing three very interesting studies looking at the accuracy of 10-year risk prediction using traditional risk factors with, and without, measurement of the SNPs in the 9p21 locus.

5.2 The 9p21.3 locus

The 9p21.3 locus is the first risk variant associated with CAD that was published and has since then been confirmed in many other studies [64, 65]. The identified rs10757274 SNP comprises an A>G transition increasing the risk for individuals associated with an estimated 20% increase in CHD risk in heterozygotes and 40% increase in CHD risk in homozygotes [66]. This is equivalent to non-genetic risk factors such as smoking [67].
The risk allele is carried by 75% of the European population and has also been found in other ethnic groups including Japanese [68], Korean [69], Chinese [70], Pakistani [71] and Indian [72], but interestingly not among Africans [73].
The mechanism by which 9p21.3 increases the risk for CAD is unknown and is independent of known risk factors [64, 65].

5.2.1 The 9p21.3 genomic region

The rs10757274 SNP is part of a cluster of linked SNPs at chromosomal location 9p21.3 that are associated with coronary artery disease, myocardial infarction, stroke, abdominal aortic aneurysm and intracranial aneurysm [64, 65, 66, 74, 75]. It is located in a gene desert spanning about 50 kb which contains a long noncoding RNA (lncRNA) of 126,000 bps, referred to as ANRIL (antisense noncoding RNA in the INK4 locus) or CDKN2BAS (CDKN2B antisense RNA) [76, 77].

Adjacent to the 9p21.3 locus is the INK4/ARF locus that contains the genes CDKN2A and CDKN2B, encoding cyclin-dependent kinase inhibitors, and MTAP, encoding methylthioadenosine phosphorylase. The CDKN2A, CDKN2B, and MTAP genes are important targets in tumor biology because loss of the INK4/ARF locus is a frequently found in the development of cancer. Moreover the Chr9p21 region has also appeared as a risk locus in genome-wide association studies for several cancers, including glioma, basal cell carcinoma, breast cancer, and nasopharyngeal carcinoma.

The lncRNA is transcribed into several alternate transcripts and expression of the 9p21.3 risk allele is consistently associated with higher expression of ANRIL but lower mRNA expression of the nearby genes CDKN2A andCDKN2B [76, 77, 78].

It has been shown that several conserved sequences in this region contain enhancer elements and can regulate the surrounding genes indicating a possible role of this locus in affecting CAD via these genes [77, 79].

5.3 CVD risk prediction using the 9p21.3 risk variant

In the past years several studies have investigated the potential clinical value of the association of Chr9p21 with CVD risk prediction. This was done by analyzing the effect on risk prediction after adding Chr9p21 to the currently available models of risk prediction such as the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III), FRS, Reynolds Risk score and ARIC Cardiovascular Risk Score (ACRS). These three studies that all focused on the effect of risk prediction after the addition of the rs10757274 SNP into their risk prediction models (Table 2).

Reference	Study cohort	Cohort size	Risk model using CRF	Significant improvement in risk score	Significant improvement in reclassification
Talmud et al [84]	NPHS II	2742	FRS	No	Yes
Brautbar et al [86]	ARIC	9998	ACRS	Yes	Yes
Paynter et al [80]	WHS	21.129	Reynolds score	No	No

Table 5. Summary of 9p21.3 studies
Summary of studies that evaluated the effect of adding the 9p21.3 genotype to their risk prediction models.

5.3.1 CVD risk prediction by addition of the 9P21.3 genotype

In the first published study performed by Paynter et al they used data from the Women's health study (WHS), which was started in 1992 [80]. The WHS recruited female health professionals in the United States who had no major chronic disease at the beginning of the study and followed them for 10 years for incident myocardial infarction, stroke, coronary revascularization, and cardiovascular death [81, 82]. Of these women, 23 226 were genotyped for the rs10757274 polymorphism in the 9P21.3 locus, but they only included the 22 129 white women for their analysis. Subsequently they assesed the effect of the addition of rs10757274 SNP to the ATP III risk score and the Reynolds Risk Score [83].

Of the 22.129 selected women 26.2% had no risk (G) alleles at rs10757274, 49.5% had 1 risk allele, and 24.3% had 2 risk alleles. The number of risk alleles only had a significant association with a family history of premature myocardial infarction (13% mortality rate for 1 or 2 risk alleles vs 11% for no risk alleles) and a slight association with a history of diabetes. But there was no association with smoking, age, blood pressure or any biomarkers as described before.

Next they analyzed the affect of addition of the rs10757274 SNP in prediction scores in both risk prediction tests performed. They observed almost identical results in their analyses using either the ATP III or Reynolds Risk Score prediction models. Thus unfortunately addition of the genetic variation only

slightly improved the classification of risk prediction in a model based on ATP and did not improve classification using the Reynolds Risk Score (Table 6).

Risk Factor	ATP III risk score	ATP III risk score + genotype	Reynolds Risk Score	Reynolds Risk Score + genotype
Age	4.092 ± 0.287 (<0.001)	4.108 ± 0.287 (<0.001)	0.074 ± 0.005 (<0.001)	0.074 ± 0.005 (<0.001)
Blood Pressure	3.578 ± 0.381 (<0.001)	3.569 ± 0.381 (<0.001)	3.653 ± 0.353 (<0.001)	3.648 ± 0.353 (<0.001)
Total Cholesterol	1.174 ± 0.198 (<0.001)	1.173 ± 0.198 (<0.001)	0.997 ± 0.200 (<0.001)	0.996 ± 0.200 (<0.001)
HDL cholesterol	-1.114 ± 0.143 (<0.001)	-1.117 ± 0.143 (<0.001)	-0.978 ± 0.145 (<0.001)	-0.979 ± 0.145 (<0.001)
Current smoker	0.888 ± 0.091 (<0.001)	0.887 ± 0.091 (<0.001)	0.880 ± 0.092 (<0.001)	0.876 ± 0.092 (<0.001)
History of diabetes	1.340 ± 0.110 (<0.001)	1.335 ± 0.110 (<0.001)	-	-
Family History of MI	-	-	0.423 ± 0.100 (<0.001)	0.423 ± 0.100 (<0.001)

Table 6. Comparison of Cardiovascular Risk Prediction Models with and without 9p21.3 Genotype
The Reynolds and ATP III risk scores with and without genotye information are shown for each risk factor.

In the second study Talmud et al used the Framingham risk algorithm to determine the effect of adding the genotype to the risk predciton algorithm [84]. They used the available data from the Northwick Park Heart Study II (NPHS-II). This is a CVD study of 2742 healthy middle-aged men (50–64 years old) recruited from 9 UK general practices that were followed for an average of 14 years, with 270 CHD events [85]. They analyzed the effect of combining the rs10757274 SNP data to the Framingham risk algorithm and again this did not significantly increase the risk prediction.

Interestingly however, when they modeled the effect on CHD risk of up to 10 hypothetical, randomly assigned gene variants, with allele frequencies and risk similar to those of rs10757274 they did see a significant improvement. The addition of 1 further SNP with similar characteristics increases the risk association significantly (P <0.03), whereas the inclusion of 2 or more SNPs had a greater effect (P<0.001), with the addition of further SNPs having smaller incremental effect. However, whether this improvement is clinically significant is not known as the number of individuals with multiple independently segregating risk alleles is likely to be small.

Genotype	AA (no risk allele)	AG (1 risk allele)	GG (2 risk alleles)
CHD Risk	5.9	8.5	9.5
Model 1	1.00	1.40 (1.02-1.92)	1.60 (1.12-2.28)
Model 2	1.00	1.38 (1.00-1.90)	1.57 (1.10-2.25)
Model 3	1.00	1.58 (1.09-2.28)	1.96 (1.31-2.94)

Table 7. Comparison of Cardiovascular Risk Prediction Models With 9p21.3 Genotype
Model 1: adjusted for age and general practice.
Model 2: adjusted for age, smoking, blood pressure, chlesterol, triglycerides and BMI.
Model 3: adjusted for age, smoking, blood pressure, chlesterol and calculated baseline HDL.

5.3.2 Reclassification of CVD risk by addition of the 9P21.3 genotype

The above discussed studies from Paynter et al and Talbud et al showed that addition of the rs10757274 SNP to their risk prediction models did not significantly increase the risk prediction score, although this SNP has been strongly associated with an increased risk for CVD. Therefore Talbud et al and other studies from Brautbar et al looked at the effect of addition of the 9P21.3 genotype to the ability of using this genotype to classify individuals into risk categories by determining the number of men correctly reclassified. Based on their CRF score individuals are divided into risk categories to estimate their risk of CVD in the coming 10 years. In the study by Talbud et al men were divided into those with a 10-year CHD risk of <5%, 5%–10%, 10%–20%, and >20% risk. After addition of the p21.3 genotype, 21.9% of the men were reclassified, of which a striking 63% moved into more accurate prediction categories.
The improvement in reclassification by the addition of the rs10757274 SNP was confirmed by the other study performed by Brautbar et al. Here they assed the addition of 9p21 allele to the ARIC Cardiovascular Risk Score (ACRS) [86]. The study used 9,998 white participants from the Atherosclerosis Risk in Communities (ARIC) study. When they added the 9p21 allele to the traditional RF, 12–13% in the

intermediate-low (>5% to ≤10% 10-year risk) and intermediate-high (>10% to ≤20% 10-year risk) categories were reclassified in both ACRS and FRS models.

It is clear from these studies that despite having a strong correlation with an increased risk for CVD the addition of the rs10757274 SNP in the 9p21.3 locus does not significantly approve the risk prediction score in several well established risk prediction models. When combined with multiple SNPs the risk prediction score becomes better, but this approach is not feasible since it is not likely for a patient to carry more then 5 of these SNPs. Interestingly the addition of the genotype to the classification of individuals in risk categories did improve by taking the genotype into account.
Addition of 9p21 genotype information might therefore influence treatment based on lifestyle modification and initiation of targeted drug therapy.

6. Discussion and conclusions

6.1 Application of NGS in the clinic

By applying massive parellel sequencing reactions, next generation sequencing has had an enormous impact on the field of genetics. The new techniques that are being developed are more accurate in detecting mutations and can supply physicians with a lot more detailed, and sometimes unwanted, genetic information about their patients compared to the currently used techniques. This is making NGS very suitable for clinical diagnostics and genetic testing or screening.
NGS could be useful in a whole scala of diagnostic tests and can help to identify the genetic cause, and improve treatment, of many disorders. It can be applied for the analyis of DNA, RNA and even epigenetic patterns like DNA methylation [7]. Some technical applications of NGS that are promising for clinical application are targetetd re-sequencing, whole exome sequencing and whole genome sequencing. Depending on the demands of the test each of these will have their benefits and drawbacks.
Before NGS can be routinely applied in the clinic there are some important challenges that need to be overcome, the first one obviously being the high costs.

The current development of new third generation sequencing machines will make NGS more attractive for routine diagnostic labs. NGS will become cheapier, more reliable and easy to use in the clinic.
A second challenge is the analysis and storage of NGS date. Specifically trained personnel is required and there is the need for new rules and laws for the storage and management of the data. Skilled personal needs to be trained to analyze the data in a reliable manner and ofcourse physicians and clinicians need to be able to understand this data.
Whole exome sequencing will probably be the first most widely used NGS based technique applied in the clinic. Since this is the most cost-effective way to detect disease causing mutations and the data analyis is fairly simple compared to whole genome sequencing. It is clear that the implementation of NGS technologies in the clinic will no doubt lead to a whole new era of diagnostic testing.

6.2 Risk prediction for CVD using genetic markers

Because of the high impact on worldwide mortality rates, CVD are a group of diseases for which it would be very interesting to perform routine genetic screening. If the risk for an individual to develop a CVD could be reliably predicted at a young age, effective precautions could be made to prevent disease onset.
CVD is, as many common traits, a group of complex polygenic disorders that are caused and influenced by many different factors. Some of the risk factors for CVD can be controlled, like smoking, physical activity and keeping a healthy diet, however some like genetics and age cannot. These factors together influence the disease outcome.
To estimate the capacity of NGS to predict disease a recent study by Roberts et al used monozygotic twins to determine how well they could establish a specific level of genetic risk for disease [89]. In their study they found that the maximum capacity of WGS to identify individuals that have a clinically significant risk lies at 24 different diseases. Their analysis also revealed that when individulals are tested for one of these 24 diseases wil receive negative test results for 23 out of the 24 while they still hve a significant chance in developing this disease [58].
Their date once more confirm that it is still very difficult to predict a person's risk to develop disease based on genetic data. More extensive knowledge about specific disease associated SNP's and associated environmental factors could be very beneficial.

6.2.1 Knowledge of Chr9p21 doesn't improve CVD risk prediction

At present it is possible to test for most Mendelian forms of CVD based on a familial history. The results are used to reliably establish familial CVD disorders and are succesfully used for treatment. For common forms of CVD however no testing is currently being applied and the current knowledge is not good enough to be able to predict CVD on the basis of genetics.

Using GWAS, and lately using NGS, multiple loci have been found to be associated with an increased risk for CVD. However these all have a low effect rate and it is not yet possible to reliable predict disease from genetics alone. Using GWAS 33 loci were found to be specifically associated with myocardial infarction and stroke. One of the highest associated loci being Chr9p21.3.

Since Chr9p21 has been intensively studied in the past years it is a very interesting candidate to use in combination with the existing CVD risk prediction models. However from the several studies discussed in this thesis we can conclude that addition of this genotype to risk prediction model such as the FRS, Reynolds risk score and ARCS do not have any significant effect. One reason for this could be that the SNP is located in a gene desert and only indirectly influences the surrounding genes complicating the analysis. Another interesting keyplayer might be the long noncoding RNA ANRIL, which is transcribed from this locus, the expression of which could be affected by SNPs in the locus. Unfortunately the function of most long non-coding RNAs is still a mystery [90].

Interestingly the addition of the Chr9p21.3 SNP did have a positive effect when assigning people to the right risk group. Because drug dosages are defined dependent on the risk classification, assigning people to the right risk group can greatly affect treatment

6.2.2 Combining genomic variants improves risk prediction

Why the effect of addition of the rs10757274 SNP to the risk models is so small, while it has such a strong assocation with CVD could also be caused by the fact that multiple genomic variants are playing a role in complex traits like CVD. Talmud et al in their study showed that addition of multiple SNP's greatly improves the risk predicition signficance [84]. This was also studied in a recent combined analysis of the prospective FINRISK and Malmö Diet and Cancer studies using a score of Chr9p21 and 12 additional genetic markers identified in GWASs [91]. However, adding this score to conventional risk factors unfortunately also did not lead to a significant improvement of risk prediction. A possible reason for this result might be that most of the markers used in the genetic score had only very modest effect sizes [91].

Consequently genetic risk prediction may only be an added value when more genetic markers or markers with greater effect sizes are combined. This greatly reduces the clinical usability of disease prediction based on genetic markers because it will be difficult to find all these SNPs together in one individual. Therefore it is still very important to take all the modifiable risk factors into account when prediciting risk for cardiovascular disease.

6.3 Ethical and legal issues of predictive genetic screening

The use of NGS technologies in the clinic will generate a lot more detailed and sometimes unwanted data after perfoming genetic tests. Therefore it is important to optimize certain aspects of the current counselling sessions for them to be compatible with the implications of NGS based technologies.

First the informed consent, which is now mostly based on giving the patient a clear understanding of the facts, implications, and future consequences of the test performed. However with NGS technologies another important factor will be the additionally acquired data and how to handle this. It will be difficult for a counselor to realize before hand all the consequences from the information that will be gained from a genetic screening experiment. One proposal is to adjust the informed consent into a more generic consent [60].

Secondly due to the additional information acquired in NGS based tests the right not to know will also play a bigger role. It will be important to have rules set up for especially the physician indicating when he or she can overcome this right in the context of health safety concerns of the patients and their families.

Predictive genetic screening can have a severe impact on an indivdual's life path after knowing he or she has a certain risk to develop a disease, which can lead to genetic discrimination. The current laws and regulations for genetic testing are not sufficient to cover all aspects associated with genetic screening and need to be adjusted and specified.

6.3 Clinical implication of genetic testing for CVD

CVD are the leading cause of death worldwide and form a heterogeneous group of diseases with different underlying genetic mechanisms. This heterogenous nature makes it difficult to perform accurate risk prediction based on genetics alone. Current risk prediction models only take the modifiable risk factors into account and it would be interesting to combine those models with the addition of genetic variants. However from several studies it has become clear that until now we are not capable of providing an accurate risk prediction based on genetic data combined with the current used risk prediction models.
In combination with the psychological consequences of these tests on the people that are tested and the incomplete view of all ethical and legal aspects it is not yet feasible to implement routine predictive genetic screening for CVD. Genetic testing based on family history is definately very helpful and the acquired genetic variant data will be of great value in improving this field.

7. References

1. Tian Q, Price ND, Hood L. Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. J Intern Med. 2012 Feb;271(2):111-21.

2. Kute, T; Lack CM, Willingham M, Bishwokama B, Williams H, Barrett K, Mitchell T, Vaughn JP. Development of Herceptin resistance in breast cancer cells. Cytometry 2004 57A (2): 86–93.

3. Sanger F. The Croonian Lecture, Nucleotide sequences in DNA. Proc R Soc. Lond B Biol Sci 1975;191:317e33.

4. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Raymond C, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blöcker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ; International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature. 2001 Feb 15;409(6822):860-921.

5. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. Science 2001, Jun 5;292(5523):1838.

6. James S Ware, Angharad M Roberts, Stuart A Cook. Next generation sequencing for clinical diagnostics and personalised medicine: implications for the next generation cardiologist. Heart 2012;98:276-281

7. Chandra Shekhar Pareek, Rafal Smoczynski and Andrzej Tretyn. Sequencing technologies and genome sequencing. J Appl Genet. 2011 November; 52(4): 413–435.

8. Sobreira NL, Gnanakkan V, Walsh M et al. Characterization of complex chromosomal rearrangements by targeted capture and nextgeneration sequencing. Gen Res 2011: 21 (10): 1720–1727.

9. Desai AN, Jere A. Next-generation sequencing: ready for the clinics? Clin Genet. 2012 Jun;81(6):503-10.

10. Welch JS, Westervelt P, Ding L et al. Use of whole-genome sequencing to diagnose a cryptic fusion oncogene. JAMA 2011: 305 (15):1577–1584.

11. Syed F, Grunenwald H, Caruccio N. Optimized library preparation method for next-generation sequencing. Nat Methods App Note 6 2009:i–ii.

12. Parkinson NJ, Maslau S, Ferneyhough B et al. Preparation of high quality next generation sequencing libraries from picogram quantities of target DNA. Genome Res 2012: 22 (1): 125–133.

13. Turner EH, Lee C, Ng SB, Nickerson DA, Shendure J. Massively parallel exon capture and library-free resequencing across 16 genomes. Nat Methods 2009: 6: 315–316.

14. Xi Lin, Wenxue Tang, Shoeb Ahmad, Jingqiao Lu, Candice C. Colby, Jason Zhu, Qing Yu. Applications of targeted gene capture and next-generation sequencing technologies in studies of human deafness and other genetic disabilities. Hearing Research, Volume 288, Issues 1–2, June 2012, Pages 67–76

15. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloğlu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP (10 November 2009). "Genetic diagnosis by whole exome capture and massively parallel DNA sequencing". Proc Natl Acad Sci U S A 106 (45): 19096–19101.

16. Vissers LE, Fano V, Martinelli D, Campos-Xavier B, Barbuti D, Cho TJ, Dursun A, Kim OH, Lee SH, Timpani G, Nishimura G, Unger S, Sass JO, Veltman JA,Brunner HG, Bonafé L, Dionisi-Vici C, Superti-Furga A. Whole-exome sequencing detects somatic mutations of IDH1 in metaphyseal chondromatosis with D-2-hydroxyglutaric aciduria (MC-HGA). Am J Med Genet A. 2011 Nov;155A(11):2609-16.

17. Hoischen A, van Bon BW, Gilissen C, Arts P, van Lier B, Steehouwer M, de Vries P, de Reuver R, Wieskamp N, Mortier G, Devriendt K, Amorim MZ, Revencu N,Kidd A, Barbosa M, Turner A, Smith J, Oley C, Henderson A, Hayes IM, Thompson EM, Brunner HG, de Vries BB, Veltman JA. De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. Nat Genet. 2010 Jun;42(6):483-5.

18. Joep de Ligt, M.Sc., Marjolein H. Willemsen, M.D., Bregje W.M. van Bon, M.D., Ph.D., Tjitske Kleefstra, M.D., Ph.D., Helger G. Yntema, Ph.D., Thessa Kroes, B.Sc., Anneke T. Vulto-van Silfhout, M.D., David A. Koolen, M.D., Ph.D., Petra de Vries, B.Sc., Christian Gilissen, Ph.D., Marisol del Rosario, B.Sc., Alexander Hoischen, Ph.D., Hans Scheffer, Ph.D., Bert B.A. de Vries, M.D., Ph.D., Han G. Brunner, M.D., Ph.D., Joris A. Veltman, Ph.D., and Lisenka E.L.M. Vissers, Ph.D. Diagnostic Exome Sequencing in Persons with Severe Intellectual Disability. N Engl J Med 2012; 367:1921-1929, November 15, 2012

19. Shyr D, Liu Q. Next generation sequencing in cancer research and clinical application. Biol Proced Online. 2013 Feb 13;15(1):4.

20. Welch JS, Westervelt P, Ding L et al. Use of whole-genome sequencing to diagnose a cryptic fusion oncogene. JAMA 2011: 305(15): 1577–1584.

21. Sboner A, Mu XJ, Greenbaum D, Auerbach RK, Gerstein MB. The real cost of sequencing: higher than you think! Genome Biol 2011:12 (8): 125.

22. Schadt EE, Turner S, Kasarskis A (2010) A window into thirdgeneration sequencing. Hum Mol Genet 19:R227–R240

23. Allender, S.S. et al. (2008) European cardiovascular disease statistics 2008 edition, European Heart Network: Brussels, Belgium

24. American Heart Association (2008) Heart Disease and Stroke Statistics – 2008 Update American Heart Association: Dallas, Texas

25. World Health Organization (2003) The World Health Report: 2003: shaping the future, World Health Organization: Geneva

26. Nederlandse Hartstichting. Hart- en vaatziekten in Nederland 2012. Cijfers over risicofactoren, ziekte en sterfte. December 2012

27. Arking, D.E.; Chakravarti, A. Understanding cardiovascular disease through the lens of genome-wide association studies. Trends in genetics, Volume: 25 Issue: 9 (September 2009), pp: 387-394

28. WHO. 2008b. The global burden of disease: 00 update. Geneva: World Health Organization.

29. Beaglehole, R., and R. Bonita. 2008. Global public health: A scorecard. Lancet 372(9654):1988-1996.

30. Zakharova MY, Meyer RM, Brandy KR, Datta YH, Joseph MS, Schreiner PJ, Rao GH, Divani AA.

31. Risk factors for heart attack, stroke, and venous thrombosis associated with hormonal contraceptive use. Clin Appl Thromb Hemost. 2011 Aug;17(4):323-31.

32. Sekar Kathiresan[1,] Deepak Srivastava[4] Genetics of Human Cardiovascular Disease. Cell Volume 148, Issue 6, 16 March 2012, Pages 1242–1257

33. Christopher J. O'Donnell, M.D., and Elizabeth G. Nabel, M.D. Genomics of Cardiovascular Disease. N Engl J Med 2011;365:2098-109.

34. Marelli AJ, et al. Congenital heart disease in the general population: changing prevalence and age distribution. Circulation. 2007; 115:163–172.

35. Roberts R, Stewart AF. The genetics of coronary artery disease. Curr Opin Cardiol. 2012 May;27(3):221-7.

36. Christopher J. O'Donnell, M.D., and Elizabeth G. Nabel, M.D. Genomics of Cardiovascular Disease. N Engl J Med 2011;365:2098-109.

37. Arnett DK, Baird AE, Barkley RA, Basson CT, Boerwinkle E, Ganesh SK, Herrington DM, Hong Y, Jaquish C, McDermott DA, O'Donnell CJ; American Heart Association Council on Epidemiology and Prevention; American Heart Association Stroke Council; Functional Genomics and Translational Biology Interdisciplinary Working Group. Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group. Circulation. 2007 Jun 5;115(22):2878-901.

38. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. N Engl J Med. 2013 May 23;368(21):2004-13.

39. McConaghy JR, Oza RS. Outpatient diagnosis of acute chest pain in adults. Am Fam Physician. 2013 Feb 1;87(3):177-82.

40. Boateng S, Sanborn T. Acute myocardial infarction. Dis Mon. 2013 Mar;59(3):83-96.

41. Global Atlas on Cardiovascular Disease Prevention and Control. Mendis S, Puska P, Norrving B editors. World Health Organization (in collaboration with the World Heart Federation and World Stroke Organization), Geneva 2011.

42. Zakia Bibi. Role of cytochrome P450 in drug interactions Nutrition & Metabolism 2008, 5:27

43. Karim Ouahchi, Neal Lindeman, and Charles Lee. Copy number variants and pharmacogenomics. Pharmacogenomics, January 2006, Vol. 7, No. 1 , Pages 25-29

44. Mooser V, Waterworth DM, Isenhour T, Middleton L. Cardiovascular pharmacogenetics in the SNP era. J Thromb Haemost. 2003 Jul;1(7):1398-402.

45. Teresa Norat, Richard Bowman, Robert Luben, Ailsa Welch, Kay Tee Khaw, Nick Warehamand, Sheila Bingham. Blood pressure and interactions between the angiotensin polymorphism AGT M235T and sodium intake: a cross-sectional population study. Am J Clin Nutr August 2008 vol. 88 no. 2 392-397

46. Grosse S.D.' Rogowski W.H.' Ross L.F.' Cornel M.C.' Dondorp W.J.' Khoury M.J. Population Screening for Genetic Disorders in the 21st Century: Evidence, Economics, and Ethics. Public Health Genomics 2010;13:106–115

47. Mango R, Vecchione L, Raso B, Borgiani P, Brunetti E, Mehta JL, Lauro R, Romeo F, Novelli G. Pharmacogenomics in cardiovascular disease: the role of single nucleotide polymorphisms in improving drug therapy. Expert Opin Pharmacother. 2005 Dec;6(15):2565-76.

48. Genetics home reference. ttp://ghr.nlm.nih.gov/handbook/testing/uses

49. RIVM http://www.rivm.nl/Onderwerpen/Onderwerpen/H/Hielprik

50. AMC Amsterdam http://www.cardiogenetica.nl/index.php?page=100

51. Umans-Eckenhausen MA, Defesche JC, Sijbrands EJ, et al. Review of first 5 years of screening for familial hypercholesterolaemia in the Netherlands. Lancet. 2001;357:165–8.

52. van Langen IM, Hofman N, Tan HL, et al. Family and population strategies for screening and counselling of inherited cardiac arrhythmias. Ann Med. 2004;36 Suppl 1:116–24.

53. Otlowski M, Taylor S, Bombard Y. Genetic discrimination: international perspectives. Annu Rev Genomics Hum Genet. 2012;13:433-54.

54. Geelen E, Horstman K, Marcelis CL, Doevendans PA, Van Hoyweghen I. Unravelling fears of genetic discrimination: an exploratory study of Dutch HCM families in an era of genetic non-discrimination acts. Eur J Hum Genet. 2012 Oct;20(10):1018-23.

55. Medical Examination Act Journal of the State (in Dutch) 1997. pp. 636–642.

56. Ministerie van volksgezondheid, welzijn en sport. http://www.rbng.nl/userfiles/file/wetten/WGBO.pdf

57. Website Overheid http://wetten.overheid.nl/BWBR0011468/geldigheidsdatum_09-06-2013

58. Website Overheid http://wetten.overheid.nl/BWBR0009408/geldigheidsdatum_09-06-2013

59. Website Overheid http://wetten.overheid.nl/BWBR0005699/geldigheidsdatum_09-06-2013

60. Corrette Ploem & Wybo Dondorp, Elcke Kranendonk, Guido de Wert. Klinische toepassing van Next Generation Sequencing: juridische en ethische aspecten. December 2012, unpublished.

61. Dickert NW, Llanos A, Samady H. Re-visiting consent for clinical research on acute myocardial infarction and other emergent conditions. Prog Cardiovasc Dis. 2012 Nov-Dec;55(3):251-7.

62. Website Framing Heart Study http://www.framinghamheartstudy.org/index.html

63. Thanassoulis G, Vasan RS. Genetic cardiovascular risk prediction: will we get there? Circulation. 2010 Nov 30;122(22):2323-34.

64. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008 Feb 12;117(6):743-53.

65. Siontis GC, Tzoulaki I, Siontis KC, Ioannidis JP. Comparisons of established risk prediction models for cardiovascular disease: systematic review. BMJ. 2012 May 24.

66. R. McPherson, A. Pertsemlidis, N. Kavaslar et al. A common allele on chromosome 9 associated with coronary heart disease. Science, 316 (2007), pp. 1488–1491

67. A. Helgadottir, G. Thorleifsson, A. Manolescu et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science, 316 (2007), pp. 1491–1493

68. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, Linsel-Nitschke P, Cambien F, Hengstenberg C, Stark K, Blankenberg S, Tiret L, Ducimetiere P, Keniry A, Ghori MJ, Schreiber S, El Mokhtari NE, Hall AS, Dixon RJ, Goodall AH, Liptau H, Pollard H, Schwarz DF, 67. Hothorn LA, Wichmann HE, Konig IR, Fischer M, Meisinger C, Ouwehand W, Deloukas P, Thompson JR, Erdmann J, Ziegler A, Samani NJ. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008; 117: 1675–1684.

69. Robert Roberts, MD; Alexandre F.R. Stewart, PhD. Genes and Coronary Artery Disease. Where Are We? J Am Coll Cardiol. 2012;60(18):1715-1721.

70. K. Hinohara, T. Nakajima, M. Takahashi et al. Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. J Hum Genet, 53 (2008), pp. 357–359

71. G.Q. Shen, L. Li, S. Rao et al. Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. Arterioscler Thromb Vasc Biol, 28 (2008), pp. 360–365

72. H. Ding, Y. Xu, X. Wang et al. 9p21 is a shared susceptibility locus strongly for coronary artery disease and weakly for ischemic stroke in Chinese Han population. Circ Cardiovasc Genet, 2 (2009), pp. 338–346

73. D. Saleheen, M. Alexander, A. Rasheed et al. Association of the 9p21.3 locus with risk of first-ever myocardial infarction in Pakistanis: case-control study in South Asia and updated meta-analysis of Europeans. Arterioscler Thromb Vasc Biol, 30 (2010), pp. 1467–1473

74. J. Kumar, S. Yumnam, T. Basu et al. Association of polymorphisms in 9p21 region with CAD in North Indian population: replication of SNPs identified through GWAS. Clin Genet, 79 (2011), pp. 588–593

75. B.G. Kral, R.A. Mathias, B. Suktitipat et al. A common variant in the CDKN2B gene on chromosome 9p21 protects against coronary artery disease in Americans of African ancestry. J Hum Genet, 56 (2011), pp. 224–229

76. A. Helgadottir, G. Thorleifsson, K.P. Magnusson et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet, 40 (2008), pp. 217–224

77. Matarin M, Brown WM, Singleton A, Hardy JA, Meschia JF. ISGS investigators, Whole genome analyses suggest ischemic stroke and heart disease share an association with polymorphisms on chromosome 9p21 [Letter] Stroke. 2008;391586-9

78. O. Jarinova, A.F.R. Stewart, R. Roberts et al. Functional analysis of the chromosome 9p21.3 coronary artery disease risk locus. Arterioscler Thromb Vasc Biol, 29 (2009), pp. 1671–1677

79. Y. Liu, H.K. Sanoff, H. Cho et al. INK4/ARF transcript expression is associated with chromosome 9p21 variants linked to atherosclerosis. PLoS One, 4 (2009),

80. M.S. Cunnington, M. Santibanez Koref, B.M. Mayosi, J. Burn, B. Keavney. Chromosome 9p21 SNPs associated with multiple disease phenotypes correlate with ANRIL expression. PLoS Genet, 6 (2010),

81. Harismendy O., Notani D., Song X.. et al. 9p21 DNA variants associated with coronary artery disease impair interferon-gamma signalling response, Nature 2011 470 () 264-268

82. Nina P. Paynter, PhD; Daniel I. Chasman, PhD; Julie E. Buring, ScD; Dov Shiffman, PhD; Nancy R. Cook, ScD; and Paul M Ridker. Cardiovascular Disease Risk Prediction With and Without Knowledge of Genetic Variation at Chromosome 9p21.3. Ann Intern Med. 2009;150:65-72.

83. Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR. et al. Women's Genome Health Study Working Group, Rationale, design, and methodology of the Women's

Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women.. Clin Chem. 2008;54249-55

84. Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE. et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women.. N Engl J Med. 2005;3521293-304

85. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score.. JAMA. 2007;297611-9

86. Philippa J. Talmud1, Jackie A. Cooper1, Jutta Palmen1, Ruth Lovering2,Fotios Drenos1, Aroon D. Hingorani3 and Steve E. Humphries1,Chromosome 9p21.3 Coronary Heart Disease Locus Genotype and Prospective Risk of CHD in Healthy Middle-Aged MenClinical ChemistryMarch 2008 vol. 54 no. 3 467-474

87. Miller GJ, Bauer KA, Barzegar S, Cooper JA, Rosenberg RD. Increased activation of the haemostatic system in men at high risk of fatal coronary heart disease. Thrombaemost 1996;75:767-771

88. Brautbar A, Ballantyne CM, Lawson K, Nambi V, Chambless L, Folsom AR, Willerson JT, Boerwinkle E.Impact of adding a single allele in the 9p21 locus to traditional risk factors on reclassification of coronary heart disease risk and implications for lipid-modifying therapy in the Atherosclerosis Risk in Communities study. Circ Cardiovasc Genet. 2009 Jun;2(3):279-85.

89. Roberts NJ, Vogelstein JT, Parmigiani G, Kinzler KW, Vogelstein B, Velculescu VE..The predictive capacity of personal genome sequencing. Sci Transl Med. 2012 May 9;4(133):133ra58.

90. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013 Mar 14;152(6):1298-307

91. Ripatti S, Tikkanen E, Orho-Melander M, Havulinna AS, Silander K, Sharma A, Guiducci C, Perola M, Jula A, Sinisalo J, Lokki ML, Nieminen MS, Melander O,Salomaa V, Peltonen L, Kathiresan S. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet. 2010 Oct 23;376(9750):1393-400

92. Website AHA http://www.heart.org/HEARTORG/

Appendix 1 CVD affecting the heart

CVD Class	CVD Subclass	Clinical features
Angina pectoris	Stable angina Unstable angina Variant angina	Chest pain due to heart attack
Arrhythmias	Atrial fibrillation Heart block, including first-degree AV block, second-degree AV block, and complete AV block Premature atrial complex (PAC) Atrial flutter Paroxysmal supraventricular tachycardia (PSVT) Wolff-Parkinson-White syndrome Premature ventricular complex (PVC) Ventricular tachycardia Ventricular fibrillation Long QT syndrome	Iregular Heart beat
Cardiomyopathy	Dilated cardiomyopathy Hypertrophic cardiomyopathy Restrictive cardiomyopathy	Death of heart tissue
Congestive heart failure		Heart failure
Congenital heart disease	Atrial septal defect (ASD) Ventricular septal defect (VSD) Patent ductus arteriosus Pulmonic stenosis Congenital aortic stenosis Coarctation of aorta Tetralogy of Fallot Tricuspid atresia Truncus arteriosus Ebstein's anomaly of the tricuspid valve Transposition of the great vessels	Birth defect of the heart
Coronary artery disease (CAD), also known as heart disease, ischemic heart disease, or coronary heart disease (CHD)		Plaque formation in the arteries of the heart
Cor pulmonale		Enlargement of the right ventricle of the heart
Heart attack, myocardial infarction		Interuption of blood supply to the heart
Heart valve disease, such as:	Mitral stenosis Mitral valve regurgitation Mitral valve prolapse Aortic stenosis Aortic regurgitation Tricuspid stenosis Tricuspid regurgitation	Malfunction of the heart valves
Myocarditis		Inflammation of heart muscle
Rheumatic heart disease		Repeated inflammation of the heart
Pericarditis		Inflammation of the pericardium
Sudden cardiac death		Death by sudden heart failure
Cardiac tumor		Tumor in the heart

Table 7. Overview of CVD affecting the heart [92]

Appendix 2 CVD affecting the blood vessels

CVD Class	CVD subclas	Clinical features
Aortic aneurysm		Swelling of the aorta
Aortitis		Inflammation of the aortic wall
Atherosclerosis		Thickening and hardening of the arteries
Aortic dissection		Tear in the inner wall of the aorta
High blood pressure (hypertension),	Essential hypertension Secondary hypertension Malignant hypertension	High Blood pressure
Stroke		Loss of brain function
Other problems in arteries	Atherosclerosis of the extremities (arteriosclerosis obliterans) Arterial embolism Acute arterial occlusion, which is when a blood vessel becomes blocked Raynaud's phenomenon Arteriovenous fistula Vasculitis Thoracic outlet syndrome	
Other problems in veins, including:	Venous thrombosis Deep vein thrombosis (DVT) Thrombophlebitis Varicose veins Spider veins	
Lymphedema.		Tissue swelling caused by a defective lymhayic system.

Table 8. Overview of CVD affecting theblood vessels [92]