Rare genetic variants associated with early onset CVD
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Chapter 1

Introduction
Introduction

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in Western Societies. CVD is the most common type of heart disease and leads to myocardial infarctions [1]. CVD is mainly triggered by atherosclerosis. A combination of lipid accumulation, inflammation at the vessel wall and thrombotic reactions are underlying its pathobiology. The use of acetyl salicylic acid and statins became the core of pharmacological therapy [2]. Statins have been proven to be safe and effective in reducing low-density lipoprotein cholesterol (LDL-c) levels in clinical trials and practice [3-6], as well as in reducing morbidity and mortality [7]. Despite improvements in the therapy of atherosclerotic disease, it is still the leading cause of death in the developed world [8]. Atherosclerosis has long been identified as having an inflammatory component contributing to its pathogenesis, whereas the available therapy primarily targets hyperlipidemia and prevention of thrombosis [8]. The further development of effective therapeutic approaches is needed. This requires better understanding of the molecular mechanisms and pathophysiology of the disease.

We attempted to unravel some of those unknown mechanisms underlying the pathophysiology of atherosclerosis by performing extensive genetic screening in families with a monogenic prevalence of the disease. We collected patients with early onset CVD at a young age and a familial predisposition for CVD. It has been shown in several studies that a positive family history for early onset CVD is an independent risk factor [9] and up to 6-10% of patients with CVD are affected at a very young age [10]. A combination of classical linkage analysis and next generation sequencing was applied to identify the rare variant that is linked to the early onset CVD phenotype in those families.

CVD is caused by local injuries at the vessel wall including the triggering of the adaptive and innate immune system [11]. This process involves the infiltration of circulating monocytes [12], which play a pivotal role in a number of crucial steps. Endothelial cells begin to secret adhesion molecules, followed by adherence of leucocytes to the cell surface [13]. Trapping of LDL particles into the intima region occurs [14]. Local modifcatins, such as oxidation of lipids (ox-LDL) makes the particle more prone to induce the atherosclerotic process. These ox-LDL particles are taken up by macrophages, which then turn into foam cells. If the foam cells cannot be removed, apoptosis occurs. At this stage of inflammation more leucocytes, particularly monocytes and T-leucocytes, are attracted to the site of injury [11, 15]. Besides circulating monocytes, platelets play a critical role in the development of CVD, especially during the process of plaque formation [16]. They represent a source of inflammatory mediators [16]. After the adherence of platelets at the site of injury to the extracellular matrix, a thrombus is formed to
repair the injury [16]. Further circulating platelets and adhesive substartes stabilize the forming thrombus [16]. The further grow of the thrombus and increased inflammation through monocytes and T-lymphocytes could lead to an unstable plaque leading to its disruption and subsequent event [16,17].

Outline

Part I

In Part I of my thesis I discuss new approaches that are instrumental to identify patients at risk and may be instrumental to study the underlying pathobiology.

In chapter two the use of gene expression analysis of circulating monocytes derived from patients suffering from coronary artery disease is discussed. Evaluation of gene expression patterns in monocytes may be used as biological marker to identify individuals at risk. In chapter three the gene expression level of circulating monocytes derived from twenty-two patients with premature atherosclerosis (PAS) and twenty-four matching controls were compared. Since patients were on statin therapy we included a control group who received statin treatment. In this study we used whole genome expression profiling to identify novel players in the progression of atherosclerosis. In chapter four we analyzed the transcriptome of monocytes from healthy volunteers after a single injection with lipopolysaccharide (LPS) to induce an acute inflammatory stage. In chapter five we compared patients with a myocardial infarction but normal coronary arteries (MINCA), MI patients and matching healthy controls by measuring their carotid-Intima Media Thickness (cIMT). By comparing parameters of thrombosis such as thrombin generation and haemostatic protein measurements we evaluated unique significant characteristics to identify the MINCA subpopulation.

Part II

In chapter six the impact of GWA studies on the finding of novel loci harboring common variants, associated with the disease phenotype that will help to decipher the molecular mechanisms underlying the heritable and prevalent phenotype of CVD is discussed. The findings from the recent large meta-analyses are presented and implications for biology and clinical medicine were discussed. Furthermore an overview of the use of informative families with monogenic forms of CVD to identify novel rare variants using next generation whole exome sequencing technology is given. Families with a monogenic pattern of early onset CVD are valuable to identify novel genes and could help to get new insights into the pathophysiology of CVD. Examples of these studies are
presented in **chapters seven** and **eight** where we identified 2 novel rare variants associated with early onset CVD in a large and a small pedigree respectively. We identified 2 pedigree-specific variants, which were not present in approximately 10,000 controls and 935 patients with CVD.

In **chapter seven**, classical linkage analysis and targeted sequencing of the identified region revealed a novel variant in keratocan (KERA). KERA encodes for an extracellular protein mainly expressed in the cornea. We showed that Kera is expressed in the vasculature and associates with severeness of the plaque in a mouse model. In **chapter eight** a combination of exclusion linkage analysis and next generation sequencing (NGS) led to the identification of a pathogenic variant in MCF.2 cell line derived transforming sequence-like protein (MCF2L). MCF2L is a guanine-nucleotide exchange factor (GEF) that functions as a ‘switch’ for downstream targets. It potentially links pathways that signal through Ras-related C3 botulinum toxin substrate 1 (Rac1) and Ras homolog gene family, member A (RhoA). RhoA is known to regulate the assembly of actin stress fibers.

In **chapter nine** the methodology I used to isolate and prepare human induced pluripotent stem cells (hiPSCs) is presented. These types of stem cells have the ability to differentiate into any of the three germ layers (endoderm, mesoderm and ectoderm). I explain the optimized protocol for harvesting skin fibroblasts from index patients with early onset CVD and the reprogramming of those cells into hiPSC by using specific transcription factors. The combination of this transcription factors is associated with pluripotency of cells [18]. I explain the analysis of the pluripotent status and the ability of hiPSCs to differentiate into extraembryonic tissue, mesendoderm and neuroectoderm and explain the pitfalls of this method.

In **chapter ten** the findings of my thesis are summarized and I am giving a perspective on future strategies to identify novel causative genetic variants.
References


Introduction


