The molecular basis of early onset cardiovascular disease
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GENERAL INTRODUCTION

LETTER TO THE READER
Lectori Salutem,

We have attempted to unravel the molecular basis of a very complex disorder, cardiovascular disease (CVD). The molecular basis of CVD pertains to novel genes and related genetic mechanisms. Minute changes at a molecular level in genes can have tremendous effects on a protein, cell, individual, group and cohort level. Please note that “novel genes” in most cases does not imply the actual discovery of a gene but a novel association to one of the 21,500 genes with the disease. Only in this context can our attempts be considered successful.

As mentioned previously, genes have an impact on the disease at several levels. Mutations within a gene can cause a protein to alter its function. These mutations can be rare or common. Rare mutations are where the search for the molecular mechanisms of CVD started. These mutations tend to have a large effect on the disease state and were initially identified by studying families with multiple generations of diseased individuals. One of such disorders is Familial Hypercholesterolemia (FH). Here, due to a mutation in the LDL-receptor, it is less effective in clearing bad cholesterol from the circulation which leads to cholesterol levels in the blood to rise and cause rapid progression of atherosclerosis and consequently CVD. The onset and severity of CVD varies considerably between FH patients, even among individuals who share an identical gene defect. In chapter one and two we investigate respectively the role of maternal vs. paternal inheritance and the effect of common genetic variation on this phenotypic variability. In chapter three we assess the benefit of primary prevention by long term statin treatment in these individuals.

Not only families with FH are at increased risk of the development of early onset CVD; at our premature atherosclerosis outpatient clinic, numerous individuals were seen with early onset CVD without a known gene defect or ample risk factors. We were also frequently visited by worried, but apparently healthy first degree relatives. This has been the starting point of the next two parts of this thesis.

Part two begins with chapter four where we show that in a cohort of 22,000 individuals even apparently healthy first degree relatives of a patient with early onset CVD are at an increased risk of CVD. This probably reflects the accumulation of risk factors within families due to their shared lifestyles, but we demonstrate that it is independent of known risk factors, such as smoking or high cholesterol. This in turn indicates that other molecular mechanisms are worth identifying. At the outpatient clinic we screened the apparently healthy first degree relatives for CVD risk factors and assessed their atherosclerotic burden by using non-invasive imaging of the coronary arteries with a CT-scan. In chapter five we publish evidence that in those individuals with CT-abnormalities without a cardiovascular event yet, primary prevention with aspirin and statins is warranted. Part three describes our efforts to identify novel candidate genes and genetic variation
related to CVD by mainly studying individuals with unknown aetiology of early onset CVD. We start this section with chapter six where we review literature published so far. In an ideal world the most direct way to study genetics would be by reading the unique genetic code (DNA) of a diseased individual with CVD and compare it with a healthy individual. Three years ago this was not possible since there was no analysis or storage capacity for the data generated. Therefore at that time the big trend in genetics after initial family studies was gene expression profiling. In chapter seven and eight we apply gene expression profiling in monocytes, cells that are known to play a pivotal role in the process of atherosclerosis in different CVD associated conditions. Gradually we were able to look at certain DNA markers at fixed distances on the human DNA and determine how these markers were different between large number of cases and controls. This identified regions of the DNA that warranted the need for further studies. Only relatively common mutations with a mild effect have been identified by these strategies. We were part of all major studies in which a group of people with CVD at a young age from our outpatient clinic was used (see publication list at the end of this book). Chapter nine is a good example of our contribution to these studies.

As time progressed we no longer needed very large families due to technical innovations. This resulted in the latest trend; Next Generation Sequencing (NGS). By studying smaller families and applying NGS we looked to identify rare mutations in genes with a great effect on the disease. Much of my PhD student time was spent finding families with little risk factors and multiple cases of early onset CVD all over the Netherlands. We started with the medical records Dr. Trip had collected and cases that were seen at the outpatient clinic. Only the families that met our prescribed criteria were selected for follow up with genetic analysis. In final chapters ten and eleven, we report our findings on the first two families that were put forward for further analysis.

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