Familial hypercholesterolemia. The determination of phenotype

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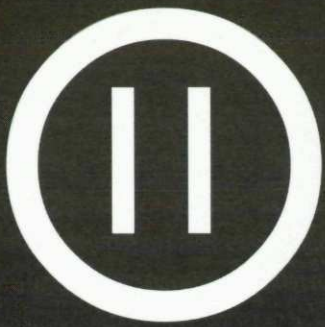

Summary

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Summary

Heterozygous Familial Hypercholesterolemia is one of the most common inherited dominant disorders, characterized by severely elevated LDL-C levels and premature cardiovascular disease. Although the cause of FH is monogenic, there is substantial variation in the onset and severity of atherosclerotic disease symptoms which is not well understood. This thesis aims to address the underlying factors that influence cardiovascular disease risk, with a special emphasis on the contribution of genetic variation.

In general, additional atherogenic risk factors, of environmental, metabolic and genetic origin, in conjunction with the LDL-receptor defect, are presumed to influence the clinical phenotype in FH. **Chapter 2** presents a review of recent developments in this field and summarizes the current knowledge on these topics. Age, gender, LDL-C levels and a positive family history for premature atherosclerosis are established determinants for early and severe events. Concerning potential additional metabolic factors, the roles of lipoprotein(a), HDL-C and triglycerides are interesting, but study results have been equivocal. With respect to genetic influences, the LDL-receptor mutation type indeed constitutes a determinant of the FH phenotype. The potential contribution to risk of other genes is certainly intriguing. However, so far, only case-control studies with rather small numbers of patients and polymorphisms have been carried out and further studies with better designs and larger cohorts are warranted to confirm current results, which formed the rationale for this thesis.

GIRaFH study

In order to gain more in-depth knowledge of the underlying genetic variations of the clinical phenotype, we designed the 'GIRaFH-study' (Genetic Identification of Risk Factors in Familial Hypercholesterolemia). From the central DNA and Biobank of the department of Vascular Medicine at the Academic Medical Center of the University of Amsterdam, that currently contains DNA samples of more than 9300 severely hypercholesterolemic patients collected from Lipid Clinics throughout the Netherlands, we randomly selected a cohort of 4000 severely hypercholesterolemic patients from 27 different hospitals.

A team of especially trained data-collectors visited the different hospitals and reviewed all patients' records. A total of 2400 patients of 18 years and older, that fulfilled strict clinical criteria for FH, and of whom written informed consent was obtained, were included in the study. Subsequently, extensive information on demographics, classical risk factors, laboratory parameters, and CVD endpoints was acquired by reviewing the patients' medical records and the use of questionnaires. Additional information was sought from general practitioners

and hospitals that patients had visited formerly. Dubious CVD endpoints were presented to an independent adjudication committee. Genotyping, using a multiplex assay, was performed by Roche Molecular Systems, CA, USA. Complete genotypes were obtained of 2092 patients.

The collection of this very large, well-defined FH cohort provided the possibility to describe the contribution of classical risk factors, as described in Chapter 3. The cohort of 2400 FH patients represented 112.943 person-years, during which 782 (32.6%) patients had at least one cardiovascular event. Male gender, smoking, hypertension, diabetes mellitus, low HDL-C, and Lp(a) proved to be significant and independent risk factors on multivariate Cox regression. Since earlier reports had yielded equivocal results, the influence of these parameters, although expected to be important, was not clear at the start of this study. Interestingly, these classical risk factors contributed only 18.7 % to the occurrence of CVD in our FH patients. Consequently, we hypothesized that other, still unknown and possibly genetic, factors must have contributed to atherosclerotic disease in these patients. Therefore, we studied the role of additional susceptibility genes in **Chapter 6 and 7.**

We used a set of established clinical diagnostic criteria to define FH in our GIRaFH study. Nevertheless, analysis of our cohort revealed significantly different clinical and laboratory profiles between those patients with and those without a known LDL-receptor mutation, as described in **Chapter 4**. Patients clinically diagnosed as having FH but yet without a known mutation, are characterized by a somewhat lower total cholesterol, lower LDL-C, higher triglyceride and HDL-C levels, a significantly higher prevalence of hypertension, and higher glucose levels. These findings suggest that among those without a known LDL-receptor mutation, patients with other causes of dyslipidemia may be present.
Clinical criteria, such as LDL-C cut-off levels, the presence of tendon xanthoma and arcus lipoides, and a positive family history for premature CAD are discussed. Unfortunately, none of these characteristics is sensitive and specific enough to be used as definite FH marker. Advances in the application of DNA diagnostic techniques will hopefully soon favor the ultimate decisive criterion, namely, the genetic test. Genetic testing in FH is discussed to be important for accurate cardiovascular risk assessment and treatment of FH patients, and for proper definition of study subjects in research with FH patients.

In the process of designing the GIRaFH study we encountered a deficit in the literature on guidelines for the design, execution and reporting of retrospective studies using medical records. Therefore, we decided to develop our own guidelines using recommendations from the literature and our own experience with the GIRaFH study. In **Chapter 5** we present this set of guidelines, which incorporates a number of strategies for accurate data collection and inconsistency reduction, and demonstrate their application. Recommended strategies

include the performance of a pilot study to reconsider and adjust all elements of a study design, the use of tested case record forms and handbooks with data collection guidelines and the performance of interobserver studies. Correct interpretation of clinical outcomes documented in the medical records often necessitates an independent adjudication committee to prevent bias in outcome definition. Data collection should be centrally monitored, and double data entry into a central database, and a consistency check of the final data set should be applied.

In **Chapter 6** we investigated the contribution to CVD risk of 65 polymorphisms in 36 candidate genes in this large GIRaFH cohort. We identified six polymorphisms, involved in thrombosis, lipid metabolism, potentially anti-oxidative pathways and essential hypertension, that were related with the occurrence of CVD. Strikingly, the G20210A polymorphism of the prothrombin gene was very strongly related to increased CVD risk. In addition, five other polymorphisms were associated with CVD: Met235Thr in the angiotensinogen gene, Thr347Ser in the apolipoprotein A4 gene and Gly460Trp in the alpha-adducin gene with increased risk, and in contrast, the Ser311Cys substitution in the paraoxonase 2 gene and the C1100T variant in the apoC3 gene with decreased risk.

In **Chapter 7** the relationship between the same polymorphisms in these candidate susceptibility genes and mortality in the parents of 1473 GIRaFH participants was analysed. Remarkably, polymorphisms in three genes, namely those encoding apolipoprotein CIII, the β_2-adrenergic receptor and apolipoprotein B, were associated with excess mortality, while polymorphisms in the genes encoding tumor necrosis factor beta and G-protein B3 subunit were related to a better life expectancy. When compared to the CVD analyses in **Chapter 6**, the apolipoprotein C3 gene was detected as significant risk contributor in both analyses. In **Chapter 3**, we could confirm a major role of low HDL-cholesterol levels in the development of CVD in FH. Intrigued by this important role of reverse cholesterol transport, we set out to investigate the underlying genetic determinants of plasma HDL-cholesterol levels in our GIRaFH cohort in **Chapter 8.** We found that the combined effects of genetic variations at 25 candidate gene loci explained 12.5 % of the variation of HDL-C levels in our FH patients. When five covariates (HDL-C gender, smoking, alcohol use, BMI and concomitant beta-blocker use) were incorporated into the model, the main-effects model explained 20.2%, and the combined-effects model 32.5% of HDL-cholesterol variation.

In **Chapter 9** we determined the relationship between hyperhomocysteinemia, the MTHFR-genotype and the development of atherosclerosis in 981 FH patients. FH patients with established CVD had higher homocysteine levels than those without CVD. The accompanying odds ratios suggested that hyperhomocysteinemia could indeed be an independent predictor

of atherosclerotic cardiovascular disease in these patients. However, whereas those with the MTHFR TT-genotype, a cause life-long of hyperhomocysteinemia, exhibited significantly increased homocysteine levels, this was neither reflected in CVD rates nor in carotid IMT. Even more remarkable was the fact that homocysteine levels were not related to IMT measurements in any way. These latter findings weaken the concept of risk conferred by hyperhomocysteinemia in FH and questions the role of elevated homocysteine levels in these patients. In addition, these findings are supported by the fact that increased homocysteine levels were also not a risk factor in the large GIRaFH cohort, as described in **Chapter 3**.

Paraoxonase (PON-1) is a high-density lipoprotein (HDL) associated enzyme that may protect against atherosclerosis. In **Chapter 10** we studied the effects of plasma PON-1 levels and six functional polymorphisms on clinical manifestations of cardiovascular disease (CVD) in 187 patients with Familial Hypercholesterolemia. FH patients with CVD had significantly lower PON-1 levels than patients without CVD. Although the PON-1 genotype was a major determinant in the variation of PON-1 levels, this did not contribute to CVD risk. Our findings support the hypothesis that PON-1 might protect against CVD. The role of the genotype is as yet not well established. In **Chapter 6** we also studied the roles of two polymorphisms in the PON-1 gene and did not find an association with the occurrence of CVD as well. A large cohort study on the contribution of PON-1 genotype and the influence of environmental factors to CVD risk is required to elucidate their possible interaction.

Concluding remarks

- Male gender, smoking, hypertension, diabetes mellitus, low HDL-C, and Lp(a) are significant and independent risk factors for the occurrence of CVD in FH.
- In heterozygous FH, polymorphisms in the genes encoding prothrombin, angiotensinogen, apolipoprotein A4 and alpha-adducin are associated with an increased CVD risk. Polymorphisms in the paraoxonase 2 gene and the apoC3 gene are associated with a decreased CVD risk.
- Overall-mortality risk of patients with Familial Hypercholesterolemia is certainly also based on additional genetic risk factors for cardiovascular disease.
- Increased mortality is associated with polymorphisms in the genes encoding apolipoprotein CIII, the β_2-adrenergic receptor and apolipoprotein B, while, in contrast, polymorphisms in the genes encoding tumor necrosis factor beta and G-protein B3 subunit are associated with a better life expectancy in FH.
- The APOA1-C3-A4-A5 gene cluster seems to play an important role in the occurrence of

CVD and mortality in FH.

- The use of standardised guidelines for data collection enhances the quality of research data.
- Genetic testing for FH diagnosis is important for clinical practice and for research purposes involving FH patients.
- Genetic and environmental factors explain at least 20 % of plasma HDL-cholesterol levels
- Homocysteine and cardiovascular disease are associated in FH, but hyperhomocysteinemia seems to be a consequence rather than a cause of this disease process.
- Increased paraoxonase-1 plasma levels seem to protect individuals with Familial Hypercholesterolemia against atherosclerosis. The relationship between the underlying genotype and the occurrence of CVD is not clear, pointing at the influence of environmental factors on plasma paraoxonase-1 concentrations.

Clinical and research perspectives

Our results might have a number of implications for the clinician. Until today, the ultimate goal in treating FH patients is sharply lowering LDL-C with cholesterol-lowering medication. Our study was not designed to detect the influence of LDL-C levels to atherogenesis, but from earlier studies it is clear that increased LDL-C levels play a key role in the development of CVD in FH. Consequently, we emphasize that statin therapy should remain central in the treatment of patients with heterozygous FH. However, the findings of the research described in this thesis teach us that it is also important to carefully monitor classical risk factors such as smoking, hypertension and diabetes mellitus, and that drug and lifestyle interventions should be applied accordingly. Lipoprotein(a) levels should be determined in all FH patients for a better risk stratification. Special attention should be paid to patients with low HDL-cholesterol levels, in whom treatment should be aimed at increasing these levels. Currently, this means adhere to life-style advices: refrain from smoking, reduce weight and enhance daily exercise. New therapies which positively affect HDL-C plasma levels and reverse cholesterol transport might become available in near future and might be useful for these FH patients at the highest risk. The routine measurement of homocysteine levels or the MTHFR-genotype is premature since the consequences are not clear and we first have to await the results of large endpoint trials. Diagnosing FH on basis of clinical criteria leads to incorrect qualification of a significant number of patients. Therefore, genetic testing should be recommended for appropriate diagnosing.

The results of our large genetic studies form a step forward in the unraveling of genetic mechanisms underlying CVD in FH. Considering the limitations of association studies in general, we suggest the results of our polymorphism studies should first be replicated, also

in prospective studies of large and well-defined FH populations, in which genetic and environmental modifiers should be carefully controlled. We identified six polymorphisms that were related to CVD risk and, surprisingly, five polymorphisms that were associated with total mortality in our large cohort. All cause mortality is by definition very heterogeneous, but in case of FH it is very likely that the majority of deaths is caused by cardiovascular disease. We hope that our results will enhance further research into the underlying pathophysiological mechanisms involved in these relationships. Notably, in this regard, the APOA1-C3-A4-A5 gene cluster deserves extra attention, since polymorphisms in this cluster were found to be associated with CVD as well as mortality.

The long-term clinical implications of our genetic studies, however, are intriguing. Genotyping individuals for sets of polymorphisms could be helpful in improving cardiovascular risk assessment of the individual FH patient. In addition, these genetic markers might be used to institute individually tailored drug therapy.

In this regard, and in line with our identified polymorphisms, an interesting new focus of research could be the performance of randomized clinical trials in which FH patients, stratified according to genotype, are treated with drugs such as oral anticoagulation therapy or anti-platelet drugs (prothrombin gene variant), antihypertensive drugs (polymorphisms in angiotensinogen gene, the α-adducin gene and the G-protein B3 subunit gene) and fibrates and PPARα-agonists, that act via modulation of apoC3 and apoA1 genes (APOA1-C3-A4-A5 gene cluster).

Overall, our results constitute a firm basis for further research and form a step forward in the unraveling of the underlying mechanisms of CVD in FH.