Familial hypercholesterolemia in childhood: diagnostics, therapeutical options and risk stratification
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Summary
The studies described in this thesis provide insight into the diagnostics, therapeutical options, and additional risk factors for cardiovascular disease (CVD) in children with familial hypercholesterolemia (FH). The three main issues that are addressed are:

1. The most accurate diagnosis for FH in children
2. The efficacy and safety of statin treatment and other therapeutical options
3. Additional risk factors for premature CVD in childhood FH

Part I: Diagnostics of Familial Hypercholesterolemia in Children

Children with FH are characterized by elevated low-density lipoprotein cholesterol (LDL-C) levels from birth onwards, although they rarely have clinical symptoms of atherosclerosis or physical characteristics of FH. Therefore, the most accurate diagnostic tool to identify these children is the detection of an LDL-receptor mutation. However, DNA sequencing is not available for all physicians, hence the measurement of LDL-C levels to screen for FH in children. We identified the most optimal cut-off point of LDL-C levels for the diagnosis of children with FH. Furthermore, the effect of other risk factors for premature atherosclerosis was evaluated.

Chapter 1 gives an overview of the current knowledge and research in children with FH. In Chapter 2 we investigated a large cohort of children who were referred to our pediatric lipid clinic for FH diagnosis. We assessed the most optimal LDL-C level to diagnose FH in children with a parent in which the diagnosis of FH was unequivocal. We demonstrated that children with a LDL-C level above 3.50 mmol/L had a post-test probability of 98% of predicting the presence of a LDL-receptor mutation. However, lipoprotein (a) (Lp(a)) levels more than 300 mg/L and HDL-cholesterol levels less than 1.00 mmol/L were associated with a positive family history for CVD. These findings might point to those children with the highest risk for premature atherosclerosis.

In Chapter 3 we examined morphological changes of the arterial wall in a cohort of children with FH. We assessed the intima media thickness (IMT) by B-mode ultrasound measurements of the carotid arterial wall in 201 children with a definite molecular diagnosis of FH and 80 unaffected siblings. Children with FH were characterized by significantly thicker carotid artery measurements than their healthy siblings.
suggests that, even in young FH patients, pre-atherosclerotic wall abnormalities are present. Furthermore, we established the most important contributors to these arterial wall changes. We demonstrated that age, gender and LDL-C levels were important determinants for increased IMT in these children. These findings underscore the need for early LDL-lowering intervention.

The hypothesis of chapter 4 was that children with FH provide a better model to perform genotype–phenotype analysis than FH adults. Furthermore, we investigated the relationship between LDL-receptor genotypes and lipoproteins and their relationship with the occurrence of parental CVD. In a linear mixed model, familial factors explained more than 50% of the variance in LDL-C levels among FH children in contrast to the 9.5% of the variance in the adult FH cohort. Therefore, we could confirm that FH children provide a better model than adults to analyze the relationship between the type of LDL receptor mutation and lipoprotein concentrations. Our second objective was to investigate the relationship between LDL-receptor genotypes and lipoproteins in these children and the relationship with parental CVD. We demonstrated that the type of mutation did not significantly contribute to the variation of CVD risk in their parents, but carriers of one specific mutation clearly had a less increased CVD risk. This N543H/2393 del 9 mutation resulted in a less atherogenic profile as compared to other mutations. However, the variation in lipid profile poorly explained the differences in CVD risk between carriers of other mutations. Therefore, future research in FH patients should focus on the identification of novel additional risk factors in the pathogenesis of CVD.

Chapter 5 is a case report of a boy with extremely high LDL-C levels and tuberous xanthomas. His consanguineous parents had normal cholesterol concentrations, which suggested an autosomal recessive disorder rather than autosomal dominant familial hypercholesterolemia. The diagnosis of autosomal recessive hypercholesterolemia (ARH) was confirmed by the presence of a mutation in the phosphotyrosine binding domain of a putative adaptor protein. ARH has a comparable clinical phenotype to that of classical homozygous familial hypercholesterolemia caused by defects in the LDLR gene, but it is more variable and generally less severe. In addition, patients with ARH are more responsive to lipid lowering therapy, as in this case, where an LDL-lowering effect of more than 60% was obtained with atorvastatin and ezetimibe.
Part II: Therapeutical Options in Children with Familial Hypercholesterolemia.

In children with FH, the first line of treatment is dietary therapy, but compliance and efficacy are poor. National Cholesterol Education Program (NCEP) guidelines for children and adolescents recommends drug therapy in children older than 10 years whose LDL-C levels are above 4.1 mmol/L when other risk factors are present, or above 4.9 mmol/L when no other risk factors are present.

The NCEP recommends bile acid-binding resins as pharmacological treatment in FH children. However, the lipid-lowering efficacy of resins is modest and again compliance is poor. In adult patients with FH, the first choice in pharmacological treatment consist of HMG-CoA reductase inhibitors (statins), which have been showed to reduce the incidence of CVD in adults. In recent years, several randomized trials have shown that statins are also effective in reducing LDL-C levels in children and seem safe, at least in the short term.

Chapter 6 provides an overview of the effects, mechanisms of action, and side effects of various cholesterol-lowering modalities that are used or considered for use in the pediatric population. The following agents are discussed: bile acid-binding resins, statins, fibrates, nicotinic acid, and ezetimibe. There is an increasing number of options for pharmacological treatment of dyslipidemia in children and adolescents. Clinicians who use these medications in pediatric patients should be familiar with their indications, dosing, and side effects. However, data on long term safety and efficacy are still lacking. Therefore, it is essential that long-term efficacy and safety of (new) pharmacological agents is continuously assessed in order to gain confidence in the use of these therapies in children with FH.

In Chapter 7 we review the current knowledge about statin therapy in children. Early initiation of statin therapy seems to be the right therapeutic option with regards to improvement of endothelial dysfunction, regression of intima-media thickening, and prevention of future cardiovascular events. But again, data on long term efficacy and safety in children with FH are not available and long term follow-up studies are needed to confirm safety of statin therapy in children with FH. Chapter 8 describes a two-year randomized placebo controlled clinical trial, evaluating the efficacy and safety of pravastatin therapy in children with FH. The primary efficacy outcome of
this trial was defined as the change from baseline in mean carotid IMT between pravastatin treated children and the placebo group after two-year follow-up. The principal safety outcomes were deviations of growth and sexual maturation as well as changes of levels of muscle and liver enzymes. We found an IMT regression of 0.010 mm in the pravastatin treated group and a progression of 0.005 mm in the placebo group, which indicates that two-year statin treatment significantly decreased mean carotid IMT as compared to the placebo. With respect to safety outcome, we observed no significant changes or deviations in sexual maturation or growth, and pravastatin was well tolerated in these children. From these data we might conclude that two-year pravastatin treatment is efficacious and safe. However, it is unknown what the consequences of early statin treatment are for IMT progression and safety later in life. In Chapter 9, we evaluated the children who participated in the open label trial described in chapter 8, after a mean follow-up period of 4.3 years. All children received pravastatin at the end of the above mentioned trial. In this study, 195 children were able to visit our pediatric lipid clinic for a follow-up visit. We evaluated carotid artery IMT, lipoprotein levels, and safety parameters such as growth and sexual maturation and liver and muscle enzymes. None of the children had complaints of cardiovascular disease or events during statin treatment. Furthermore, none of the children deviated from normal sexual maturation as compared to the age and sex matched healthy controls. Using multivariate analysis, we showed that months of statin use, the age at the start of statin use, gender, LDL-cholesterol at the start of statin use and combined IMT at the start of statin use were significant determinants of carotid artery IMT in adolescents and young adults. These data suggest that early statin treatment is an important determinant of carotid artery wall thickness at an older age, which underscores the need for early statin treatment in children. Although the current safety data support treatment of FH children older than 8 years, it remains to be investigated if FH children can also be treated with statins even before the age of 8.

In Chapter 10 we analyzed the relationship between LDL-receptor genotype and response to pravastatin treatment in children with FH using carotid intima-media thickness (IMT) to measure efficacy. A total of 49 different mutations were detected in 193 children with heterozygous FH. We found 17 null alleles in 75 children, 14 receptor-defective mutations in 80 children, and 18 mutations with undetermined residual function in 38 children. After adjustment for baseline LDL-C levels, the
difference in mean carotid IMT between carriers of null alleles and receptor-defective mutations was 0.018 mm, which was significant. Mean IMT of the bulb and the internal carotid artery segments after the two-year statin period tended to be higher in children with null alleles but this did not reach statistical significance. In conclusion, we show that LDL-receptor genotype was associated with carotid artery IMT in FH children. Although the reduction of LDL-C levels modulated by pravastatin treatment tended to be less in carriers of null alleles, we observed no significant difference in change of carotid artery IMT during the trial between the two LDL-receptor genotype groups. However, at baseline and after two-year treatment, carotid IMT and lipid profile were more unfavorable in children with null alleles compared to children with receptor-defective mutations. These findings imply that selection of null alleles identifies children with the highest cardiovascular disease risk who might benefit by more aggressive as well as earlier lipid lowering treatment.

In chapter 8 and 9 we showed that statins improved IMT in FH children. Statin therapy has also been shown to restore endothelial function in these children. Endothelial dysfunction is an early reversible stage in the development of atherosclerosis, and it is detectable before morphological changes are present. Since statins have not been approved for use in children in Europe, we studied the effect of alternative lipid lowering agents on endothelial function in Chapter 11. Plant sterols or stanols are incorporated into food products and they lower cholesterol levels by inhibiting cholesterol absorption in the small intestine. We examined the effect of plant stanols on lipids and endothelial function in pre-pubertal children (7-12 years) with FH. The daily intake of food products enriched with 2.0 g stanols decreased LDL cholesterol by 9.2% as compared to placebo. However, this reduction of LDL-C did not improve endothelial dysfunction, as measured by flow-mediated dilatation (FMD). This lack of improvement in endothelial function was possibly due to insufficient reduction of LDL-C, since a previous trial with statin treatment in FH children, clearly showed an improvement of FMD with a mean LDL-cholesterol reduction of 40%. The results of this trial advocate more aggressive lipid lowering treatment, by means of statins or possibly by combining stanols with other lipid-lowering compounds.

Previous studies in adults have demonstrated that several additional factors, besides high levels of LDL-cholesterol contribute to a higher risk for CVD. Two of such additional risk factors may be LDL-C particle concentrations as well as elevated levels of oxidized LDL (OxPL), which both contribute to foam cell formation during atherogenesis. However, data on these additional risk factors in children with FH is sparse. We evaluated the concentrations of LDL particles and oxidized levels of LDL in these children and compared those with their unaffected siblings in Chapters 12 and 13. In addition, we evaluated whether these risk factors are modulated by statin treatment. In Chapter 12 we showed that children with FH have significantly higher concentrations of VLDL and LDL particles numbers and lower levels of HDL particles when compared to healthy controls. Furthermore, pravastatin significantly reduced the atherogenic LDL particle concentration by 19.6% in the FH children as compared to placebo, which was mainly caused by a decrease in large LDL and to a lesser degree by the decrease of small dense LDL. In Chapter 13 we investigated the various OxPL markers in children with FH and their unaffected siblings and we evaluated the effect of pravastatin on plasma oxidized LDL levels. FH children had significantly higher concentrations of OxPL/ApoB as compared to the controls. Pravastatin significantly increased OxPL per ApoB particle by 19.4 % and Lp(a) levels by 11.2 % as compared to placebo. This suggests that pravastatin treatment results in OxPL enrichment of ApoB particles. These findings suggest that the clearance of OxPL from the circulation in response to pravastatin may be an important mechanism mediating the prevention of premature atherosclerosis in hypercholesterolemic children.

Conclusions

The results presented in this thesis indicate that:

- An LDL-C level above 3.5 mmol/L in children with a parent with a certain diagnosis of FH is the best cut-off point for the diagnosis of FH.
- The use of pravastatin is effective and safe in hypercholesterolemic children. The data in this thesis provide evidence that children with FH should be treated with statins as early as possible, to prevent progression of atherosclerosis.
Recommendations

Several recommendations for future research can be made on the basis of the results described in this thesis. Conventional statin treatment in children with FH is deemed safe and efficacious, at least in the short term. Although statins are effective at reducing IMT values in FH children, their LDL-lowering effectiveness in terms of reaching LDL goals has not been consistently demonstrated. Therefore, aggressive lipid-lowering trials should be conducted to evaluate the safety and the efficacy of reaching lower LDL-cholesterol goals in these children. Moreover, therapeutical options could include combination therapy, as with statins and ezetimibe, a cholesterol absorption inhibitor. Furthermore, FH children are characterized with lower levels of HDL-cholesterol as compared to their healthy siblings and low levels of HDL contribute to the progression of atherosclerosis. A novel cholesteryl ester transfer protein (CETP) inhibitor in combination with pravastatin was safe and efficacious in increasing HDL-C in patients with type II dyslipidemia. In addition, Niaspan an extended release nicotinic acid, effectively raises HDL-C with concomitant beneficial effects on TG and LDL-C. It was demonstrated that this drug can be combined safely with statins in adult patients. However, studies are needed in children with FH to confirm the safety and efficacy of combination therapy with ezetimibe and statins, CETP inhibitors and extended release nicotinic acid before their use is advocated.

Finally, we clearly demonstrated that the age at the start of statin use is a strong predictor for carotid IMT in adolescence. Therefore, studies are needed to confirm the efficacy and safety of statin use in FH children even before the age of 8.

References


