Dyslipidemia in the Young: From Genotype to Treatment
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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General discussion

This last chapter describes the current and future challenges regarding the treatment of cardiovascular diseases (CVD) in clinical practice, that emerged from the data presented in this thesis. As a final point I will discuss the factors driving dyslipidemia based on epidemiological and genetic studies presented in this thesis as well.

Challenges in the prevention and treatment of cardiovascular disease

First challenge: Identifying young individuals in need of lipid-lowering treatment

Chapter 1 discusses multiple pharmaceutical interventions that are currently available to reduce low-density lipoprotein cholesterol (LDL-c) levels in plasma. The first challenge in the prevention and treatment of CVD is therefore not how to reduce LDL-c, but how to effectively identify those individuals in whom LDL-c lowering is useful. Who will most likely benefit from lipid-lowering therapy is summarized in guidelines that are based on randomized controlled trials, observational studies and subsequent meta-analyses. The American\textsuperscript{110} and European\textsuperscript{40} guidelines both distinguish several major patients groups that benefit from statin treatment (summarized in Table 1).

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Table 1. Comparison between the major patients groups that could benefit from statin treatment based on European\textsuperscript{40} and American guidelines.\textsuperscript{110}

Abbreviations: CVD, cardiovascular disease; LDL-c, low-density lipoprotein cholesterol.

Important for the main topic of my thesis – treatment of dyslipidemia in the young – is that when applying guidelines to a population-based sample, one will see that older individuals with a less severe cardiovascular risk profile suffer from increased estimated 10-year CVD risk, compared to younger individuals with a more severe cardiovascular risk profile.\textsuperscript{215, 226} Moreover, almost all individuals younger than 50 years of age have a definite low 10-year risk of CVD. This is a direct consequence of the fact that the current risk calculations tools are governed by age. However, individuals could simultaneously have

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a calculated low short-term risk (i.e., 10-year risk of CVD) but also suffer from a high lifetime risk of CVD, for example due to genetic predisposition, extremely elevated single risk factors or lifelong exposure to multiple elevated risk factors.

Several alternative approaches have been proposed, complementary to existing guidelines, to assist in the identification of young individuals, including an individualized benefit approach, lifetime risk calculators, and age- and sex-specific risk thresholds. These methodologies have not been implemented in European and American guidelines because of the lack of data for accurate lifetime risk estimation, lack of evidence of the efficacy of lifelong preventive pharmaceutical interventions in terms of lowering LDL-c and cardiovascular events, and absence of clinical endpoint trials in young individuals (<40 years of age).

Another shortcoming of current guidelines, in my opinion, is applying a fixed LDL-c cutoff to identify individuals eligible for statin treatment (i.e., ≥ 4.9 mmol/l) when short-term CVD risk is low. This is surprising when already in the 1980s, and in Chapter 4, it has been shown that LDL-c increases with age and that there were pronounced gender differences. For example, the 99th percentile of young males aged between 18 and 20 years, is 4.2 mmol/l, compared to 5.8 mmol/l in middle aged (45–49 year) males. Thus, an LDL-c of 4.0 mmol/l in a young individual is much more extreme than an LDL-c of 5.0 mmol/l in middle-aged individuals. As a result, these guidelines will fail to identify most young individuals with extremely elevated LDL-c in comparison to their peers. These young individuals are likely to have an increased lifetime risk, but longitudinal prospective data is currently unavailable.

Why appropriate identification of young individuals with increased LDL-c is of utmost importance, is illustrated by the concept of cumulative burden of LDL-c. This parameter quantifies an individual’s exposure to LDL-c levels over his or her lifetime. It is estimated that exposure to an LDL-c burden (calculated by multiplying the average LDL-c concentration by years e.g., 2.5 mmol/l x 60 = 150 mmol) of approximately 155 mmol is sufficient to develop CVD. The question remains if it is realistic and reliable to estimate the cumulative burden of LDL-c in primary prevention setting. It is, however, hypothesized and partly proven that persons with high LDL-c levels, for example ≥ 90th percentile for age and gender, will keep their percentile ranking over time and have increased cardiovascular risk. When taking this into account, individuals with lifelong LDL-c above the 95th percentile (corrected for age and gender, Chapters 4 and 5) will reach an LDL-c burden of 155 mmol by age 38 (data not shown). This is sixteen years earlier compared to individuals with lifelong median LDL-c levels (corrected for age and gender). By contrast, individuals with lifelong LDL-c below the 5th percentile (corrected for age and gender, Chapters 4 and 5) will cross this threshold by age 75. These figures show the importance of using age-specific LDL-c cutoff values to identify young individuals with hypercholesterolemia, instead of using a fixed LDL-c cutoff of 4.9 mmol/l.
Although this may sound simple, there are several important barriers: (1) cholesterol measurements early in life are advocated but are not common practice; (2) plasma LDL-c levels are not a fixed entity but a dynamic trait influenced by age, gender and environment. Chapters 4 and 5 provide a clear insight in this matter: why wait to treat a female of 32 years of age with an LDL-c level of 4.0 mmol/l (≥ 95th percentile for age and gender), when it is clear that her LDL-c will exceed 4.9 mmol/l fifteen years later in life. Chapter 7 illustrates that the use of age-specific LDL-c cutoff levels is an efficient tool to identify individuals with genetic predisposition to FH. In this study, we examined factors that are associated with LDL-c levels in young and apparently healthy females. There was clearly a higher prevalence of deleterious mutations in young females with LDL-c levels ≥ 4.9 mmol/l compared to other reports (17% vs. 2.5% and 1.7%). The most likely explanation for this finding is the use of age- and gender-specific LDL-c cutoff levels; and (3) the most important difficulty to overcome the implementation of age- and gender-specific cutoff values is the availability of evidence-based clinical trial data showing that lipid-lowering treatment in the young with increased LDL-c levels is indeed beneficial and outweighs potential harmful effects. Naturally, such evidence would result in guidelines adjustments. Clinical judgement is needed in situations when hard evidence is (not yet) available. Starting lipid lowering drugs in these young hypercholesterolemic individuals should gain momentum and become standard practice of physicians and pediatricians that are managing these patients.

Second challenge: Implementation and adherence to guidelines
Chapters 2 and 3 demonstrate the huge gap between clinical practice and guidelines: 77% of the individuals in primary prevention and 31% in secondary prevention, eligible for statins, do not report statin treatment (Chapter 2). However, 66% of the statin prescriptions in primary prevention are reported by individuals considered not eligible for statin treatment (Chapter 3).

Chapter 2 shows that undertreatment in primary prevention is most often present in individuals with a low estimated 10-year CVD risk, but with TC/HDL-c > 8, which requires immediate treatment initiation according to the Dutch guideline. Undertreatment is more common in men in comparison to women. Interestingly, for secondary prevention the opposite is true: females were more likely to be undertreated. In both primary and secondary prevention individuals with diabetes mellitus were most often treated according to guidelines. Undertreatment is not exclusive to the Netherlands, undertreatment in primary prevention has also been observed in e.g. Denmark (52%),228 the United Kingdom (~70%),119 and the USA (80%).

Chapter 3 describes that two-thirds (66%) of the statin prescriptions in Lifelines is not in line with guidelines, and could thus be considered as overtreatment. Also in the United Kingdom (~60%), and the USA (69%), overtreatment with statins was a common finding.
Although Lifelines data cannot be used to understand the underlying reasons for the observed gap between guidelines and clinical practice, one could speculate about possible causes. The first possible cause is unawareness of (primary care) physicians and that physicians seem to be reluctant to change existing treatment practices when guidelines are changed.\(^{236}\) It seems likely that treatment decisions are made according to outdated versions of guidelines.\(^{237}\) The Dutch guidelines are developed by the Dutch Institute for Health Care Improvement and the Dutch College of General Practitioners (NHG). The NHG has a distinct department dedicated to the implementation of guidelines. They develop e-learning modules, group courses, protocols for nurse practitioners, and websites for patients (www.thuisarts.nl). In 2010, a study of 523 general practitioners (GPs) showed that in almost all GP practices the Dutch guideline is available, either printed or online. Most GPs assess cardiovascular risk in patients with CVD or DM. However, only 50\% of the GPs were found to perform cardiovascular risk assessment in patients with a high 10-year cardiovascular risk in absence of CVD or DM (source: Vitale Vaten). A systematic review evaluating successful strategies for guideline implementation suggested that guideline implementation strategies should have three components: education for health care professionals (e.g. physicians and nurses), information for patients, and organizational changes.\(^{238}\) In the Netherlands, despite using the suggested strategies, disparity between guidelines and clinical practice remains, as illustrated by the Lifelines study (Chapters 2 and 3).

A second cause of the poor implementation and adherence to guidelines could be the gap between patients’ preferences and physicians’ judgement. For example, long-term therapeutic compliance of patients is poor.\(^{239}\) It is possible that statin treatment was started in patients at high risk of CVD, but was eventually discontinued by the patients. Or vice versa: some patients were willing to take medication while they would not benefit from it. A recent review showed that especially younger patients have low compliance. Education level and gender did not affect compliance.\(^{240}\) Patients’ beliefs and motivation about the therapy are reported to be strongly associated with compliance. Compliance is better if the patient (1) feels susceptible to the illness; (2) knows that the illness could dramatically reduce quality of life; (3) believes the intervention is effective; and (4) feels supported by the physicians. The latter can be achieved by being emotionally supportive, asking questions, spending time, making eye contact, and involving patients in treatment decisions.\(^{241}\) Involvement of patients in treatment decisions is a growing challenge for physicians. Especially since patients are becoming more assertive and demand to be involved in treatment choices. It requires time, interpersonal skills, and knowledge of physicians to effectively involve patients in treatment decisions.\(^{242}\)

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In conclusion, our and other studies call for improved awareness of CVD in clinical practice, improvement of guidelines especially for those individuals with low short-term but high lifetime CVD risk, and a better focus on therapeutic compliance by involving patients in treatment decisions.
Third challenge: Fight undertreatment of familial hypercholesterolemia

It has been estimated that familial hypercholesterolemia (FH) accounts for 5% of the premature myocardial infarctions (age < 60 years) in the general population. This seems to be a small percentage, but this disease appeals to me most. As discussed in Chapter 1, FH is the most common monogenic disorder in humans, causing increased plasma LDL-c levels. The prevalence of FH is estimated to be 1 in 200 – 250. Patients with FH usually develop premature CVD, however, once identified and properly treated, the progression of atherothrombotic complications can be attenuated. In most countries less than 1% of the patients are diagnosed. However, due to active screening in the Netherlands many individuals with FH have been identified. After a successful small pilot program as “proof of principle” from 1994 to 2000, the national screening was supported by the Dutch Ministry of Health from 2001 to 2014. To date > 35,000 individuals with FH have been identified. Based on a prevalence of 1 in 250, it can be estimated that 68,000 individuals suffer from FH in the Netherlands. So approximately 50% of the patients are identified in the Netherlands, but worldwide a large number of patients with FH remain undiagnosed and most likely untreated. Worldwide, the Dutch Lipid Clinic Network (DLCN) criteria, are considered the most reliable clinical diagnostic criteria for FH. These criteria categorize individuals based on LDL-c levels and family history into a definite, probable or possible diagnosis of FH. In individuals with a definite/probable score, molecular genetic testing is recommended. After finding a mutation in the index patient (first family member diagnosed with FH), the potentially affected family members are actively recruited and tested for mutations. The so-called cascade approach calls for testing of affected individuals off-spring only. No need to test children of FH family members that are not mutation carriers. This type of screening is extremely efficient and affordable. On average, after identifying one new index patient, eight undiagnosed and untreated relatives with the same mutation were identified. This approach saves lives and families, and is cost-effective.

An alternative approach to identify index cases, is universal screening of for example young children. Total cholesterol levels could be measured when children are seen for standard immunization or could be included in the metabolic screening using neonatal heel prick. This was already suggested in the early nineteen eighties, and it has been tested in 10,000 neonates in the United Kingdom, Slovenia, and more recently in the United States. Although the false positive rates were high, they conclude that this screening method is feasible and cost-effective. Universal screening could certainly help early identification and possible early treatment of pediatric dyslipidemia.

In adult universal screening the question is which cutoff values should be used to conduct additional diagnostic testing. This is especially difficult in the young. In the Copenhagen General Population Study an LDL-c cutoff of 4.4 mmol/l discriminated best between carriers and non-carriers of FH mutations. This threshold was however not adapted for age and gender, which should be preferred, in my opinion, as previously discussed.
Recently, a new prediction model, based on all participants from the Dutch FH screening program, was developed to estimate the probability of being an FH mutation carrier in one of the candidate genes. In this model age and LDL-c are used as continuous predictors, therefore more applicable for young persons. When applying the calculator to the Lifelines cohort, younger individuals with LDL-c ≥ 4.9 mmol/l have indeed a higher chance to be classified as carrying an FH mutation compared to older individuals with LDL-c ≥ 4.9 mmol/l (data not shown).

The data presented in this thesis call for an improvement of the identification and treatment of individuals with FH. Even in the Netherlands, where the identification of FH has been a priority for many years, FH remains undertreated: Chapter 2 shows that more than 95% of the individuals with low 10-year CVD risk and TC/HDL-c ratio > 8 do not report statin treatment (Lifelines data; based on n = 70,292). The prevalence of TC/HDL-c ratio > 8 in individuals with low 10-year CVD risk was 1.250. Although TC/HDL-c ratio is generally not used for the diagnosis of FH, these are likely to be individuals with severely increased total cholesterol and LDL-c levels. Chapter 4 shows a high prevalence of severe hypercholesterolemia in adults: of 133,540 adult Lifelines participants free of CVD and without lipid-lowering drugs, 256 (prevalence 1:500) individuals have LDL-c levels ≥ 6.5 mmol/l (data not shown). Chapter 7 describes the prevalence of monogenetic causes of FH: 18 out of 8,071 children (prevalence 1:450) have LDL-c levels above 4.9 mmol/l, indicative of FH.75

In my opinion, the consequences of the global burden of FH need much more attention. Worldwide millions of children and adults are unaware of their life-threatening disease and suffer from preventable and not seldom morbidity and mortality from CVD. The reference ranges presented in Chapters 4 and 5 could be helpful in identification of possible FH affected individuals.

### Additional origins of dyslipidemia

#### Non-LDL dyslipidemia

Non-LDL dyslipidemia is the umbrella term for low HDL-c, high triglyceride levels and increased remnant cholesterol levels. Although current guidelines provide ample recommendations for the treatment of hypercholesterolemia, treatment of non-LDL dyslipidemia is hardly discussed. However, non-LDL dyslipidemia is also strongly associated with CVD:137, 138, 240 Until now reported prevalence rates are based on small population studies of mainly elderly individuals,137, 138, 240 but Chapter 6 describes the prevalence rates
and the factors associated with non-LDL dyslipidemia based on the Lifelines cohort. To our best knowledge, we show for the first time in a large population-based cohort study that non-LDL dyslipidemia is prevalent in the young, with a prevalence rate up to 30% in men between 35 and 55 year of age. We furthermore show that obesity, smoking and diabetes mellitus were strong determinants of non-LDL dyslipidemia. This study indicates the need of public health efforts to reduce non-LDL dyslipidemia.

Hypo- and hypercholesterolemia in young women
To investigate the genetic and environmental factors associated with extreme LDL-c levels, we randomly selected 121 young women with hypocholesterolemia (LDL-c ≤ 1st percentile for age and gender) and 119 young females with hypercholesterolemia (LDL-c ≥ 99th percentile for age and gender) (Chapter 7). Interestingly, in two-thirds of the women with hypercholesterolemia we identified a genetic component (16% monogenic and 50% polygenic). Previous investigations into the origin of hypercholesterolemia predominantly focused on patients who were referred for a clinical evaluation due to symptomatic hypercholesterolemia. Our study shows, to the best of our knowledge, for the first time that low LDL-c levels in a healthy general population is mainly driven by genetic components. A calculated healthy lifestyle score did not differ from the selected controls. The opposite is true regarding hypercholesterolemia. In 38% of the women (17% monogenic and 21% polygenic) we identified a genetic origin for hypercholesterolemia. Furthermore, women in whom no genetic origin of hypercholesterolemia could be identified were more often classified as having an unfavorable lifestyle and had a much more detrimental metabolic risk profile in comparison to a control group: higher triglyceride levels, lower HDL-c levels and much higher BMI levels. Our data support that close monitoring of young females with unfavorable lifestyle (e.g. obesity or smoking) is warranted. Especially, because a detrimental lifestyle is associated with severe hypercholesterolemia which almost equals LDL-c levels of heterozygous FH.

STAP1, a novel candidate gene of FH?
Remarkably, in 10 – 40% of the patients with clinically diagnosed FH, a molecular defect cannot be found. Further studies into the molecular origin of FH in these cases may increase our understanding of LDL metabolism. For example, the discovery of PCSK9 through classical linkage analysis in families with hypercholesterolemia, was the start of the development of a new and very effective pharmaceutical intervention to further reduce plasma LDL-c levels in high-risk individuals on top of statin therapy. In 2014, mutations in signal transducing adapter protein 1 (STAP1) were found to be associated with FH in four families. Braenne et al analyzed genetic variants of FH candidate genes in 25S families...
with premature CVD and a positive family history of CVD. A novel mutation in \textit{STAP1} was associated with the FH phenotype.

To further investigate the possible role of \textit{STAP1} in cholesterol metabolism, we developed a \textit{Stap1} knockout mouse model using CRISPR/Cas9 as described in Chapter 8. \textit{Stap1} knockout mice fed chow or a high-fat-high-cholesterol diet, did, however, not show altered plasma cholesterol levels, compared to wild-type littermates. Reasons for the conflicting results with earlier findings in man are discussed in Chapter 8. In short, our mouse model mimics loss-of-function mutations (no \textit{STAP1} protein expression), but the mutations described in humans could render a gain-of-function. It is also possible that mice are simply a poor model for studying how \textit{STAP1} affects human biology. The \textit{STAP1} mutations found in man could also be a chance finding. The respective linkage analyses results may have suffered from incorrect assignments due to the large overlap in LDL-c levels between FH and non-FH individuals. GWAS have neither validated \textit{STAP1} as a novel LDL candidate gene\textsuperscript{214, 215} while further recent sequencing studies have not identified novel \textit{STAP1} mutations\textsuperscript{216-218}. We also were unable to find \textit{STAP1} mutations in 119 healthy young females with severe hypercholesterolemia (Chapter 7). In fact, one of the mutations described by Fouchier et al in the context of hypercholesterolemia\textsuperscript{216} was found in a woman with hypcholesterolemia (Chapter 7, data not shown). This raises the question whether mutations in \textit{STAP1} are causally related to FH.

However, until now we have focused on plasma lipid levels and future experiments will include bone marrow transplantation of \textit{Stap1} deficient mice into \textit{Ldlr} knockout mice to investigate the possible role of \textit{STAP1} in the development of atherosclerosis from a more immunological angle (\textit{STAP1} is mainly expressed in B cells).

**Conclusions**

The data presented in this thesis call for: (1) global improvement in the identification of hypercholesterolemia in young individuals by using age- and gender-specific plasma LDL-c levels. These individuals have an increased lifetime risk of CVD – especially those suffering from FH – and personalized advice on lifestyle changes and cholesterol lowering medication should be considered; (2) promotion of the understanding and stimulation of using cardiovascular risk prevention guidelines; and (3) further studies to explore novel pathways implicated in disturbances of LDL metabolism as well as investigate genetic targets like \textit{STAP1} that ultimately could lead to improved diagnosis and treatment of dyslipidemia.

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