

# Treatment and long term follow-up of children and young adults with familial hypercholesterolemia

Gisle Langslet  
Doctor philosophiae thesis



Lipid Clinic, Oslo University Hospital

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Lipid Clinic,

Department of endocrinology, morbid obesity and preventive medicine,

Oslo University Hospital





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## **Preface and acknowledgements**

This thesis has emerged from my work since 2002 at the Lipid Clinic, Rikshospitalet, Oslo University Hospital. The Lipid Clinic was started in 1984 by pediatrician Leiv Ose, to care for children with familial hypercholesterolemia and their parents. In my work at the Clinic I have had the privilege to follow these families. My main task has been to be responsible for performing clinical trials with new lipid lowering drugs. In addition, I have followed up children and adults in the regular outpatient clinic. My professional background is mainly from family medicine, having worked for 20 years as a family physician before I was employed at the Lipid Clinic. I am very grateful to Leiv Ose for having employed me, giving me the opportunity to explore this, for me, new field of medicine in the final part of my professional career, and for guiding me into the world of lipidology and clinical trials. Special thanks also to my colleague since 2002, Kjetil Retterstøl, for having taught me the fundamentals in lipidology and for always fruitful, inspiring and humorous discussions. For many years, Martin Prøven Bogsrud has been very important to me as a colleague, research fellow and discussion partner, and he has undertaken the task to appoint opponents for this thesis. Since 2014, Martin has been the leader for the newly established National Advisory Unit on familial hypercholesterolemia (NKT for FH). The National Advisory Unit has directly supported my work by employing me part-time and by establishing the treatment registry which our paper V is based on. Kirsten Holven, who is head of research in the National Advisory Unit is an important discussion partner, research partner, has provided significant feedback on this thesis and has been guiding me in the submission process. Arne Svilaas, colleague at the Lipid Clinic since 2002, has been an important discussion partner and provided valuable feedback. All the above mentioned persons have served as a counselling group in the work with the thesis.

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Last, but not least, special thanks to the children and parents having used their time to participate in our clinical trials over many years, enduring blood sampling and procedures.

To my children, grandchildren, other family and friends, I apologize for having been so busy for the last couple of years, and promise to do better in the years to come.



## **Abbreviations**

ABCA1: ATP binding cassette subfamily A member 1

AE: adverse event

AHA: American Heart Association

ALT: alanine aminotransferase

apoA1: apolipoprotein A1

apoB: apolipoprotein B

apoB-100: apolipoprotein B-100

AST: aspartate aminotransferase

ATP: adenosine triphosphate

BAS: bile acid sequestrants

BMI: body mass index

CAD: coronary artery disease

CETP: cholesterol ester transfer protein

CHARON: hyperCholesterolemia in cHildren and Adolescents taking Rosuvastatin Open label

CHD: coronary heart disease

CI: confidence interval

cIMT: carotid intima media thickness

CK: creatine kinase

CTT: Cholesterol Treatment Trialists

CVD: cardiovascular disease

DHCR7: 7-dehydrocholesterol reductase

DISC: Dietary Intervention Study in Children

DLCN: Dutch Lipid Clinic Network

EAS: European Atherosclerotic Society

ECG: Electrocardiogram

EMA: European Medicines Agency

FDA: Food and Drug Administration

FH: heterozygous familial hypercholesterolemia

FMD: flow mediated dilation

FOURIER: Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk

4S-study: Scandinavian Simvastatin Survival Study

HDL: high-density lipoprotein

HDL-C: high-density lipoprotein cholesterol

HMG-CoA: hydroxy-methylglutaryl-coenzyme A

HoFH: homozygous familial hypercholesterolemia

HPS2-THRIVE: Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events

IMT: intima-media thickness

LDL: low-density lipoprotein

LDL-C: low-density lipoprotein cholesterol

LDLR: low-density lipoprotein receptor

LDLRAP1: low density lipoprotein receptor adaptor protein 1

LLT: lipid lowering therapy

mAbs: monoclonal antibodies

MEDPED: Make Early Diagnosis to Prevent Early Deaths

MI: myocardial infarction

MLPA: multiplex ligation-dependent probe amplification

mRNA: messenger ribonucleic acid

MTP: microsomal triglyceride transfer protein

NCEP: National Cholesterol Education Program

NHLBI: National Heart, Lung and Blood Institute

NICE: National Institute for Health and Care Excellence

NPC1L1: Niemann-Pick C1-Like 1

PCSK9: proprotein convertase subtilisin/kexin type 9

PDAY: Pathobiologic Determinants of Atherosclerosis in Youth

POSCH: Program on the Surgical Control of the Hyperlipidemias

PPAR: peroxisome proliferator-activated receptors

REVEAL: Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification

RNA: ribonucleic acid

SD: standard deviation

SMR: standardized mortality ratio

SPC: Summary of Product Characteristics

SREBP: sterol regulatory element-binding protein

STRIP: Special Turku Coronary Risk Factor Intervention Project for Babies

TC: total cholesterol

TS: Tanner stage

UCCG: Unit for Cardiac and Cardiovascular Genetics

UK: United Kingdom

ULN: upper limit of normal

US: United States

WHO: World Health Organization

## List of papers

### Paper I

Efficacy and safety of rosuvastatin therapy in children and adolescents with familial hypercholesterolemia: Results from the CHARON study.

Braamskamp MJ, **Langslet G**, McCrindle BW, Cassiman D, Francis GA, Gagné C, Gaudet D, Morrison KM, Wiegman A, Turner T, Kusters DM, Miller E, Raichlen JS, Wissmar J, Martin PD, Stein EA, Kastelein JJ. *J Clin Lipidol*. 2015 Nov-Dec;9(6):741-50. doi: 10.1016/j.jacl.2015.07.011

M.J.A.M.B. and G.L. contributed equally to the work.

### Paper II

Effect of Rosuvastatin on Carotid Intima-Media Thickness in Children with Heterozygous Familial Hypercholesterolemia: The CHARON Study.

Braamskamp MJAM, **Langslet G**, McCrindle BW, Cassiman DM, Francis GA, Gagne C, Gaudet D, Morrison KM, Wiegman A, Turner T, Miller E, Kusters DM, Raichlen JS, Martin PD, Stein EA, Kastelein JJP, Hutten BA. *Circulation*. 2017 Jul 25;136(4):359-366. doi: 10.1161/CIRCULATIONAHA.116.025158. Epub 2017 Jun 7.

### Paper III

A 3-year study of atorvastatin in children and adolescents with heterozygous familial hypercholesterolemia.

**Langslet G**, Breazna A, Drogari E. *J Clin Lipidol*. 2016 Sep-Oct;10(5):1153-1162.e3. doi: 10.1016/j.jacl.2016.05.010.

#### **Paper IV**

Long-term follow-up of young adults with familial hypercholesterolemia after participation in clinical trials during childhood.

**Langslet G**, Bogsrud MP, Halvorsen I, Fjeldstad H, Retterstøl K, Veierød MB, Ose L. *J Clin Lipidol.* 2015 Nov-Dec;9(6):778-85. doi: 10.1016/j.jacl.2015.08.008.

#### **Paper V**

Treatment goal attainment in children with familial hypercholesterolemia: A cohort study of 302 children in Norway.

Bogsrud MP, **Langslet G**, Wium C, Johansen D, Svilaas A, Holven KB. Accepted for publication in *Journal of Clinical Lipidology* 29 Nov 2017. Available online 6 Dec 2017. DOI: 10.1016/j.jacl.2017.11.009

G.L and M.P.B contributed equally to the work.

# 1. Introduction

## 1.1. History of cholesterol and atherosclerosis

Atherosclerosis has been found in preindustrial populations living more than 4000 years ago in different geographical regions (1).

Cholesterol was first identified in gallstones in the 1700s. The name is made up from “chole” (bile) and “steros” (solid). The molecular formula of cholesterol was established in the late 1880s and its complicated structure, consisting of four rings was eventually described by Wieland and Windaus, who won the Nobel Prize in Chemistry in 1927 and 1928 for their work (2). Cholesterol was detected in atherosclerotic arteries in the 1800s. The name atherosclerosis is made up from “athero” (gruel) and “sclerosis” (hardening). The term was introduced by the French pathologist Jean Lobstein in 1829 (3), and was used in 1904 by the German pathologist Felix Marchand, who suggested that it was responsible for most of the obstructive processes in the arteries (4). In 1908, Ignatowski found a possible connection between cholesterol-rich food and atherosclerosis (5) and in 1910 Windaus showed that atheromatous lesions contained 6 times more free cholesterol and 20 times more esterified cholesterol than the normal arterial wall (6). In 1913 the Russian pathologist Anitschkow, in a pioneering experimental work, showed that rabbits fed cholesterol-rich food rapidly developed atherosclerosis, demonstrating early lesions in the form of fatty streaks, as well as more advanced lesions (7, 8). His findings had no impact in the medical community at that time, when atherosclerosis was rather regarded as a normal ageing process. It was not until the late 1940s and 1950s that research on cholesterol and atherosclerosis got wind in the sails. In 1950 Gofman et al. showed that the cholesterol containing lipoproteins could be separated in two groups by ultracentrifugation; high-density lipoprotein (HDL) and low-density lipoprotein (LDL), and that individuals having had myocardial infarction had higher levels of LDL particles in blood compared with those without infarction (9). In 1952 it was shown that intake of vegetable-containing food and limited intake of fat from animal sources led to decreased cholesterol levels, and that it was the unsaturated fatty acids in fat from vegetable sources that were the main cause of the cholesterol reduction (10, 11).

Beginning with the Framingham study from 1957, many subsequent epidemiological studies have shown a connection between high serum cholesterol and coronary heart disease (12-15). In Norway, this has been shown in a cohort of 45 000 individuals examined in the 1970s and followed-up for mortality 25 years later (16). Relative risk of death from coronary heart

disease (CHD) increased by 30% per 1 mmol/L increase in total cholesterol level, and risk of death increased exponentially (log linearly) from a level of 4 mmol/L, with no lower threshold.

In autopsy studies of American soldiers killed in the Korean and Vietnam wars, “fatty streaks” (accumulation of lipid laden macrophages) and “fibrous plaques” (increase of lipid laden macrophages and ingrowth and proliferation of smooth muscle cells) were found in the coronary arteries and aorta (17, 18).

In autopsies of about 500 persons dying accidentally or from natural reasons, Holman et al. in 1958 found fatty streaks in the aorta from below 3 years of age and fibrous plaques beginning between 10-20 years of age, increasing in incidence from 20-30 years of age (19). More recently, in the Pathobiologic Determinants of Atherosclerosis in Youth (PDAY) and the Bogalusa Heart Study (20-22), fatty streaks have been found in the aorta and coronary arteries early in life, and fibrous plaques were found in adolescents and young adults. In the PDAY and Bogalusa studies, the risk factors high body mass index (BMI), high blood pressure, smoking and lipid levels were strongly associated with the incidence and extent of arterial lesions.

Also, from the natural history of heterozygous familial hypercholesterolemia (FH), and especially homozygous familial hypercholesterolemia (HoFH), it became evident that high cholesterol in early life was linked to higher morbidity and mortality from cardiovascular disease later in life. It was, however, not immediately recognized that the very high cholesterol levels in FH also pointed to cholesterol as a risk factor for cardiovascular disease (CVD) in non-FH patients, when cholesterol levels were only moderately increased (23).

## 1.2 History of familial hypercholesterolemia

FH was for a long time designated Müller-Harbitz disease, due to the work done by the Norwegian internist Carl Müller and pathologist Francis Harbitz (24).

The clinical entity of FH was first systematically described by Carl Müller (25, 26). In several reports starting from 1937 until 1939, when he published a seminal work describing 17 families with xanthomatosis, hypercholesterolemia and cardiovascular disease, with a pattern of an inborn error of metabolism and monogenetic autosomal dominant inheritance in four



generations. He postulated that xanthomatosis was the underlying cause, that the condition occurred with a high frequency in the population, and that causal and prophylactic treatment could be effective

From 1925 to 1938 Francis Harbitz described findings on autopsy in patients with xanthomatosis and sudden death. He found foam cells and more marked changes in arteries than in senile atherosclerosis (27).

Before Harbitz' and Müller's work there had been reports of cases of xanthomatosis and cardiovascular disease from different authors, including Fagge in 1873, Lebzen and Knauss in 1889, Török in 1893, and Raeder in 1936 who reported a family with hypercholesterolemia and xanthomatosis (24).

The inheritance pattern of FH was further elucidated by Khachadurian in 1964, describing the clinical heterozygous and homozygous forms of the disease, concluding that it was inherited as a dominant disorder with incomplete penetrance (28). In the extreme form in HoFH, subjects acquired aortic valve disease, especially aortic stenosis, and CHD in the first or second decade of life (29). In 1991, in a cohort of patients with FH followed prospectively in the United Kingdom (UK) it was reported that FH carried a nearly 40-fold increased risk for CHD (30).

The genetic causes of FH have been clarified from the 1970s and onwards. Brown and Goldstein showed that FH was caused by defects in the gene encoding the LDL-receptor (*LDLR*) at the surface of liver cells, resulting in decreased uptake of LDL from blood to the liver and increased serum LDL-cholesterol (LDL-C) levels (31). In 1985 they received the Nobel Prize for their research on cholesterol metabolism.

Later, autosomal dominant hypercholesterolemia has also been found to be caused by mutations in the genes encoding apolipoprotein B-100 (*APOB*), and the glycoprotein proprotein convertase subtilisin/kexin type 9 (*PCSK9*). ApoB is the protein component of LDL, which binds to the LDLR. The R3500Q mutation in *APOB*, often referred to as familial defective ApoB, was described in the late 1980s (32, 33). It affects the binding domain for LDLR, resulting in reduced binding of LDL to the receptor and increased LDL-C levels (33). The glycoprotein PCSK9 was discovered in 2003, and shortly afterwards it was reported that "gain of function" mutations in *PCSK9* could cause FH (34, 35).

PCSK9 is synthesized and secreted to the circulation by the liver. It is involved in the degradation of the LDLR, binding to the receptor on cell surfaces, targeting it for degradation intracellularly in the lysosomes (36, 37). LDLRs which are not bound to PCSK9 are recirculated to the cell surface and reused for uptake of LDL particles, up to 150 times (38). Increased levels of PCSK9 results in increased LDLR degradation and increased LDL-C levels.

In 2001 an extremely rare form of severe hypercholesterolemia, autosomal recessive hypercholesterolemia (ARH), was described and shown to be caused by mutations in the gene encoding the LDL-receptor adaptor protein 1 (*LDLRAP1*), a protein required for efficient endocytosis of the LDLR-LDL complex in hepatocytes (39, 40).

Mutations in *LDLR* are, by far, the most common cause of FH, accounting for approximately 95% of cases in Norway. Mutations in *APOB* and *PCSK9* accounts only for approximately 3% and 2% of cases respectively (41). Of note is that mutations in *LDLR*, *APOB* and *LDLRAP1* are “loss of function mutations”, while mutations in *PCSK9*, causing FH, are “gain of function” mutations.

### 1.3 Diagnosis of FH

Historically, diagnosis of FH has been done clinically and still, in most countries, FH is usually diagnosed clinically (42). Markedly elevated plasma cholesterol in combination with extensor tendon xanthomas and/or early cardiovascular disease in the patient or close relatives have been regarded specific for FH. Lipid deposits that occur in adult FH patients are rarely found in children. Validated sets of diagnostic criteria have been developed (Make Early Diagnosis to Prevent Early Deaths [MEDPED], Simone Broome and the Dutch Lipid Clinic Network [DLCN]) (30, 43, 44). From the 1990s, molecular or genetic testing has emerged, adding to the knowledge of the nature of FH, and making the diagnosis more precise.

Inheritance of one defect allele in the autosomal dominant genes *LDLR*, *APOB* and *PCSK9*, typically results in approximately 50% reduced clearance of LDL-C from the circulation. In the autosomal recessive *LDLRAP1*, a defective allele must be inherited from both parents to affect LDL-C levels.

Mutations associated with elevated cholesterol levels are designated as pathogenic or causative. Mutations not affecting LDL uptake, and not associated with elevated cholesterol

levels, are designated non-pathogenic or non-causative. Pathogenic mutations can be further classified as defective, when there is some residual LDLR function, or negative when there is no residual LDLR function. Especially in HoFH, having defective as compared with negative mutations, typically results in lower LDL-C levels, better response to lipid lowering therapy (LLT) and better prognosis.

Worldwide, more than 1700 pathogenic mutations in *LDLR* have been identified (45), and in Norway more than 240 mutations have been identified (41). Only one of the identified mutations in *APOB*, R3500Q, is considered to be pathogenic. Mutations in *PCSK9* and *LDLRAP1* are much less prevalent and consequently only a small number of pathogenic mutations have been identified.

It has been thought that the prevalence of FH in most Western populations was around 1 in 500 inhabitants, and the corresponding prevalence of HoFH 1 in 1 000 000 (46), although in certain populations with specific founder mutations, the prevalence has been shown to be higher (44). In recent years, in population and cohort studies, a higher prevalence of FH mutations, between 1 in 200 and 1 in 300, has been found (47-50). In the Netherlands the prevalence of HoFH has been estimated to be approximately 1 in 300 000 (51). In Norway, based on the number of known, living HoFH patients, the prevalence of HoFH is approximately 1 in 500 000.

A small number of individuals having pathogenic mutations known to cause FH, have normal cholesterol levels. This may be caused by loss-of-function mutations in genes regulating production or uptake of apoB containing lipoproteins, or other unknown gene variants affecting LDL-C metabolism (52). As reported by Khera et al., if, in addition to a FH-mutation, it is required that there is a markedly elevated LDL-C level, with thresholds of  $\geq 3.4$  mmol/L or  $\geq 4.9$  mmol/L, the prevalence of FH were 1 in 301 and 1 in 853, respectively (49).

Since FH is an autosomal dominant disease, except for the rare recessive *LDLRAP1* mutation, the probability is 50% for a child of a parent with FH to inherit the disease, and second degree relatives will have a 25% chance of inheriting the affected allele. Screening close relatives of a person already diagnosed with FH, designated as an index case or a proband, is known as family cascade screening. First-degree relatives of the proband is screened, either by lipid profile, or if genetic testing has been done, in addition for the proband mutation. In Norway most children with FH have been diagnosed through cascade screening, having one parent diagnosed with FH. Family cascade screening has been done in Norway and the Netherlands

since the mid-1990s, and is an effective method to identify affected individuals. In spite of this, the total number of individuals with genetically verified FH in Norway as per Dec 2017 is around 7800 (41), or about one half to one third of an estimated total number of 15-25000 affected individuals in the population.

## 1.4 Disease risk in FH

### 1.4.1 Before statins

In 1969 Slack reported a prevalence of ischaemic heart disease in 104 males and females with Fredrickson type II-hyperbetalipoproteinemia of 51.4% and 12.2%, respectively by age 50, and 85.4% and 53.3%, respectively by age 60 (53). Probably most of these patients had FH as they had xanthomas and elevated cholesterol levels.

Stone et al., in 1974, reported prevalence of coronary artery disease (CAD) in 116 kindred with Fredrickson type II hyperbetalipoproteinemia, compared with unaffected family members; 52% of males and 32.8% of females were affected by age 60 years, unaffected males lagging 20 years behind (54). Probably, many had FH as the diagnostic criterias used for type Fredrickson II hyperbetalipoproteinemia were elevated LDL-C, and either a similarly affected first degree relative or tendon xanthomas.

In 1991 the Simone Broome Register Group reported standardized mortality ratio (SMR) in a cohort of 282 men and 244 women aged 20-74 years with FH in the United Kingdom (UK) during 1980-1989. Fifteen of 24 deaths were due to CHD, resulting in an overall SMR of 3.86 (SMR=1.00 for the normal population), with no significant difference between men and women, and with the highest ratio of 96.86 at age 20-39 years. SMR for death for all causes was 1.83, and was also highest at age 20-39 years (SMR 9.02). The authors conclude that FH is associated with a substantial excess mortality from CHD in young adults, but may not be associated with a substantial excess mortality in older patients (30).

### 1.4.2 After statins

The introduction of hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, around 1990 revolutionized the treatment of FH with prospects for significant improvements in the prognosis of the disease. These hopes have only partly been confirmed

in registry and cohort studies from many countries, including the United Kingdom, Netherlands, Spain, Denmark, and recently from Norway (55). Compared with the pre-statin era, the risk of CVD has been substantially reduced, but a considerable increased risk remains, even in treated FH, especially when treatment starts late in life and possibly also, according to a Danish population study, when treatment and follow-up is done outside specialized clinics (55-57).

In 1999 the Simone Broome Register Group reported results from a cohort of 605 men and 580 women aged 20-79 years with FH, followed prospectively from 1980 to 1995. In women aged 20-39 years, fatal coronary disease occurred in 2 individuals, versus an expected rate of 0.02, i.e. the relative risk of a fatal coronary event was increased 125-fold. In men aged 20-39, fatal coronary disease occurred in 6 individuals, versus an expected rate of 0.12, i.e. the relative risk was increased 48-fold. However, due to the low number of events, confidence intervals are large; 15-451 in women and 17-105 in men. With increasing age, the relative risk decreased, but the absolute risk increased. There was a decline in the relative risk for coronary mortality in patients aged 20-59 years, from an eight-fold increased risk before 1992 to 3.7 thereafter ( $P=0.08$ ) (58).

Recently, Mundal et al. reported findings in a Norwegian cohort of 4688 FH-patients. In Norway all genetic testing for FH is done by the Unit for Cardiac and Cardiovascular Genetics (UCCG) at Oslo University Hospital. All individuals with a verified molecular genetic diagnosis of FH in the period 1992-2010 in the UCCG Registry were linked to the Norwegian Cause of Death Registry. Compared with the Norwegian population, CVD mortality was significantly higher in the UCCG Registry in all age groups younger than 70 years with a SMR 2.29 (CI 1.65 to 3.19) in men and women combined (2.00 in men and 3.03 in women). Of note, it was shown that the mean age at inclusion in the genetic registry was 54.8 years for those who died, compared with 33.6 years for the registry in total, indicating that delayed diagnosis and statin treatment is a major issue in those who died. No significant differences were found in all-cause mortality or cancer mortality. The authors conclude that despite prescription of lipid-lowering drugs, FH patients still have significantly increased CVD mortality compared with the general Norwegian population (59).

In a follow-up of the same Norwegian registry, published in 2016, 5518 patients with genotyped FH during 1992-2013 were included. CVD was the most common cause of death (42.3%). Mean age at CVD death was 64.5 years (range 33-91). CVD mortality was

significantly higher in FH patients compared with the general Norwegian population under 70 years of age. SMR was highest in the 20-39 years age group; 4.12, decreasing to 0.77 for those over 80 years. For total CVD deaths occurring out of hospital, SMR was 12.35 for those aged 20-39 years (60).

In another study from the same registry, 5538 patients with verified genotyped FH were linked to data on all Norwegian CVD hospitalizations. Mean age at first hospitalization was 45.1 years, with no sex differences (61), compared with 64.9 years in the general population in the same time period (61).

In a Norwegian cohort of deceased FH-patients, it was recently shown that 93% had established CVD at the time of death, and 69% had experienced myocardial infarctions (62).

In a Danish general population cohort from Copenhagen, not subject to the biases inherent in the FH-registry studies, 33% of those with definite or probable FH (according to the DLCN criterias) had CAD. Only 48% of subjects with FH admitted to taking cholesterol-lowering medication. The odds ratio for CAD off cholesterol-lowering medication was 13.2 (10.0-17.4) in definite/probable FH compared with non-FH subjects, after adjusting for age, gender, body mass index, hypertension, metabolic syndrome and diabetes, and smoking. The corresponding adjusted odds ratio for CAD in FH subjects on cholesterol-lowering medication was as high as 10.3 (7.8-13.8) (57).

Recently, Khera et al. reported findings in a cohort study among 20485 CAD-free control and prospective cohort participants. Only 24 (1.7%) of participants with an LDL-C level  $\geq$  190 mg/dl (4.9 mmol/L) had an FH-mutation. The risk of CAD was 22-fold increased when an FH-mutation was found in combination with an LDL-C level  $\geq$  190 mg/dl compared with a 6-fold increased risk in subjects with the same LDL-C level, but with no FH-mutation, both groups compared with a reference group with LDL-C <130 mg/dl and no mutation (49).

## 1.5 History of treatment of children and young adults with FH

### 1.5.1 Diet and lifestyle

In his paper “Angina in Hereditary Xanthomatosis” from 1939, Carl Müller noted that the cholesterol content of the blood could be reduced by a diet poor in cholesterol. He wrote: “*I have ordered a diet poor in cholesterol (no yolk of egg, butter, cream, fat milk or animal fat*

*in general)...” and further: “The treatment may be of prophylactic value to persons with a hereditary predisposition” (25).*

In a report from Segall et al. in 1970, 13 children with FH, aged 2-15 years, were treated with a diet reduced in saturated fats. Serum total cholesterol (TC) was reduced by mean 18-24% after mean treatment periods of 3-7 months (63).

Obarzanek et al. in 2001, published results from the Dietary Intervention Study in Children (DISC), a long-term study of efficacy and safety of a cholesterol-lowering dietary intervention in 663 children 8 to 10 years of age with elevated LDL-C (approximately mean 3.4 mmol/L at baseline). The children were randomized either to dietary intervention or usual care, with mean 7.4 years follow-up. The intervention promoted adherence to a diet low in total and saturated fat and low in cholesterol (28% of energy from total fat, <8% from saturated fat, up to 9% from polyunsaturated fat, and <75 mg cholesterol/1000 kcal per day). After one year and 3 years there was a small, but significant reduction in LDL-C of 0.13 mmol/L ( $P<0.001$ ) and 0.09 mmol/L ( $P<0.02$ ), respectively. After 5 years, however, the difference was non-significant. There were no differences in height, serum ferritin, sexual maturation or BMI (64).

Another prospective randomized trial from Finland, the Special Turku Coronary Risk Factor Intervention Project for Babies (STRIP) was published in 2007. A “heart healthy” low-saturated-fat, low-cholesterol diet, including increased intake of fruits, vegetables and whole grain products, was introduced to 540 intervention infants at 7 months of age, and compared with 522 control children who received an unrestricted diet (65). Skimmed milk was recommended from 1 year of age. Dietary advice was supervised by a dietician and given continuously during follow-up. From 7 years of age, the advice was increasingly directed at the child. Saturated fat intake, TC and LDL-C values were lower ( $P<0.001$ ) in the intervention than in control children during the 14 years of follow-up. The intervention effect on TC was larger in boys than in girls, and was significant only in boys. The absolute serum cholesterol difference between the intervention and control boys was 0.2 mmol/L (5%). Importantly, the 2 study groups showed no difference in growth, BMI, pubertal development, or age at menarche. The authors conclude that repeated dietary counseling remains effective in decreasing saturated fat and cholesterol intake and serum cholesterol values until at least 14 years of age.

Concerns have been expressed that a diet lower in saturated fat might interfere with growth and development in children (66). The STRIP study shows that these concerns are unfounded. However, without proper counseling from qualified nutritionists, a diet very low in fat and low in calories may result in failure to thrive (67). In children with FH, where diet is of greater importance than in the general population, qualified dietary counseling is especially important.

Theoretically, using combinations of cholesterol-lowering foods in one diet (portfolio diet), including ingestion of soy, viscous fibers, plant sterols and nuts, can reduce LDL-C by approximately 30% (68). In a 6-month randomized study in 351 adult hyperlipidemic patients, a cholesterol-lowering portfolio diet reduced LDL-C by approximately 10% compared with a low saturated fat diet (69)

In a small, recently published Norwegian study, among 10 children with FH, aged 5-18 years, TC and LDL-C levels were reduced by 16% and 22% respectively, at follow-up after dietary advice (70).

Until the early 1970s, dietary treatment with reduction of total fat, replacing saturated fat with unsaturated fat and restricted cholesterol intake was the main therapy for children with FH, and is still a cornerstone in the treatment. In Norway, dietary advice by clinical nutritionists has been an integrated part of the care for FH-patients since the start of the Lipid Clinic in Oslo in 1984.

The dietary and lifestyle measures implemented in the FH-population may explain the reduced cancer mortality and mortality from other causes in this group (71).

### 1.5.2. Partial ileal bypass

In 1964 it was reported, in experiments on rabbits and pigs, that blood cholesterol could be substantially lowered by surgically bypassing 40-50% of the small intestine (72). Also, in humans having undergone partial ilectomy for other reasons, it was shown that a substantial reduction of TC and LDL-C could be achieved. In 1963 the first ileal bypass procedure was performed to lower plasma cholesterol in a patient with hypercholesterolemia (72).

In 1970, Buchwald et al. published a report on 6 children with FH and one child with HoFH treated with partial ileal bypass. (29). At 3 months follow-up TC was reduced by mean 33%



in the children with FH and by 16% in the child with HoFH, still remaining at 16% at two-year follow-up. All were given vitamin B12 injections every second month. None of the children had diarrhea. Growth and sexual development in the 16 year-old homozygous patient was normal at two years follow-up.

In view of the limited treatment options in the 1960s it is interesting to read Buchwald's rationale for this invasive intervention. He describes the homozygous condition as follows:

*“The patient is commonly in his teens or younger when first discovered to manifest the hyperlipidemia trait; arcus senilis is often present and subcutaneous and tendon xanthomas are evident. Atherosclerotic fundic changes may be seen, and the patient may well be incapacitated by severe exertional angina pectoris. An early death from myocardial infarction in these individuals is often to be expected. A unique and characteristic lesion of these patients is acquired aortic valvar stenosis and/or occasionally, aortic valvar insufficiency, both resulting from heavy accumulation of proliferative atherosclerotic plaques in the valve and adjacent aortic wall. Similar plaques commonly involve the orifices of the coronary arteries.”*

In 1990 results from the Program on the Surgical Control of the Hyperlipidemias (POSCH) was published. The study randomized 838 patients having survived a myocardial infarction, either to ileal bypass surgery by bypassing of the distal third of the small intestine by an end to side ileocecostomy, or to a control group without surgery. The participants were followed-up in mean 9.7 years. In the surgery group LDL-C was reduced by 37.7%, and overall mortality and mortality due to CHD were reduced, but not significantly. Death due to CHD and nonfatal myocardial infarction (MI), however, were significantly reduced, with a relative risk reduction of 35%. The principal side effect was diarrhea. Kidney stones and gallstones and bowel obstruction were also increased in the surgery group. No mention is made in the publication of differences in body weight between the surgery group and control group (73). Five years after the trial end, however, overall mortality and mortality from CHD were significantly reduced in the surgery group (74).

With the advent of statins, ileal bypass surgery to treat elevated cholesterol became obsolete.

### 1.5.3 Bile acid sequestrants

Bile acid sequestrants (BAS) are large polymers which bind bile acids in the ileum. The non-absorbable complex is excreted in the faeces. Consequently, hepatic cholesterol levels fall, and to preserve intracellular cholesterol homeostasis, this leads to increased synthesis of cholesterol, increased expression of hepatic LDLR, increased LDL-C uptake to the liver, and subsequently reduced plasma LDL-C levels (75). Due to lack of intestinal absorption, BASs have been considered safe to use in children. Gastrointestinal side effects and non-palatability are, however, limiting their use.

The BAS cholestyramine was developed in the late 1950s. In 1959 it was reported that it reduced serum cholesterol in humans by an average of 20% (76). Another BAS, colestipol, was introduced in the beginning of the 1970s. Colesevelam, a second-generation BAS, with a higher affinity and binding capacity for bile salts, and lower rates of gastrointestinal side-effects was approved in the United States in 2000 and in Norway in 2004.

Early reports of treating adults with cholestyramine are from 1961, treating hypercholesterolemia and pruritus in primary biliary cirrhosis and pruritus in jaundice (77, 78). From the mid 1960s cholestyramine was used in the treatment of adults with FH (79).

The first report of cholestyramine use in children seems to be by Horan et al. in 1964, who treated two siblings with FH in a 3 week course with 15 grams daily resulting in lowering of plasma cholesterol levels by 12 to 44% from baseline. The authors note that there was a tendency for triglycerides to increase during treatment and concludes that “Cholestyramine warrants further trial in children with familial hypercholesterolemia with careful attention to their effect on other blood lipids and on acid-base balance and other possible sources of toxicity” (80).

In 1973 West et al. reported use of cholestyramine in 19 children with FH, in doses of 8-24 g/day up to 20 months, resulting in mean serum cholesterol reduction of 36%. Growth rates were normal, but serum folate levels were reduced in all patients (79).

In 1980 the same investigators reported results from follow-up of 35 children with FH up to 8 years after having started cholestyramine treatment. There was a progressive decrease in compliance with therapy over time; only 55% remained on treatment after 6 years and only 48% after 8 years. Long-term compliance was significantly better in those starting treatment

before age 10. Plasma-cholesterol was lowered in all children taking cholestyramine, mean reductions in plasma-cholesterol ranging from 26 to 44% (81).

Glueck et al. also in 1973 reported use of cholestyramine and diet in the treatment of 36 children with FH, of which 20 received cholestyramine. However, drug adherence was satisfactory in only half of the patients (82).

These early reports were non-randomized studies without control groups. In 1996 two randomized, placebo-controlled studies with BAS in children with FH were published by Tonstad et al. In the first study, in 72 children aged 6-11 years, cholestyramine 8 grams per day reduced LDL-C by 16.9%, compared with 1.4% increase in the placebo group. There were no effects on growth. Compliance was low, however, of 36 children each in the active treated and placebo groups, 22 and 26, respectively, completed the one-year study (83). In the second study, in 66 children aged 10-16 years, colestipol 10 g/day was compared with placebo for eight weeks, followed by a one-year open phase. LDL-C was reduced by 19.5% in the colestipol group, compared with 1.0% increase in the placebo group. Again, compliance was rather low; after one year two thirds of the participants remained in the study, of whom half took  $\geq 80\%$  of the prescribed dose (84).

Efficacy and safety of the newer, tablet formulated colesevelam, was evaluated in children with FH in a randomized placebo-controlled, double blind trial, published in 2010. 194 children aged 10 to 17 years were randomized 1:1:1 to placebo, colesevelam 1.875 g/day, or 3.75 g/day for 8 weeks. Thereafter, all received open-label colesevelam 3.75 g/day for 18 weeks. After 8 weeks LDL-C was significantly reduced by 6.3% and 12.5% in the low- and high-dose colesevelam groups respectively, and the treatment effects were maintained during the open-label period. The most common drug-related adverse events (AEs) were gastrointestinal, including diarrhea, nausea, vomiting, and abdominal pain. No clinically meaningful changes in hormones, vitamins, and clotting factors were noted. Among subjects completing the study (89.2%) the changes in height-velocity were as expected in normal maturation. Compliance in the randomization period was good, at 85% for all treatment groups (85).

In 2002, McCrindle et al. reported a randomized crossover open-label trial of combination therapy with colestipol and pravastatin in 36 children and adolescents with FH or familial combined hyperlipidemia. The regimens included colestipol 10 g/day (10 pills) versus a combination of colestipol 5 g/day with pravastatin 10 mg/d (six pills). As expected,

acceptability was better with the combination regimen, but compliance was suboptimal (approximately 60%) with all medication components. Also, as could be expected mean relative LDL-C lowering was significantly better with the combination regimen (-17% versus -10%  $P=0.045$ ) (86).

With the availability of statin therapy, also for pediatric patients, from the late 1990s, treatment with BASs gradually decreased, due to efficacy, tolerability and compliance issues.

#### 1.5.4 Plant stanols and sterols

Sterols are an essential constituent of cell membranes in animals and plants. Cholesterol, the sterol of mammalian cells, is synthesized by the cells. Phytosterols are synthesized by plants, the most common being sitosterol, campesterol and stigmasterol. Phytosterols cannot be synthesized by humans and are poorly taken up in the human intestine. In the early 1950s it was observed that ingested plant sterols could decrease serum cholesterol. The mechanism of action is thought to be by inhibition of cholesterol absorption, the effect of ingesting 2 g sterols/day approximating  $\approx 10\%$  LDL-C reduction. In 4 studies with normocholesterolemic and hypercholesterolemic children, including children with FH, ingestion of 1.6-3 g plant sterols/day, have been shown to reduce LDL-C by 6-15% (87-90). Some concerns remains, however, over the possible long-term effects on fat-soluble nutrient levels of plant stanol/sterol addition to the diet (91).

#### 1.5.5 Fibrates

Chlorophenoxyisobutyrate was synthesized in the 1950s, after it was discovered that farm workers exposed to an insecticide, phenyl ethyl acetic acid, had remarkably low plasma cholesterol. The substance, a fibric acid derivative, was named clofibrate, or Atromid-S, and the trade name for the marketed drug was “Atromidin” (92). Clofibrate acts through peroxisome proliferator-activated receptors (PPARs), a group of nuclear receptor proteins, or transcription factors, regulating the expression of several genes involved in lipid and lipoprotein metabolism, including hydroxy-methylglutaryl-coenzyme A (HMG-CoA) reductase (*HMGCR*), Niemann-Pick C1-Like 1 (*NPC1L1*) and Adenosine triphosphate (ATP) binding cassette genes of different subfamilies (*ABCA1*, *ABCB4*, *ABCG5* and *ABCG8*) (93). There are three classes of PPARs, PPAR-alfa, PPAR-delta (also named beta) and PPAR-gamma.

Fibrates are PPAR- $\alpha$  agonists. Other PPAR-agonist drugs, activating PPAR- $\delta$  and  $\gamma$ , have been in development for treatment of hyperlipidemia and diabetes, but have invariably failed, except for the anti-diabetic PPAR- $\gamma$  agonist pioglitazone (“Actos”), due to side effects related to the diverse effects of PPAR stimulation in a variety of tissues.

Segall et al. reported in 1970, to have treated six children with FH with clofibrate in addition to diet, resulting in an additional decrease in serum TC from mean 22% to 33%. In addition, one child with HoFH was treated from 9 years of age with a combination of diet, clofibrate 500 mg two times daily and cholestyramine 32 g/day. During the two years of follow-up, TC was reduced by 32% and her xanthomas decreased strikingly (63).

West et al. in 1975 reported to have treated 9 children with FH with clofibrate in addition to a fat restricted diet. Diet alone reduced TC by mean approximately 20%, and addition of clofibrate resulted in an additional 10% decrease. By 2 ½ years, however, none of the children remained on the drug for different reasons (lost to follow-up, stopped taking drug on own initiative or on doctor’s advice owing to lack of compliance with the dietary component of the regime). The authors conclude that clofibrate no longer is recommended for treatment of children with FH (94).

Wheeler et al. reported in 1985 a six month, double blind, crossover, placebo-controlled trial with bezafibrate in 14 children with FH, aged 4-15 years. TC was reduced by mean 22% compared with placebo and HDL-cholesterol (HDL-C) increased moderately. The authors conclude that bezafibrate might be a useful adjunct to treatment in children with FH (95).

In addition to niacin, clofibrate was the only lipid-lowering agent available in the early 1960s, and was used in the treatment of hypercholesterolemia and FH in adult patients until the late 1970s. After the publication of the large World Health Organization (WHO) primary prevention trial in 1978, showing a significant excess of deaths in clofibrate treated patients compared to control patients (92), the use of clofibrate fell abruptly. It was gradually replaced by other fibrates, bezafibrate, fenofibrate and gemfibrozil, and used primarily in the treatment of elevated triglycerides.

Fibrates have not been used to any large extent in the pediatric FH-population.

### 1.5.6 Probucol

Probucol, (4,4-[Isopropylidenedithio]-Bis[2,6-di-t-Butylphenol]) was originally synthesized as an antioxidant for use in rubber manufacturing, including airplane tires. In the search for new cholesterol lowering agents it was subsequently investigated by the Dow Chemical Company in the late 1960s (96). In 1969 it was reported to decrease serum cholesterol and phospholipid concentrations in 6 hospitalized patients, of which two women had type II hypercholesterolemia (97). Probucol's hypocholesterolemic effect in mice, rats and monkeys was described in a report by Barnhart et al. in 1970 (98). The mechanism of action is still unclear. It is thought to have an effect on cholesterol catabolism, increasing bile acid secretion. Probucol also has an independent antioxidant effect. It lowers TC by about 10-20%, usually with no effect on triglycerides. It also lowers HDL-C by 20-30%, possibly by enhancing reverse cholesterol transport. It has marked effects on cutaneous and tendinous xanthomas, with regression often seen after 2-3 months of therapy and in some cases disappearance of xanthomas after one year of therapy. This effect is thought to be due to inhibition of the oxidative modification of LDL, inhibiting foam cell formation and also enhancing release of cholesterol from macrophages (99). Probucol is given orally as a tablet, usually dosed 500 mg two times daily. It is usually well tolerated, diarrhea and other gastrointestinal symptoms being the most common side effects. In Western countries the drug was used from the late 1970s until 1995, when it was withdrawn in the US due to elongation of QT-intervals in electrocardiograms and possible ventricular arrhythmias, and also due the lowering of HDL-C. Large randomized clinical trials with Probucol, demonstrating effect on clinical cardiovascular endpoints have not been done. Smaller studies have shown conflicting results on surrogate vascular endpoints (96, 100). In a cohort study from Japan, in patients with FH, there was a reduced hazard ratio for cardiovascular events in secondary prevention, but no effect in primary prevention. There were, however, large differences in baseline characteristics between exposure and non-exposure patients (101). In Japan the drug has been used extensively since the mid 1980s and in 2009 it was still being used by at least 60 000 patients (96). Its use in Norway has mainly been in HoFH and FH patients with large xanthomas, and use in children has been limited to HoFH patients with large xanthomas.

### 1.5.7 Nicotinic acid

Nicotinic acid is the oldest known drug used to lower cholesterol and has been used since the 1950s (102). It reduces cholesterol modestly, lowers triglycerides and increases HDL-C. The mechanism of action is to a large degree unknown. The most important side effect is flushing, limiting its use. In a large secondary prevention study from the 1970s, the Coronary Drug Project, niacin did not reduce coronary or total mortality after 5 years (103), but at follow-up 9 years after the completion of the study, the mortality was reduced in the niacin treated group (104).

In a retrospective review of 21 hypercholesterolemic children aged 4 to 14 years, receiving niacin between 1980 and 1991, TC and LDL-C was reduced by 23 and 30%, respectively. Side effects with flushing, headache and elevation of transaminases were reported (105). The authors conclude that although niacin treatment in children seems to be efficacious, adverse effects are common, and that until further studies demonstrates long-term safety, “niacin treatment should be reserved for the closely-supervised treatment of severe hypercholesterolemia by a lipid-specialist.”

Recently, in the Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) study, among participants with atherosclerotic vascular disease, the addition of extended-release niacin-laropiprant to statin-based LDL-C lowering therapy did not significantly reduce the risk of major vascular events, but did increase the risk of serious AEs, i.e. worsening of diabetes and new-onset diabetes, gastrointestinal, musculoskeletal and skin disturbances as well as infections and bleeding (106).

A Cochrane systematic review of randomized controlled trials from 2017 concludes that benefits from niacin therapy in the prevention of cardiovascular disease events are unlikely (107).

Niacin has never been used on a larger scale in the treatment of children with FH.

### 1.5.8 Thyroid hormones

The effects of thyroid hormone on cholesterol levels have been known for many years, hyperthyroidism being accompanied by lowering, and hypothyroidism by elevation of serum cholesterol levels. Also, when hypothyroidism is treated with thyroid hormone

replacement, cholesterol is lowered to normal levels. The effect is probably mediated through up-regulation of *LDLR* (108).

Müller in his work in 1939 noted that thyroid preparations had been used by Koch and Westphal to treat high cholesterol and he adopted this practice. Treatment studies with desiccated thyroid were performed in the 1950s and resulted in fall in cholesterol levels, but a large number of patients experienced side-effects with tachycardia, angina pectoris, diarrhea, weight loss and/or insomnia (108).

Thyroid hormones have not been used in the treatment of children with hypercholesterolemia to any extent.

### 1.5.9 Statins

The pathway for cholesterol synthesis in the body were worked out in the 1950s including the rate-limiting step and major point of regulation of the biosynthesis, i.e. the reduction of HMG-CoA to mevalonate by HMG-CoA reductase, and in 1964 Bloch and Lynen were awarded the Noble Prize for this work (2). HMG-CoA reductase inhibitors, known as statins, act by inhibiting this rate-limiting step in the cholesterol synthesis. The reduction of cholesterol synthesis and drop of intracellular cholesterol levels in the liver cell results in up-regulation of nuclear transcription factors (sterol regulatory element-binding proteins [SREBP]), leading to an increase in LDLRs, increased uptake of LDL-C from the circulation and reduced plasma LDL-C (109).

The first statin, ML-236B (mevastatin or compactin) was discovered in the early 1970s by Akira Endo and his coworkers at the laboratory of Sankyo in Japan. It was not marketed due to concern about toxic effects in dogs. The first commercially available statin was lovastatin (“Mevacor”), developed by Merck Sharp & Dohme and marketed in 1987 in the United States (US) and available from 1989 in Norway.

The landmark study, Scandinavian Simvastatin Survival Study (4S-study), published in 1994, was a breakthrough for cholesterol-lowering treatment with statins. The 4S-study was a double-blind, randomized, placebo-controlled study to evaluate the effect of cholesterol lowering with simvastatin versus placebo on mortality and morbidity in patients with CHD. LDL-C was lowered by 35% in the group who received simvastatin 20-40 mg daily. Over 5.4



years, all-cause mortality was reduced by 30% and coronary mortality by 42%, compared with placebo, without significant AEs (110).

After the 4S-study, several statin-trials have been conducted in adults, both in primary and secondary prevention. The results from these studies have been summarized in meta-analyses by the Cholesterol Treatment Trialists' (CTT) Collaboration, concluding that statin therapy reduces the 5-year incidence of myocardial infarction, coronary death, coronary revascularisation, and stroke by about 20% and total mortality by about 10% per mmol/L reduction in LDL-C, in men as well as in women. The absolute benefit is related to the individuals' absolute risk of an event, and to the absolute reduction in LDL-C achieved (111-113). It can be anticipated that some of the participants in these trials have had FH, but dedicated randomized cardiovascular endpoint trials in FH-populations have never been, and will never be performed, for ethical reasons.

The first report of statin treatment in children was from Stein in 1989, who treated six children with severe FH with lovastatin 80 mg/day or simvastatin 40 mg/day, achieving a reduction in LDL-C by 41% compared with diet alone (114). Ducobu et al. in 1992 treated 32 hypercholesterolemic children below 17 years with simvastatin 5-40 mg/day for at least 24 months with a 37% mean reduction in LDL-C from baseline. No clinically relevant changes in liver transaminases, alkaline phosphatase or creatine kinase (CK) were observed. Height and weight were recorded in only a subset of patients with no recognizable deviations from their growth percentiles at baseline (115).

Interestingly, Athyros et al. in 2002 reported to have treated 16 children with FH, aged 10–17 (median 13) years with atorvastatin 10–40 mg/day (mean dosage 23 mg/day) in addition to cholestyramine, for a period of 3 years. The efficacy and safety outcomes were serum LDL-C reduction, somatic, mental and social development as well as statin-related side effects. At baseline, mean LDL-C was  $276 \pm 31$  mg/dL (7.2 mmol/L). Diet reduced LDL-C by 4.6%. Cholestyramine contributed a 16% reduction in LDL-C levels. With atorvastatin treatment, LDL-C was further reduced by 45%, and an LDL-C treatment goal of <130 mg/dL (3.4 mmol/L), or <100 mg/dL (2.6 mmol/L) for two patients with a positive exercise tolerance test, was reached by all participants. Somatic, mental and social development of subjects were not affected and no statin-related AEs were recorded (116).

The first double-blind, randomized, placebo-controlled statin study in children with FH was published in 1996, investigating treatment with pravastatin 5-20 mg daily versus placebo over

12 weeks in 72 children aged 8 to 16 years. The authors concluded that pravastatin was well tolerated and that adverse events were mild and equally distributed among the three treatment groups. LDL-C levels were significantly reduced by 32.9% compared with placebo (117).

In the period from 1999 to 2015, several randomized, placebo-controlled studies in children have been performed investigating treatment with lovastatin, simvastatin, atorvastatin, pravastatin, rosuvastatin and pitavastatin (118-123).

In 2007 two meta-analyses of randomized placebo-controlled statin studies in children were published, and in 2014 a Cochrane systematic review was published, with an update in 2017.

The first meta-analysis by Arambepola et al. assessed 8 trials published between 1996 and 2005, in 947 children aged 8-18 years, for periods of 6-96 weeks with an estimated 850 person-years follow-up (124). Statins used in the studies were pravastatin, lovastatin, simvastatin and atorvastatin. There were no differences in clinical or laboratory adverse reactions between placebo and active treatment. Statins lowered LDL-C by 32.5%, increased HDL-C 3.4%, and lowered triglycerides 3.0%.

The second meta-analysis by Avis et al. comprised six studies published between 1996 and 2005, evaluating pravastatin, lovastatin, simvastatin and atorvastatin therapy in 798 children aged 8 to 18 years, with 12 to 104 weeks of treatment (125). Of the 8 papers included in the Arambepola meta-analysis, 2 were excluded in the Avis meta-analysis due to duplicate reports (de Jongh et al. 2002) (126), and lack of safety data (Couture et al. 1998) (127). LDL-C reduction ranged from 21% for lovastatin 40 mg to 39% for atorvastatin 10-20 mg. TC and apoB were significantly reduced, whereas HDL-C and apolipoprotein A1 (apoA1) were significantly increased by statin therapy. No statistically significant differences were found between statin- and placebo-treated children with respect to the occurrence of adverse events, sexual development (risk ratio of advancing  $\geq 1$  stage in Tanner classification), muscle toxicity, or liver toxicity. There was a minimal difference in growth in favor of the statin group (0.33 cm; 95% confidence interval [CI]: 0.03 to 0.63). In four of the studies, hormone levels were measured. The different studies reported no increase, small increases, and small decreases in dehydroepiandrosterone levels in the statin treated groups. For luteinizing hormone a small decrease in the placebo group in one study was reported. In other studies, no differences were found. The authors note that normal fluctuations in hormone levels in puberty and during day- and nighttime may have influenced the measurements, and that the differences were too small to have any clinical relevant effect on growth and maturation.

They conclude that statin therapy in children with FH is efficacious and without untoward effects on safety, but that further studies should assess lifelong safety.

The Cochrane systematic review from 2014 included 8 randomized, placebo-controlled studies published between 1996 and 2010, comparing statin with placebo in a total of 1074 children with FH, aged 7 and up to 18 years. Median follow-up time was 24 weeks, with a range from six weeks to two years. The review included the studies in the Arambepola and Avis meta-analyses, and in addition a study with rosuvastatin published in 2010 (128). Statins reduced the mean LDL-C concentration at all time points, with mean relative reductions in LDL-C concentration at the end of follow-up varying from -21% to -41%. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as CK levels, did not differ between treated and placebo groups at any time point. The risk of myopathy and clinical adverse events were very low and similar in both groups. The effect of statins on puberty, assessed by Tanner staging, was only reported in three studies (119-121). Estimated risk ratios for an increase in Tanner stage were similar between statin and placebo groups at six months (2 studies), at one year (one study), and at two years (one study). Height and weight measurements were also done in the studies, but due to short follow-up in many of the studies, conclusions were difficult to draw. The authors conclude that statin treatment is an efficient lipid-lowering therapy which seems to be safe in the short term, but that long-term safety is unknown. In the 2017 update of the Cochrane systematic review, a study with pitavastatin, published in 2015, was included, increasing the number of studies to 9, including 1177 children between 6 and 18 years of age. The conclusions were the same as in the 2014 report. However, the authors assessed the evidence for no increase in risk of myopathy or ALT and CK elevations to be of low quality (129).

#### 1.5.10 Ezetimibe

Ezetimibe is a cholesterol absorption inhibitor which acts by blocking the sterol transporter NPC1L1 protein in the small intestine, thereby inhibiting uptake of dietary and biliary cholesterol. The reduced cholesterol delivery to the liver results in up-regulation of LDL-receptor expression, increased uptake of LDL-C from the blood to the liver, and lowering of LDL-C levels. LDL-C is typically reduced by 15-20%, both when used alone and in combination with a statin. Ezetimibe was approved by the US Food and Drug Administration in 2002 and marketed in Norway in 2003. The effect of ezetimibe on CVD was not proven

until 2015, in the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), showing significant effect on a composite cardiovascular endpoint with a combination of simvastatin and ezetimibe versus simvastatin alone (130).

In a prospective, multicenter placebo-controlled study in 248 children with FH, aged 10-17 years, ezetimibe in combination with simvastatin provided an additional 16% reduction in LDL-C, compared with simvastatin monotherapy after 33 weeks, with no safety issues. There were no between group differences in growth or maturation, no effect on the menstrual cycle in girls and hormone levels were within normal ranges. (131). Another study, published in 2015 investigated ezetimibe monotherapy versus placebo in 138 hypercholesterolemic children (FH or other severe hypercholesterolemia) aged 6-10 years. In this study ezetimibe reduced LDL-C significantly by 27% after 12 weeks, with a safety profile similar to studies in older children, adolescents and adults (132). In 12 of the participants the pharmacokinetics of ezetimibe were investigated and found to be similar between children  $\geq 6$  years and adults

#### 1.6 Markers of early atherosclerosis and inflammation

Increased intima-media thickness (IMT) is regarded as a sign of early atherosclerosis and a marker for increased risk of cardiovascular events (133, 134). In 1994, Marciullo et al. reported significantly higher maximum IMT in the common carotid artery in 46 children (mean age, 7.4 years) with TC  $\geq 6.4$  mmol/L, as compared with 48 children (mean age, 6.4 years) with TC < 6.4 mmol/L (0.50 vs 0.47 mm,  $P=0.007$ ) (135).

In 1996 Tonstad et al. and Lavrencic et al. reported similar findings in children with FH as compared with normolipidaemic healthy subjects, matched for sex and age (136, 137). Tonstad et al. reported findings in 61 boys and 29 girls 10 to 19 years old with FH and 30 control subjects. Mean IMT in the far wall of the carotid bulb was greater in the FH group than in the control subjects; 0.54 mm vs 0.50 mm ( $P=0.03$ ). Carotid artery plaque was present in 10% of the children with FH and in none of the control subjects, and children with plaque had higher mean cholesterol-years score than children without plaque (136).

In 2004, Wiegman et al. found a greater carotid IMT (cIMT) before the age of 10 in children with FH, compared with their unaffected siblings (138), and in a placebo-controlled pravastatin study in the children with FH, two years of pravastatin therapy induced a small, but significant regression of cIMT compared with placebo (121).

In a meta-analysis by Narverud et al. of eight studies comparing cIMT measurements in children with FH with a healthy control group, cIMT was significantly thicker in the FH groups with a weighted mean difference of 0.06 mm ( $P=0.02$ ) (133).

Impaired flow mediated dilation (FMD), measured as the percentage FMD (%FMD) in the brachial or superficial femoral artery, has been regarded as a measure of endothelial dysfunction, and an indirect marker of early atherosclerosis (133). %FMD of the brachial artery was reported by de Jongh et al. in a substudy of a randomized placebo-controlled study with simvastatin in 28 simvastatin treated, and 22 placebo treated participants. At baseline, %FMD was impaired in children with FH versus non-FH controls. In the simvastatin group, after 28 weeks of treatment, FMD increased significantly by 3.9% in the treated group, compared with 1.2% in the placebo group (126). There are, however, conflicting results on FMD measurements in children, possibly due to small study populations and different measuring techniques (133).

Elevated levels of inflammatory markers with relevance to atherogenesis have also been demonstrated in children with FH (133).

### 1.7 History of guidelines for the treatment of children with FH

The first recommendations from health authorities to prevent atherosclerosis appeared in 1957, when the American Heart Association (AHA) recommended that total fat should amount to 25-30% of calories in the diet and noted that “The possibility remains that the kind, rather than the amount of fat in the diet is responsible for atherosclerosis” (139). In 1961, they recommended the “prudent diet” for all Americans, with 25-35% of calories from total fat and to substitute vegetable oils and polyunsaturated fatty acid for saturated fatty acids (23, 140).

The first pediatric guidelines for treating dyslipidemia were developed by the National Heart, Lung and Blood Institute (NHLBI) National Cholesterol Education Program (NCEP) and published in 1992, following a similar first guideline for adults published in 1988 (141, 142). These guidelines were important in raising the awareness of FH in children and adolescents. It was not until 2011 that they were substituted by a new guideline with comprehensive evidence review (143).

In parallel, several other organizations developed guidelines incorporating new evidence. European consensus reports on FH, and children with FH, were published in 2013 and 2015 respectively (144, 145), and the National Institute for Health and Care Excellence (NICE) clinical guideline for the UK was published in 2008, with an update in 2016 (146).

Evidence grading systems have been developed by the American Academy of Pediatrics divided into “Evidence quality grades” with four levels (A to D), and “Definitions for Evidence-based statements” with 4 levels (Strong recommendation, recommendation, optional and no recommendation). In addition a category has been added for recommendations under exceptional situations in which evidence cannot be obtained, but clear benefits or harm are evident (147). This system has been used in the development of the NHLBI guideline from 2011 (148).

A summary of different guidelines and recommendations is given in table 1.

Table 1. Guidelines/recommendations/consensus statements for treatment of children with FH

Guideline/consensus	Year	Recommendation	Choice of drugs	Treatment goal
The American Academy of Pediatrics. National Cholesterol Education Program: Report of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents (149)	1992	<p>Low-fat diet from age 2 years.</p> <p>Consider drug therapy in children aged 10 years and older if, after trial of diet therapy:</p> <ol style="list-style-type: none"> <li>1) LDL cholesterol remains <math>\geq 190</math> mg/dL, or:</li> <li>2) LDL cholesterol remains <math>\geq 160</math> mg/dL, and:               <ol style="list-style-type: none"> <li>a. There is a positive family history of premature CVD (before 55 years of age), or:</li> <li>b. Two or more other CVD risk factors are present in the child</li> </ol> </li> </ol>	<p>Bile acid sequestrants (resins).</p> <p>Niacin, HMG CoA reductase inhibitors, probucol, gemfibrozil, d-thyroxine, paraminosalicylic acid and clofibrate are not recommended as routine drugs.</p> <p>If not adequate response to diet plus resins, referral to a lipid specialist for consideration of nicotinic acid therapy</p>	LDL cholesterol < 130 mg/dL or ideally lower, to near < 110 mg/dL
Management of hyperlipidaemia: guidelines of the British Hyperlipidaemia Association (150)	1993	<p>Low-fat, low-cholesterol diet after age 5 years.</p> <p>In FH families where the clinical onset of CHD is early, it may be necessary to prescribe drug therapy in children but most physicians would wait until after puberty</p>	Resins	No treatment goals given
Familial Hypercholesterolemia Report of a WHO consultation (151)	1997	<p>No clear consensus on treatment of children. The practice of the Lipid Clinic in Oslo is referred to</p> <p>Low-fat diet from age 5</p>	<p><i>Boys:</i></p> <p>Low risk: Resin at age 15, statin at age 18</p> <p>Moderate risk: Resin at age 12, statin at age 18</p> <p>High risk: Resin at age 7, statin at age 15</p> <p><i>Girls:</i></p> <p>Low risk: Resin at age 18</p> <p>Moderate risk: Resin at age 15</p> <p>High risk: Resin at age 12, statin at age 18</p>	No treatment goals given
Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. International Panel on Management of Familial Hypercholesterolemia (44)	2004	<p>Diet/lifestyle from age 2-3</p> <p>Drug therapy could be considered in the presence of major risk factors from 10 years of age in boys, and after puberty in girls, but in low-risk subjects should be delayed to 18 years of age in men and 30 years of age in women</p>	<p>Statin</p> <p>Add resin or ezetimibe if not at target</p> <p>Consider niacin if still not at target</p>	<p>Optimal LDL-C &lt; 4.1 – 2.6 mmol/L, depending on 10-year risk for adults</p> <p>No goals given for children</p>
Drug Therapy of High-Risk Lipid Abnormalities in Children and Adolescents: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee, Council of Cardiovascular Disease in the Young, with the Council on Cardiovascular Nursing (152)	2007	<p>Consider drug therapy in children 10 years of age (usually wait until menarche for females) and after a 6-12-month trial of fat- and cholesterol-restricted dietary management, if LDL level remains <math>\geq 4.90</math> mmol/L or <math>&gt; 4.10</math> mmol/L with additional risk factors</p> <p>High-risk children:</p> <p>May lower the recommended cutpoint LDL-C level for initiation of drug therapy, lower the desired target LDL-C levels, and in selected cases, may prompt consideration for initiation below the age of 10 years</p>	<p>Statin first-line</p> <p>Niacin may be considered for selected patients</p>	<p>LDL-C: minimal &lt; 3.35 mmol/L ideal &lt; 2.85 mmol/L</p>

Table 1 continues. Guidelines/recommendations/consensus statements for treatment of children with FH

Guideline/consensus	Year	Recommendation	Choice of drugs	Treatment goal
American Academy of Pediatrics Lipid Screening and Cardiovascular Health in Childhood (153)	2008	Gender-neutral recommendations: Nutritional counselling and low fat diet from 2 years of age  For patients 8 years and older with an LDL concentration of $\geq 190$ mg/dL (or $\geq 160$ mg/dL with a family history of early heart disease or $\geq 2$ additional risk factors, pharmacologic intervention should be considered	No specific recommendation given  Statins Resins Possibly ezetimibe	Initial goal to lower LDL concentration to $< 160$ mg/dL  Targets of 130 mg/dL or 110 mg/dL may be warranted when there is a strong family history of CVD, especially with other risk factors
National Institute for Health and Care Excellence (NICE) Clinical guideline (CG71) (146)	2008 Update July 2016	Nutritional advice from a healthcare professional with specific expertise in nutrition (no specific age specified)  Consider drug at the age of 10 years, taking into account: Their age, the age of onset of coronary heart disease within the family, and the presence of other cardiovascular risk factors, including their LDL-C concentration  In exceptional instances, with high risk, consider offering lipid-modifying drug therapy before the age of 10 years	Statins, low doses  In high risk, consider a higher dose of statin than is licensed for the age group, and/or more than one lipid-modifying drug therapy	No treatment goals given  (For adults, 50 % reduction of untreated LDL-C is recommended)
Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents NHLBI guideline (143)	2011	From age 1-2 years: Diet with saturated fat at $\leq 7\%$ of calories and dietary cholesterol limited to 300 mg/day  Age 10 years and older: If LDL-C remains $\geq 190$ mg/dL, or $\geq 160$ mg/dL in high risk conditions, after a 6-month of lifestyle/diet management, statin therapy should be considered  High-risk, aged 8-9 years: If LDL-C is 190 mg/dL after a trial of lifestyle/diet management statin therapy might be considered	Statins Resins	Decrease the LDL-C level to the $< 95$ th percentile ( $\leq 130$ mg/dL)
Pediatric aspects of Familial Hypercholesterolemias: Recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia (154)	2011	Diet and lifestyle. Consider to start treatment at the age of 8 years or older	Statins Resins	$\geq 50\%$ reduction in LDL-C or LDL-C $< 130$ mg/dL  There is a need in treatment of pediatric FH for balance between increased dosing and potential for side effects vs. achieving goals  More aggressive LDL cholesterol targets should be considered for those with additional CHD risk factors



Table 1 continues Guidelines/recommendations/consensus statements for treatment of children with FH

Guideline/consensus	Year	Recommendation	Choice of drugs	Treatment goal
Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: Guidance for clinicians to prevent coronary heart disease. Consensus statement of the European Atherosclerosis Society (144)	2013	Diet from age 2, including dietary advice from a certified dietitian/nutritionist. Dietary recommendations similar to those given to adults with FH  Cholesterol-lowering drugs should be strongly considered starting at age 8–10 years	Statins  Ezetimibe  Resins	LDL-C <3.5 mmol/L. Presence of very high LDL-C or additional cardiovascular risk factors may lower the target or the age at initiation of statin therapy
Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. European Atherosclerosis Society Consensus Panel (Review) (145)	2015	Diet and lifestyle  Cholesterol lowering drugs from age 8-10 years	Statins  Ezetimibe  Resins	LDL-C level <3.5 mmol/L (130 mg/dL) from age 10 years, or ideally 50 % reduction from pre-treatment levels for children 8–10 years, particularly in those with high-risk conditions or other major risk factors
Integrated guidance on the care of familial hypercholesterolaemia from the International FH Foundation (155)	2015	Diet and lifestyle  Drug therapy considered at age 8-10 years and ideally before 18 years. Boys and girls should generally be treated at similar ages, although depending on risk, boys with FH could be considered for earlier treatment with statins  Children, between the ages of 8 and 10 years, with proven FH on a suitable diet and LDL-cholesterol >4.0 mmol/L on two occasions should be started on low-dose statin monotherapy  After the age of 10 years, children with proven FH on a suitable diet and LDL-cholesterol >3.5 mmol/L on two occasions should be started on statin monotherapy	First line: Statin  Second line: Ezetimibe and resin	LDL-cholesterol targets in this age group need not be as intense as for adults  Age 8-10 years: LDL-C <4.0 mmol/L  Age >10 years: LDL-C <3.5 mmol/L

## 1.8 Current treatment of children with FH

### 1.8.1 Diet and lifestyle

Diet and lifestyle measures are first-line treatment for all FH-patients. Establishing healthy eating habits from young age is important for later adherence. According to the most recent European guidelines (144, 145), children with FH should be treated with a cholesterol lowering diet from 2 years of age, limiting total fat to 30% and saturated fat to 7% of energy intake, and limiting cholesterol intake to 200 mg/day. According to the latest US Guidelines from 2011 (143), exclusive breast feeding is recommended up to 6 months of age and a cholesterol lowering diet from 1-2 years of age, limiting total fat to 30% of energy intake, saturated fat to 7-10% of energy intake and cholesterol intake to below 300 mg/day. A high fiber intake, including whole grain cereals, a high intake of fruit and vegetables, and limited sugar sweetened beverages and foods are also recommended. The main sources of saturated fat in the diet are from processed meat such as sausages and minced beef, and from dairy products with a high fat content. Recommended foods are fish, and fish products, lean meat and poultry and lean dairy products. To encourage a healthy lifestyle is important in the counselling of children and adolescents with FH, i.e. avoiding or quitting of smoking, avoiding overweight, and to exercise regularly.

### 1.8.2 Drug treatment

According to the latest European and US guidelines, drug treatment in FH should be considered from 8-10 years of age with the aim of reducing the LDL-C level by at least 50% between age 8 and 10, and to obtain LDL-C <3.5 mmol/L from age  $\geq 10$  years (144, 145, 154). In high-risk cases with severely elevated LDL-C levels, eventually with severely elevated lipoprotein (a) levels, and/or family history of early CVD, treatment should be started or considered from 8 years of age.

The previous recommendation in the AHA guideline from 2007 (152), to discriminate in age between boys and girls at start of drug treatment, i.e. that girls should not be started on treatment until after menarche, has been abandoned. In the most recent guidelines it is the level of cardiovascular risk, not the level of pubertal development, which is emphasized when assessing at which age treatment should be started.

An overview of lipid lowering drugs approved for pediatric use in Norway is given in table 2.

Table 2. Lipid lowering drugs approved for pediatric use in Norway

Drug	Approved age	Dose	SPC or «Felleskatalog»	Other comments
Atorvastatin	From 10 years	10 - 80 mg	Limited data in children with FH 6-10 years of age. Not indicated for treatment in children below 10 years of age. Recommended starting dose 10 mg. Doses can be increased up to 80 mg  Pharmacokinetics: clearance in pediatric patients (6-17 years) similar to adults	Paper III adds information about safety and efficacy in children aged 6-15 years (included in the SPC). Commonly used in Norway
Rosuvastatin	6 - 9 years 10 - 17 years	5 - 10 mg 5 - 20 mg	Doses above 10 mg not investigated in children 6-9 years. Doses above 20 mg not investigated children 10-17 years. The 40 mg tablet is not suited for treatment of children. Not recommended for use in children below 6 years  Pharmacokinetics: Exposure in children (6-17 years) appeared to be similar or lower than in adults	Paper I adds safety, efficacy and pharmacokinetics information, (included in the SPC) Commonly used in Norway
Simvastatin	10 - 17 years	10 - 40 mg	Limited experience with use before puberty	
Pravastatin	8 - 13 years 14 - 18 years	10 - 20 mg 10 - 40 mg	No clinical data for children below 8 years of age. Doses >20 mg not investigated in children below 14 years of age	Two years randomized placebo-controlled data in children 8-17 years. Low potency  Commonly used in the Netherlands. By tradition not used to any degree in Norway
Fluvastatin	Above 9 years	20 - 80 mg	Not investigated in children below 9 years of age	
Lovastatin	10 - 17 years	10 - 40 mg	Safety and efficacy not sufficiently established	
Ezetimibe	Above 6 years	(10 mg)	Safety and efficacy in children aged 6-17 have not been established. No dosing recommendations can be given. Children above ≥6 years of age can be treated. Treatment should be initiated by a pediatrician. No data in children below 6 years	Cholesterol absorption inhibitor  Used occasionally in elder children, usually in combination with statins
Cholestyramin	No specific age indicated	Initial dose: (Body weight in kg × adult dose)/70. Dose can be increased		Resin. Powder to be mixed with liquid. Seldomly used today due to tolerability, efficacy and compliance issues
Cholestipol	No specific age indicated	Daily dose 0,25 - 0,5 g/kg body weight, divided in 2-4 doses		Resin. Powder to be mixed with liquid. Seldomly used today due to tolerability, efficacy and compliance issues
Colesevelam		No dosing recommendations	Safety and efficacy in children 0-17 years of age have not been documented	Resin. Tablet. Seldomly used due to tolerability, efficacy and compliance issues

Pitavastatin: Pitavastatin is not a registered drug in Norway and is not used in Norwegian pediatric patients

Treatment is initiated with a statin in a low dose. Depending on age, effect and tolerance, the dose can be up-titrated to reach treatment goals. Up-titration should not be done until at least 3 months on stable dose and good compliance. Usually only moderate doses of statins are

given, i.e. atorvastatin 20 mg, rosuvastatin 10 mg or simvastatin 20-40 mg. For children below 10 years of age, usually the lowest doses are given.

Instead of higher dose statin treatment, the cholesterol absorption inhibitor ezetimibe can be used in addition to statins, especially in older children. The dose-response curve for statins is not linear, the LDL-C lowering effect being greatest at the initial dose. Additional doubling of statin doses yields only approximately 6% additional LDL-C lowering, while 10 mg ezetimibe will lower LDL-C by an additional 15-20%, equivalent to a tripling of the statin dose.

In HoFH, due to the extremely high risk of CVD, treatment with statins and ezetimibe should be initiated at diagnosis, and LDL-apheresis should be started as early as technically and practically possible, and not later than 8 years of age (156). If there is residual LDLR activity, treatment with PCSK9 monoclonal antibodies (mAbs) can be effective in lowering LDL-C levels.

The European consensus panel recommend that liver transaminases, CK and creatinine levels should be measured before starting drug treatment and that lipid levels, liver transaminases, weight, growth, physical and sexual development should be monitored during treatment. CK levels should be measured if musculoskeletal symptoms are reported and fasting glucose and/or random glycated haemoglobin should be measured every 6 months in obese children and children with impaired glucose tolerance (145).

## **2. Aims of the thesis**

To obtain more knowledge about the safety, tolerance, efficacy and compliance of longer term cholesterol lowering therapy, mainly statins, in children and young adults with FH. Further to describe the current status of treatment and follow-up of children and young adults with FH in Norway, in order to guide future optimal care of young FH-patients.

### 2.1 Specific aims

#### Paper I

To study the efficacy, tolerability, safety and pharmacokinetics (PK) of rosuvastatin therapy over 2 years in children and adolescents with FH aged 6-17 years.

#### Paper II

In the study population in paper I, to assess the effect of 2-year treatment with rosuvastatin on cIMT, compared with untreated, unaffected siblings.

#### Paper III

To characterize the efficacy and safety of atorvastatin over 3 years and to assess the impact on growth and development in children aged 6–15 years with FH, and in an optional exploratory study to assess the impact of atorvastatin treatment on endothelial function in the brachial arteries by flow-mediated dilation (FMD).

#### Paper IV

To study long-term outcomes in young adults with FH who participated in clinical trials with lipid-lowering therapy at the Lipid Clinic, Oslo University Hospital, during childhood.

#### Paper V

To investigate if children with FH, seen at the Lipid Clinic, Oslo University Hospital in 2014, 2015 and 2016, were treated according to current recommendations.

### 3. Summary of papers

#### 3.1 Paper I

“Efficacy and safety of rosuvastatin therapy in children and adolescents with familial hypercholesterolemia: Results from the CHARON study”

The hypercholesterolemia in children and adolescents taking Rosuvastatin Open label (CHARON) study was an open label, prospective multicenter study, assessing the efficacy and safety of rosuvastatin over 2 years in children and adolescents 6-17 years of age with FH. Participants had LDL-C >4.92 mmol/L or >4.10 mmol/L with other cardiovascular risk factors (i.e. family history of premature CVD in first- or second-degree relatives). Patients were enrolled at 14 centers in the Netherlands, Canada, Belgium, Norway and the United States. The study enrolled 198 children, of which 64 was aged 6-9 years. The Lipid Clinic was the only Norwegian center and enrolled 35 participants. All subjects received rosuvastatin 5 mg daily. Based on LDL-C targets (<2.85 mmol/L), rosuvastatin could be up-titrated to 10 mg (aged 6–9 years) or 20 mg (aged 10–17 years). The intention-to-treat analysis included 197 children. The mean dose of rosuvastatin was 9.7 mg, 13.9 mg, and 14.0 mg, in the 6-9, 10-13-, and 14-17-year age groups, respectively. At 24 months, LDL-C was reduced by 43, 45, and 35% versus baseline in these age groups, respectively ( $P < .001$  for all groups). After 24 months of treatment, the percentage of patients achieving an LDL-C <2.85 mmol/L (110 mg/dL) was 38% in the 6-9 year age group, 46% in the 10-13 year age group and 28% in the 14-17 year age group. Furthermore, 64, 68, and 39%, in these age groups, respectively, achieved an LDL-C <3.36 mmol/L (130 mg/dL), and treatment adherence rates were 93, 89, and 87%.

Most AEs were mild. Intermittent myalgia was reported in 11 (6%) patients, and did not lead to discontinuation of rosuvastatin treatment. Serious AEs were reported by 9 (5%) patients, all considered unrelated to treatment by the investigators. The mean (standard deviation [SD]) z-score BMI was 0.14 (1.02) at baseline and 0.13 (1.02) at 24 months. Patients who were not already assessed as fully mature at baseline progressed in their sexual maturation during the study. There were no clinically important changes in hematology, clinical chemistry or hepatic, skeletal muscle, and renal biochemistries. Three patients had CK levels >5 x upper limit of normal (ULN), one of which had a CK level >10 x ULN; none of these patients had any associated muscle symptoms. No clinically significant abnormal findings were identified in the electrocardiogram or vital signs evaluations.

In single-dose pharmacokinetics analyses, performed in 12 patients from the youngest age group, exposure to metabolites, N-desmethyl and lactone, was lower than that to rosuvastatin, consistent with rosuvastatin being the main circulating moiety responsible for activity.

In conclusion, in patients with FH aged 6 to 17 years, rosuvastatin 5 to 20 mg significantly reduced LDL-C compared with baseline, which was sustained over 2 years. The treatment was generally well tolerated, with growth and sexual maturation remaining within normal ranges, and with no new safety signals.

### 3.2 Paper II

“Effect of Rosuvastatin on Carotid Intima-Media Thickness in Children with Heterozygous Familial Hypercholesterolemia: The CHARON Study”

The study was part of the study referred in paper I. cIMT was assessed by ultrasonography at baseline, 12 and 24 months in all patients and in age-matched unaffected and untreated siblings. cIMT was measured at 3 locations (common carotid artery, carotid bulb, internal carotid artery) in both the left and right carotid arteries. At baseline, mean ( $\pm$  SD) cIMT was significantly greater for the 195 FH children compared with the 65 unaffected siblings; 0.397 ( $\pm$ 0.049) mm and 0.377 ( $\pm$ 0.045) mm, respectively; ( $P_{\text{adjusted}}=0.001$ ). After 2 years of follow-up, the change in cIMT was lower in the FH children (0.0054 mm/year, 95% CI: 0.0030-0.0082) compared with the unaffected siblings (0.0143 mm/year, 95% CI: 0.0095-0.0192) ( $P_{\text{adjusted}}=0.002$ ). At study end, the difference in mean cIMT between FH children and their unaffected siblings was no longer significant: 0.408 ( $\pm$ 0.043) mm and 0.402 ( $\pm$ 0.042) mm, respectively; ( $P_{\text{adjusted}}=0.2$ ).

In conclusion, rosuvastatin treatment for 2 years resulted in significantly less progression of cIMT in FH children than in untreated, unaffected siblings, resulting in no difference in cIMT between the two groups after 2 years.

### 3.3 Paper III

“A 3-year study of atorvastatin in children and adolescents with heterozygous familial hypercholesterolemia”

This was an open-label, prospective, multicenter study to assess the efficacy and safety of atorvastatin over 3 years in children with FH, aged 6–15 years. It was conducted at 30 centers in Belgium, Canada, Germany, Greece, Hungary, Italy, Norway, Poland, Russian Federation, Slovakia, Spain, Switzerland, Turkey, and the US. A total of 272 subjects with genetically confirmed FH, and LDL-C  $\geq 4.0$  mmol/L were enrolled, 271 subjects were treated, of which 107 (39%) were aged 6-9 years. The Lipid Clinic was the only Norwegian center and enrolled 25 participants. Subjects were initiated on atorvastatin (5 mg or 10 mg), with doses increased to up to 80 mg based on LDL-C levels. At 36 months/early termination mean percentage reductions from baseline in LDL-C were 43.8% for subjects at Tanner stage (TS) I and 39.9% for TS  $\geq$ II. There was no evidence of variations in the lipid-lowering efficacy of atorvastatin between the TS groups (I vs  $\geq$ II), or in subjects aged  $<10$  vs  $\geq 10$  years (figure 2 in the paper). A total of 52% of subjects in both age groups attained an LDL-C of  $<3.35$  at month 2-3, and this goal attainment remained above 50% for the duration of the study. The mean weighted doses of atorvastatin (21.4 and 23.0 mg) and mean maximum doses (28.5 and 29.5 mg) were similar in the subjects at TS I and  $\geq$ II. Few subjects (18, 6.6%) received atorvastatin 80 mg during the study.

The mean height of both male and female subjects was above the 0 z-score lines on the gender-specific WHO height for age charts. The mean height in males followed the 1 z-score line. In both males and females, the mean increase in height over the 3-year study followed the same trajectory as the WHO reference group. The TS shift from baseline during the 3-year trial was consistent with the normal trajectory for maturation and development (figure 1 in the paper).

The overall incidence of all-causality AEs was similar in the TS I and TS  $\geq$ II groups. Overall, 6 (2.2%) subjects discontinued because of AEs. A total of 24 (8.9%) subjects had their dose reduced or temporarily discontinued because of treatment-related AEs. All the treatment-related AEs were mild or moderate in intensity. There were no obvious trends, or dose-related trends, in the incidence of laboratory abnormalities. No subjects had AST or ALT levels  $>3$  x ULN. Overall, 23 subjects (8.6%) had CK levels  $>2$  x ULN. The incidence of this abnormality was much greater in the TS  $\geq$  II (17; 12.9%) than in the TS I group (6; 4.4%). One subject, a 14-year old male, was reported with increased CK ( $>10$  x ULN) as a serious AE. This was thought to be related to hard physical exercise.



A total of 73 subjects from four centers were included in the FMD substudy. The Lipid Clinic did not participate in this part of the study. The FMD profile showed no discernable trends in either male or female subjects with the mean percentage dilation exhibiting little change over the duration of the study.

In conclusion, atorvastatin over 3 years was efficacious, had no impact on growth/maturation, and was well tolerated in children and adolescents with FH aged 6–15 years.

### 3.4 Paper IV

“Long-term follow-up of young adults with familial hypercholesterolemia after participation in clinical trials during childhood”

Participants in 6 pediatric clinical trials that took place at the Lipid Clinic between 1999 and 2008 were interviewed in 2011 or in 2013/2014. Frequency of medical consultations, use of lipid-lowering therapy (LLT), lipid levels, side effects, diet, tobacco use, and emotional issues were investigated, using information from interviews, blood samples and medical records. Of the 118 individuals who participated in the trials, 67 (57%) were included. Median age was 25 years, and median time before follow-up in our study was 10 years. Forty-eight (72%) participants were using statins at follow-up, 8 (12%) were also using ezetimibe. Nineteen participants (28%) were not using any LLT. Mean LDL-C was 3.68 mmol/L in statin users and 6.08 mmol/L in non-users ( $P < 0.001$ ). Only 6 (9%) participants reached a treatment goal of LDL-C  $\leq 2.5$  mmol/L. Participants who attended a consultation  $\leq 2$  years before follow-up had significantly lower LDL-C compared with those who had a consultation  $> 2$  years before follow-up (4.10 and 5.17 mmol/L, respectively;  $P = 0.02$ ). Statin users had their last consultation more recently than non-users (median 1.4 and 2.2 years, respectively;  $P = 0.02$ ). Out of 65 individuals 24 (37%) had experienced side effects of the LLT, but few, 3 participants (5%), had experienced severe side effects, and 7 (11%) had discontinued LLT permanently due to side effects. The most prevalent side effects reported were gastrointestinal complaints (23%), followed by muscle and joint pain (14%) and headache (12%). There were no clinically relevant deviations in ALT, AST, CK, gamma-glutamyl transferase, glucose and glycated hemoglobin.

In conclusion, statins were underused and most patients had not reached treatment goal in this young adult population with FH. Those with recent consultations had lower LDL-C levels and were more often statin users.

### 3.5 Paper V

“Treatment goal attainment in children with familial hypercholesterolemia: A cohort study of 302 children in Norway”

Data were collected retrospectively to a treatment quality-register, from medical records of children below 18 years with a diagnosis of FH, visiting the Lipid Clinic, Oslo University hospital, during 2014-2016. To obtain treatment data, only children with at least one prior visit to the clinic were included. In 99% of the children, the diagnosis of FH was genetically verified. Mean age (SD) at diagnosis was 8.5 (3.2) years. Age at first and last visit was 9.5 (2.9) and 13.9 (2.7) years, respectively, and time followed at the clinic was 4.4 (2.7) years.

Mean pretreatment LDL-C was 5.4 (1.4) mmol/L Mean age at start of LLT was 12.5 (2.0) years, with no significant difference between girls and boys, although among children below 12 years of age, a significantly higher number of boys than girls were on LLT (17 boys and 3 girls [10% vs. 2%,  $P=0.004$ ]), despite the fact that there were no difference in pretreatment LDL-C levels in boys and girls below 12 years of age (5.1 vs. 5.4 mmol/L,  $P=0.264$ ). Only one of the treated children (a boy) was below 10 years of age. LLT was used by 177 (59%) children at their last visit, 176 were treated with statins, one child was treated with ezetimibe in monotherapy and five children used ezetimibe in addition to a statin. LDL-C in children treated with LLT was 3.6 (1.2) mmol/L (38% reduction,  $P<0.001$ ). A treatment goal of LDL-C  $\leq 3.5$  mmol/L was achieved by 43% of all children, by 58% of the children on LLT, by 71% of children on stable LLT and by 22% of children not on LLT.

In conclusion, in this cohort of 302 children with FH, the mean age of 12.5 years at initiation of LLT were above the recommend 10 years of age, and many children did not achieve the LDL-C treatment goal, even with follow-up at a dedicated lipid clinic.

An overview of the papers is given in table 3.

Table 3. Overview of papers

Study name	Year	Population	Study drug	Objective, design and duration	Outcome measures	Important findings	Paper
Efficacy and safety of rosuvastatin therapy in children and adolescents with familial hypercholesterolemia: Results from the CHARON study	2015	Children with FH Age 6-17 years (n=197, 35 at the Lipid Clinic)	Rosuvastatin 5-20 mg	Efficacy and safety. Multicenter, open label. Duration: two years	LDL-C and other lipoproteins, AEs, other lab parameters, growth, sexual maturation. Adherence to therapy	Rosuvastatin significantly reduced LDL-C. The treatment was generally well tolerated. Growth and sexual maturation remaining within normal ranges. No new safety signals	I
Effect of Rosuvastatin Therapy on Carotid Intima-Media Thickness in Children with Heterozygous Familial Hypercholesterolemia: The CHARON Study	2017	Children with FH Age 6-17 years (n=197, 35 at the Lipid Clinic). Unaffected, untreated siblings (n=65)	Rosuvastatin 5-20 mg	Part of study in paper I. Carotid IMT assessed by ultrasonography at baseline, 12 and 24 months in patients and age-matched unaffected, untreated siblings	Differences in cIMT between FH children and the unaffected siblings	At baseline cIMT significantly greater in FH children than in unaffected siblings. Two years of rosuvastatin treatment resulted in significantly less progression of cIMT in FH children compared with siblings. At study end no difference in cIMT between the two groups	II
A 3-year study of atorvastatin in children and adolescents with heterozygous familial hypercholesterolemia	2016	Children with FH Age 6-15 years (n=272, 25 at the Lipid Clinic)	Atorvastatin 5-80 mg	Efficacy and safety. Multicenter, open-label. Duration: three years	LDL-C and other lipoproteins, AEs, other lab parameters, growth, sexual maturation	Atorvastatin treatment was efficacious, had no impact on growth/maturation, and was well tolerated.	III
Long-term follow-up of young adults with familial hypercholesterolemia after participation in clinical trials during childhood	2015	Young adults with FH Age 18-30 years (n=67)	n/a	Long-term outcomes in young adult FH patients. Single center, Lipid Clinic. Interview and clinical examination. Mean 10 years follow-up	Frequency of medical consultations, use of lipid-lowering therapy, lipid levels and goal attainment, side effects, diet, tobacco use, and emotional issues	Statins underused. Most patients had not reached treatment goal. Those with recent consultations had lower LDL-C levels and were more often statin users	IV
Treatment goal attainment in children with familial hypercholesterolemia: A cohort study of 302 children in Norway	2017	Children below 18 years with FH visiting the Lipid Clinic, Oslo University hospital, during 2014-2016, with at least one prior visit to the clinic (n=302)	n/a	To characterize the lipid profile, treatment status and follow-up in a cohort of children with FH Single center, registry study. Mean follow-up 4.4 years	Use of lipid lowering therapy, diet, lipid levels, LDL-C goal attainment	Mean age 12.5 years at initiation of LLT above the recommend 10 years of age. Many children did not achieve the LDL-C treatment goal, even with follow-up at a dedicated lipid clinic	V

## 4. Discussion

### 4.1 Efficacy and safety of treatment

Efficacy and safety of rosuvastatin and atorvastatin treatment in children younger than 10 years had not previously been studied, nor had more than one year use of these drugs in children with FH been assessed in studies. Because shorter term, randomized, placebo-controlled studies with rosuvastatin and atorvastatin had been carried out previously, and due to the possible beneficial effects of early statin initiation in children, it was considered unethical to do placebo-controlled studies over 2-3 years in this high-risk population. The studies reported in paper I and paper III were therefore open-label studies.

#### 4.1.1 Efficacy of treatment on lipid levels

In previous studies, LDL-C was reduced by around 40% with submaximal doses of simvastatin and atorvastatin, and up to 50% with 20 mg rosuvastatin (119, 120, 122), i.e. that statin treatment in children is as effective as in adults (157). The LDL-C reduction with rosuvastatin and atorvastatin at around 40% in our studies is consistent with the results in these previous studies. In both the 2-year study with rosuvastatin (paper I) and the 3-year study with atorvastatin (paper III), efficacy was maintained throughout the study, and the treatment was as efficacious in those below 10 years of age, as in those above this age. The small increase in LDL-C at the end of study in the atorvastatin study (paper I) may have been due to the intention to treat analysis, with inclusion of a higher number of subjects off study medication.

Remarkably, in both studies (paper I and paper III), baseline LDL-C (6.14 versus 6.15 mmol/L) and LDL-C at study end (3.50 versus 3.47 mmol/L) were almost identical. Treatment goal attainment at study end was relatively high in both studies. In the rosuvastatin study, a goal of LDL-C <3.36 mmol/L was achieved by 39-68% of participants in the different age groups, and in the atorvastatin study more than 50% of participants achieved a treatment goal of LDL-C <3.35 mmol/L.

The findings reported in paper I and III compares well with the findings in our cohort study of children with FH at the Lipid Clinic (paper V). In this cohort study LDL-C was reduced by 38% (from 5.8 to 3.6 mmol/L) among the children on LLT. A treatment goal of LDL-C  $\leq$ 3.5

mmol/L was achieved by 58% of all children on LLT and by 71% of those on stable LLT (dose not increased at last visit).

Also, our results in paper V compares well with recently published data on 232 children from a pediatric FH register in the UK. In the UK cohort, untreated mean LDL-C was 5.67 mmol/L, 48% were on statins, and in the statin-treated children LDL-C was reduced by 35%. None of those on statins had measured plasma levels of CK, ALT and AST indicative of statin toxicity (158).

However, outside a setting of a clinical trial with motivated participants and frequent visits, or frequent follow-up of pediatric patients in a specialized lipid clinic, treatment adherence and goal attainment is much lower. In our long-term follow-up study of young adults (mean age 25 years [paper IV]) only 6 out of all 67 participants (9%) reached a treatment goal of LDL-C  $\leq 2.5$  mmol/L. A less stringent goal of LDL-C  $\leq 2.9$  mmol/L (within 15% measurement variation of 2.5 mmol) was reached by 15 out of the 48 patients on LLT (31%), a level that would not automatically lead to an increase in LLT, and these patients could be classified as receiving sufficient or close to “optimal” treatment. Mean LDL-C level among users of LLT in this study was 3.68 mmol/L, comparable to the mean level of 3.85 mmol/L among adherent statin-users in a similar long-term follow-up study from the Netherlands (159). Out of 48 participants on LLT in our paper IV, 29 (60%) were on intensive LLT (20 or 40 mg rosuvastatin or 40 or 80 mg atorvastatin, of which 7 were on additional ezetimibe), leaving room for amelioration in this young adult high-risk population.

#### 4.1.2 Efficacy of treatment on cIMT and FMD

The ultimate aim of statin treatment initiated at a young age is to reduce atherosclerosis and subsequent cardiovascular events later in life. Surrogate endpoints, e.g. cIMT in the rosuvastatin study (paper II) and change in FMD in the atorvastatin study (paper III), were therefore included in an attempt to support a putative beneficial effect on atherosclerosis of the statin treatment.

Kusters et al. reported baseline cIMT findings in the study population in paper I and II (196 children with FH and 64 non-affected siblings) (160). Mean cIMT in the children with FH was greater than that of unaffected siblings before the age of 8 years. Multivariable analyses showed that age, male sex, and presence of FH were independent predictors of cIMT.

Interestingly, after rosuvastatin treatment for 2 years, there was significantly less progression of cIMT in the children with FH, resulting in no difference in cIMT between the children with FH and their untreated, unaffected siblings at study end (paper II), supporting the value of early initiation of statin treatment in children with FH.

This normalization of cIMT thickness contrasts to some degree with the findings in a similar study from the Netherlands with pravastatin, also with a control group of untreated healthy siblings. After an initial 2-year randomized placebo-controlled treatment period, the cohort was followed-up at 4 ½ and 10 years. Pravastatin treatment, initiated down to 8 years of age, was associated with reduced progression of cIMT after 4 ½ and 10 years. After 10 years, the increase in cIMT was at the same level in the statin treated individuals with FH as in unaffected siblings, but cIMT was still higher in those with FH. A younger age at start of statin treatment was associated with lower cIMT after 10 years. The difference in the cIMT findings in the two studies could be explained by the fact that in our study, treatment was initiated down to 6 years of age and LDL-C was reduced to a larger degree, from 6.15 to 3.47 mmol/L (40% reduction), compared with an LDL-C reduction from 6.2 to 4.7 mmol/L (24% reduction) in the 2-year pravastatin study.

In adults an LDL-C level below 1.8 mmol/L has been associated with reduction of atherosclerosis assessed by intravascular ultrasound of the coronary arteries (161, 162). One could speculate that, at an early age and early stage of lipid deposition/cIMT thickening, the atherosclerotic process is more reversible, and that lowering of LDL-C, even to higher levels than those associated with regression in adults, might result in normalization of increased cIMT. Indeed, the normalization of cIMT after 2 years of rosuvastatin treatment in our study is a strong argument for initiating statin therapy at a young age, and for lowering of LDL-C levels to well below 4 mmol/L. It would be of great interest to follow our study participants, including the unaffected siblings, with future cIMT and lipid measurements to see if there will be continued normalization of cIMT in affected, as compared with unaffected individuals.

Importantly, in the Netherlands cohort, followed-up after 10 years, no one had had cardiovascular disease before 30 years of age, unlike their parents in whom 7% had had a cardiovascular event at the same age (163). This is similar to the finding in our paper IV, with approximately 10 year of follow-up after initiation of statin therapy, where no participants had cardiovascular disease.

The cIMT findings in paper II contrasts with the results of the FMD measurements in paper III, which showed no discernable trends in %FMD in either male or female subjects, possibly due to issues both in the methodology and the study design. Only a small number of patients took part in this substudy and the methodology used varied between the four centers, probably leading to inconsistent results. Also, in earlier pediatric studies with FMD measurements, there has been conflicting results, and FMD, compared with cIMT, is considered to be a more indirect marker of atherosclerosis (133).

#### 4.1.3 Safety of treatment

In paper I and III, rosuvastatin and atorvastatin therapy over 2 and 3 years, respectively, did not seem to affect sexual maturation, as judged by Tanner staging. Growth was also considered normal in both girls and boys according to z-score and WHO age-adjusted height curves in both studies. Treatment related adverse events were of mild or moderate intensity in both studies. Nor were there any clinically important changes in hematology, liver transaminases, serum glucose, skeletal muscle and renal biochemistries in either study, and no clinically significant abnormal findings were identified in the electrocardiogram or vital signs evaluations. These were prospective, clinical trials, however, with selected, motivated and closely monitored participants.

Our “Long-term follow-up of young adults with familial hypercholesterolemia after participation in clinical trials during childhood” (paper IV) is a cohort study. Experience shows that this type of studies captures other side effects than the prospective trials. In our study, although with only 67 participants, 3 participants (5%), had experienced severe side effects and 7 (11%) had discontinued lipid-lowering therapy permanently due to side effects. These results are in line with findings in cohorts of adult statin-treated patients (164, 165), but contrasts with the discontinuation rates due to adverse effects of 1.5% and 2.2%, respectively, in paper I and paper III.

Safety and efficacy of statin treatment in children younger than 8 years of age had not previously been assessed. Our studies with atorvastatin and rosuvastatin, although being open-label, adds to the knowledge about early initiation of statin therapy in children. The studies had a substantial number of participants below 10 years of age. Presumably, most of these children will continue statin treatment after study end, and it will be of importance to

follow these children into adulthood to capture possible beneficial or adverse effects of initiation of therapy before 10 years of age. This has been done by investigators in the Netherlands. Ten years after initiation of pravastatin treatment in children down to 8 years of age, there was no difference in age at menarche between 194 statin treated FH-children and a control group of 83 siblings (13.1 years versus 13.4 years). Laboratory safety parameters did not differ between individuals with FH and siblings, and growth and educational level did not differ (159, 163).

An increased frequency of diabetes associated with high-dose statin therapy in adults, especially in those with impaired fasting glucose levels, was revealed in the Jupiter study in 2008 (166), some 20 years after the first marketing of statins, a finding that has later been confirmed in meta-analyses (167, 168). Recent Mendelian randomisation studies indicate that the risk of diabetes rather may be linked to LDLR levels. The same level of increased diabetes risk is found in subjects with LDL-C lowering genetic variants in the genes encoding HMGCR and PCSK9, both ultimately acting through upregulation of *LDLR* (169, 170). This theory is supported by findings in the genetically diagnosed cohort of FH subjects in the Netherlands, where odds ratio for being diagnosed with diabetes in the FH cohort was 0.49 compared with unaffected relatives (171). A possible mechanism of action may be pancreatic  $\beta$  cell *LDLR* upregulation, resulting in increased lipid accumulation and dysfunction in the  $\beta$  cells.

Concerns about increased risk of cancer and memory loss due to statin therapy have not been substantiated, however, after nearly 30 years of statin use (71, 112).

Cholesterol is an essential constituent of cell membranes and of myelin surrounding the axons of nerve cells. It is also a precursor of steroid hormones and bile acids. Intracellular cholesterol levels are under strict homeostatic regulation; low intracellular cholesterol increases the transcription of most genes in the cholesterol synthetic pathway, and up-regulates *LDLR*, mediated by the SREBP, resulting in increased cholesterol synthesis in cells and increased uptake of cholesterol from the plasma, thereby maintaining intracellular cholesterol levels (109).

Lipoprotein metabolism in the brain is less well understood than in plasma and tissues other than brain. The blood-brain barrier prevents uptake of LDL from the plasma. The brain is therefore dependent on cholesterol synthesized locally, probably mainly in astrocytes and



neurons (172). It has been shown that statin lactones and acids pass the blood-brain barrier, but little is known about statin metabolism and effects in the brain (173). Concerns have been expressed that statin therapy in children may adversely affect cholesterol metabolism in brain cells, potentially harming the developing brain and neural and cognitive function, even though no such effects of statin therapy have been reported to date (174, 175).

The Smith-Lemli-Opitz syndrome is a rare inborn error of metabolism. It is an autosomal recessive condition caused by defects in the gene encoding 7-dehydrocholesterol reductase (DHCR7), the enzyme that reduces 7-dehydrocholesterol to cholesterol, leading to low intracellular cholesterol levels and abnormal accumulation of 7-dehydrocholesterol, which possibly could have toxic effects. The phenotype varies greatly from being a lethal disorder with multiple congenital anomalies to minor physical abnormalities and lesser cognitive and behavioral dysfunctions. Standard treatment is dietary cholesterol supplementation, but the effect is limited by the inability of cholesterol to cross the blood-brain barrier. Apparently, paradoxically, simvastatin therapy in a randomized placebo-controlled crossover study over 24 months, seemed to be safe in patients with the Smith-Lemli-Opitz syndrome (22 patients aged 4 to 18 years), improving irritability symptoms and dehydrocholesterol to sterol ratio. The mechanism of action probably being upregulation of *DHCR7* via SREBP in response to low intracellular cholesterol, increasing *DHCR7* enzyme levels with residual function (176).

There is a paucity of evidence that starting lifelong statin treatment in childhood is safe, and the need for more documentation is large. Data from randomized placebo-controlled studies with statins in children are limited to 2 years of exposure. Due to a relatively small number of participants included, these studies may be considered underpowered to detect a significant difference in safety parameters, or side effects that may occur after prolonged use. Also, these studies do not provide data on longer term safety, especially on growth, development and cognitive functions, or evidence that initiating statin treatment at an early age will prevent or diminish cardiovascular events in adult age, as compared with initiating therapy in young adult age. Starting statin therapy in childhood usually will imply a longer exposure to treatment and a greater cumulative dose, compared with treatment started in adult life, with the possibility of adverse effects becoming apparent only after a long exposure to treatment. For these reasons there is professional skepticism to early statin initiation in children (177), and it is not difficult to understand that many parents may be hesitant to expose their children to statin treatment from a young age. However, no studies in children with FH, randomized,

open-label or cohort studies have concluded that statin treatment has impacted hormone levels, growth or sexual maturation in a clinically relevant manner, neither has other serious side effects been shown.

The Cochrane investigators, in their systematic review of randomized controlled statin studies in children with FH, conclude that: “Large long-term randomized controlled trials are needed to establish the long-term safety issues of statins” (128). However, in a later paper the same first author (Vuorio) states: “To determine whether statins are sufficiently safe as a long-term therapy option when initiated in childhood, would require large and long-term randomized controlled multicenter trials with thousands of FH-children, and of at least 10 years of follow-up. We accept that this is unlikely to be attractive to the pharmaceutical industry or to government funding agencies”. It could be added that in view of the totality of evidence on the effects of elevated cholesterol and statin treatment, such studies would probably also be considered unethical to perform.

#### 4.2 Adherence to treatment

Also after statins became standard treatment, even with their ease of use and relatively low incidence of side effects, as compared with resins, adherence continues to be an important issue, in children as well as in adults.

Interestingly, Buchwald in his paper from 1970, on treating children with FH with partial ileal bypass, notes:

*“...it hardly needs to be pointed out to clinicians that asymptomatic patients on drug therapy, where immediate benefit of the drug therapy cannot be appreciated by the patient, will all too commonly deviate from their drug program or completely omit their medication.”*

Further on he says:

*“True heterozygous and homozygous familial hypercholesterolemia .....may actually present with clinical manifestations during the early years of life. This is a pediatric problem that needs to be dealt with in childhood and not a problem whose management should be deferred until the manifest onset of the complications of atherosclerotic cardiovascular disease. We should, if at all possible, treat these children prophylactically before they become*

*incapacitated and unable to pursue the normal activities of childhood and fall into a group with a statistically predictable and marked shortening of life expectancy.”*

In the rosuvastatin study (paper I), 92% of the enrolled participants completed the study. Adherence to treatment, defined as taking 80-120% of the prescribed study medication, was good, at around 90%. In the atorvastatin study (paper III) 76% of the subjects allocated to treatment completed the study. Among the subjects in Tanner stage 1, the most frequent cause of discontinuation was no longer being willing to participate, whereas for subjects at  $TS \geq 2$ , the most common reason for discontinuation was “low LDL-C” (defined as  $LDL-C < 2.59$  mmol/L) in subjects receiving atorvastatin 5 mg, which was a protocol requirement. Adherence rates for those treated were not given in paper III.

In our cohort of children with FH (paper V) specific data on adherence were not included, but based on LDL-C levels before and after initiation of LLT only 4 out of 177 (2.3%) of the children treated with LLT were found to be highly non-adherent to therapy. On the contrary, our long term follow-up study (paper IV) revealed a worryingly high number of non-adherent individuals; 16 out of 64 (25%) participants who should have used statins, were non-users. The main reasons for not using drugs were side effects and poor routines, i.e. running out of prescription, not remembering to take the drugs or not understanding the importance of taking drugs. Even though side effects were reported by 37% of the 65 participants having ever used lipid lowering drugs, only 5% (3 participants) reported severe side effects. However, 19 out of 65 (29%) reported side effects that resulted in temporary or permanent discontinuation of LLT, of which 7 (11%) had permanently discontinued LLT due to side effects.

The findings in our paper IV are in accordance with findings in other registry and observational studies in adult patients. Prevalence of statin-associated muscle symptoms in such studies has been reported in 7–29% of patients (164) and such complaints are a major reason why many patients stop taking statins (165). This contrasts with findings in randomized, controlled trials where complaints of muscle pain (and other adverse event rates) are similar in statin and placebo groups (166, 178). However, a study designed to capture the effects of statins on skeletal muscle function was published in 2013. In this study, 420 healthy, statin-naïve subjects were randomized to 80 mg atorvastatin or placebo. During 6 months of treatment 9.4% of the statin-treated and 4.6% of control subjects met the study definition of myalgia ( $P = 0.05$ ) and average creatine kinase increased by 20.8 U/L ( $P < 0.0001$ ) with atorvastatin, indicating minor muscular injury (179).

An important finding in our long term follow-up study (paper IV) was that statin users had been to their last consultation more recently than non-users (median 1.4 and 2.2 years, respectively;  $P=0.02$ ). It is our clinical experience that many patients who experience side effects have not systematically tested their statin tolerance or tried low-dose treatment. These patients need frequent consultations with discussion and advice, reassurance and guidance about the importance of treatment and adequate testing of alternative drug regimens.

In patients considered statin intolerant, more than 50% were nevertheless able to tolerate low-dose or intermittent dose statin on rechallenge in randomized, double-blinded studies (180, 181). Due to the complexity in establishing the diagnosis statin intolerance, and the risk of overdiagnosis, it is recommended that high-risk statin intolerant patients are referred to a specialist centre with experience in treating these patients (182).

In a master thesis at the University of Oslo, 11 of those 51 individuals not reached in our long-term follow-up study (paper IV), were in-depth interviewed about their thoughts of living with hypercholesterolemia and how they dealt with their condition. Those interviewed were between 26 and 35 years of age and had not visited the Lipid Clinic within median 10 years (range 3-16 years). Out of 8 individuals having an established diagnosis of FH, 4 did not use their prescribed lipid-lowering medication on a regular basis. In “Grounded theory” terms the author revealed that their strategy for living with hypercholesterolemia, could be described by the core category: “Postponing the thoughts of consequences”, with three supporting subcategories: “Normalizing the condition”, “Belittling of treatment” and “The need for autonomy”(183). FH-patients may underestimate their risk of having CVD (184). Also, younger, as compared with elder FH-patients tend to have a lower perceived risk of CVD (185, 186).

In several studies on different chronic conditions, more than 40% of patients have been found to be non-adherent to medical advice on therapy for their condition (187). Adherence to life-style regimens may be even lower, with as much as 70% of patients being non-compliant (184, 187). Non-adherence may be due to misunderstanding or misinterpreting, forgetting, ignoring or denying healthcare advice. In addition to clear communication, a good relationship with the patient, good knowledge and understanding of the patients concerns, and trust between the patient and the health care provider, are key factors in improving patient adherence (187).

### 4.3 New lipid lowering therapies

New lipid lowering therapies include PCSK9 mAbs, cholesteryl ester transfer protein (CETP) inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors and apoB messenger ribonucleic acid (mRNA)3500 antisense therapy. Of these only PSCK9 mAbs are in the process of being tested in children with FH.

#### Inhibition of PCSK9

The discovery of PCSK9 in 2003 led to the development of therapeutic mAbs that specifically bind to circulating PCSK9, neutralizing the protein and thereby inhibiting degradation of the LDLR (188). In 2015, two fully human mAbs targeting PCSK9, alirocumab and evolocumab, were approved in the US and Europe. The drugs are given as subcutaneous injections twice a month or monthly.

Treatment with alirocumab and evolocumab are highly effective, reducing LDL-C by an average of 50–60%, (189, 190). So far, in clinical trials, injection site reactions, mostly mild, have been the only side effect occurring with an increased frequency by less than 5 % in those receiving active drug, compared with placebo (191, 192). Concerns have been expressed about possible adverse effects of very low LDL-C levels with regard to neurocognitive function, hormone synthesis and fat-soluble vitamin levels. No such effects have so far been demonstrated, however. Also, little is known about the long-term effects of PCSK9-inhibition, one concern being development of anti-drug antibodies, which could cause injection site reactions, allergic reactions and reduced efficacy of the drug.

In the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial 27 500 patients with atherosclerotic CVD was treated with evolocumab or placebo, on a background of statins. Evolocumab treatment reduced the primary composite CVD endpoint by 1.5% (15% relative risk reduction) over 2.2 years (192). The results are in accordance with results in previous trials with LDL-C lowering therapy. In a substudy on neurocognitive function in approximately 2000 individuals, no adverse effect of evolocumab on neurocognitive function was found (193). Results from the cardiovascular endpoint trial with alirocumab (ODYSSEY OUTCOMES) are expected in 2018 (194).

PCSK9 mAbs are indicated as add-on therapy for those high-risk patients who do not achieve treatment goals with statins and ezetimibe, and for those, maybe 5–10% of patients, who cannot tolerate statins.

PCSK9 mAbs are not approved for treatment of children, except for evolocumab which is approved for use in children above 12 years of age with HoFH, based on results from a phase III study in HoFH patients, including children (195). Evolocumab is further evaluated in an ongoing open-label study including 14 patients below 14 years with HoFH. (196).

Clinical trials are ongoing with evolocumab as add-on to statin therapy in children with FH aged 10-17 years and with alirocumab in children aged 8-17 years.

Other approaches to inhibiting PCSK9 are in pre-clinical, Phase I and Phase II development, such as PCSK9-binding peptides and small proteins (adnectins) as well as small interfering RNA (siRNA), interfering with PCSK9 mRNA and inhibiting translation (197, 198) Also, virus-like particle-based vaccines, targeting PCSK9 have been tested in animals, inducing development of IgG antibodies which binds to circulating PCSK9 (199).

Aggressive LLT, including PCSK9 inhibition, to achieve very low LDL-C levels has been proposed to prevent or regress atherosclerosis, allowing for intermittent treatment over the lifespan of a patient, as is the case for women during pregnancies, or also in other settings where treatment could be unavailable or difficult to obtain (200).

## CETP inhibitors

CETP is a plasma glycoprotein which is produced in the liver and in adipose tissue. It exchanges cholesterol ester in HDL against triglycerides in VLDL and LDL particles. CETP inhibitors block this transfer, resulting in a large increase in HDL-C and a more modest lowering of LDL-C. Four CETP inhibitors have been in phase III development. Of these torcetrapib, dalcetrapib and evacetrapib have been discontinued, either because of toxicity and off-target effects (torcetrapib), or lack of effect on clinical endpoints (dalcetrapib and evacetrapib) (201-203). Recently, the remaining drug, anacetrapib, was shown to reduce major coronary events by 1% (relative risk reduction 9%) in adult high-risk hypercholesterolemic patients on a background of high-dose atorvastatin therapy in the Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification (REVEAL)

trial (204). However, anacetrapib accumulates in body fat. The levels of the drug in adipose tissue increases during treatment, and the drug can be detected in blood many years after discontinuation. Shortly after REVEAL results were presented, Merck announced that it would not seek regulatory approval for anacetrapib, probably due both to the modest effect on coronary events, and the unknown long-term effects of the accumulated drug, including on fertility and fetal development.

### MTP inhibitors

MTP is responsible for transferring triglycerides onto apoB100 within the liver in the assembly of very-low-density lipoprotein (VLDL), the precursor to LDL. In the intestine MTP transfer triglycerides onto apoB48 in the assembly of chylomicrons. Thus, the pharmacologic inhibition of MTP might be a strategy for reducing LDL production and plasma LDL-C levels. The MTP inhibitor lomitapide reduces LDL-C up to 40% depending on dose. The main side effects are diarrhea, fatty liver and elevated liver transaminases. To avoid diarrhea and steatorrhea, dietary fat must be kept below 10%, which can be limiting for patients. The drug is approved by the Food and Drug Administration (FDA) in the US and by the European Medicines Agency (EMA) for adjunctive treatment of adults with HoFH. In selected children with HoFH, lomitapide can be an optional, off-label, adjunctive therapy. The additional LDL-C lowering could enable longer intervals between LDL apheresis, a procedure which is time-consuming and burdensome for patients (205, 206).

### Mipomersen

Mipomersen is an antisense oligonucleotide which binds to mRNA for apoB100, thereby inhibiting the formation of apoB100. ApoB100 is a surface constituent of the lipoproteins LDL, VLDL and Lipoprotein (a). Treatment with mipomersen causes a dose dependent reduction of circulating apoB100 containing lipoproteins. The drug is given as subcutaneous injections. It is approved by FDA for the treatment of adult patients with HoFH. It is not approved by EMA due to the unfavorable safety profile (elevated liver enzymes, flu-like symptoms and injection site reactions).

## Thyroid hormone receptor agonists

Thyroid hormone receptor agonists, acting selectively through the thyroid receptor  $\beta$  in the liver, can reduce LDL-C by both LDLR dependent and independent mechanisms, and has also the potential to reduce the atherogenic lipoprotein (a), without the systemic actions mediated through the major systemic thyroid receptor, thyroid hormone receptor- $\alpha$ . The thyroid hormone receptor agonist eprotirome was discontinued from phase III development due to joint cartilage toxicity in dogs (207). Another thyroid hormone receptor agonist, MGL-3196, is currently in phase II development and tested in adult FH patients. Future possible use of thyroid hormone receptor agonists in children and young adults will probably be as a fourth line drug in a small number of patients, after statins, ezetimibe and PCSK9 mAbs.

### 4.4 Future challenges

FH is the most prevalent monogenetic disorder. In most cases FH is fully curable if diagnosed and treated early. The most important issue in FH is therefore to diagnose and start treatment early in life. Treatment, primarily with cholesterol lowering diet, statins and ezetimibe is basically simple and effective, and will probably prevent most of the cardiovascular disease seen in FH-populations to date (71, 208). Obviously, the earlier the condition is identified, the earlier treatment can be instituted and the better the chances for prevention of cardiovascular disease and complications.

But, also in those diagnosed and initiated on LLT, the drugs are underused. Treatment goals are reached in only 20-30% of adult patients (209-211). Cardiovascular, and especially CHD, morbidity and mortality, although lower than in the pre-statin era is still increased 2-3 fold and up to 10-fold in registry and general population cohorts, with no clear differences between men and women (57, 59, 61), and patients with FH having acute coronary syndromes have a >2-fold risk of coronary event recurrence within the first year after discharge, as compared with patients without FH (212).

#### 4.4.1 Diagnosis – How to find new probands?

Adult probands are often diagnosed as part of opportunistic cholesterol screening, or when heart disease occurs at a relatively young age, but regrettably too late to have prevented the



atherosclerotic disease. In Norway, the mean age at genetic diagnosis of FH is 33.7 (SD 19.0) years (213). However, age at genetic diagnosis was much higher, 57.0 years, for those who died during a registration period from 1992 to 2013 (213), underscoring the need for early diagnosis to prevent early death. When a new proband is found, family cascade genetic screening is the logical next step, as there will be a 50% probability for a first degree relative to inherit the condition. The Netherlands and Norway are world leaders in genetic family cascade screening, having practiced this since the 1990s (42). As per December 2017, approximately 7800 individuals have been genetically diagnosed with FH in Norway, of which approximately 10% are children. Almost all children diagnosed with FH are being diagnosed as part of family cascade screening. Although effective in finding affected family members, and successful in finding more FH-patients than in countries with less intensive screening activities, this approach has not succeeded in finding more than approximately 1/2 to 1/3 of the estimated 15-25 000 affected individuals in the Norwegian population.

Therefore, to find more probands, a more universal approach to screening seems warranted (214). FH fulfils most of the WHO disease criteria for screening, and universal screening for FH in childhood has been proposed by expert panels and boards in Europe and the US (143, 180, 215). There are several advantages with screening in childhood, compared with universal screening of the adult population. In childhood, and especially between ages 1 and 10, the difference in cholesterol levels between FH and non-FH individuals is greater than later in life, minimizing the number of “false positives” and “false negatives” when determining the cut-off levels for cholesterol. Also, when a child with FH is identified, cascade screening can be undertaken in the family, and for each child diagnosed, at least one adult relative could be identified (216).

The objections to universal screening have been lack of proof of cost-effectiveness, and that general pediatric population lipid screening will identify a large number of individuals with intermediate lipid values, with a potential need for retesting and specialist evaluation. The effect of intervention in children with borderline or intermediate lipid levels, without FH, is uncertain. Emotionally, for the affected families, a message that their child has a borderline lipid level with no clear diagnosis or treatment option may possibly cause more anxiety than a diagnosis of FH, where effective treatment is available. On the contrary, these children and their families will most probably benefit from general preventive lifestyle and diet advice.

Until recently, universal screening programs have not been tested or evaluated. However, since 2015, two studies of universal screening for FH in children have been published.

In Slovenia, an estimated 33 000 to 70 000 children, 5 years of age, were screened between 2009 and 2013. Of these, 272 children with TC above 6 mmol/L, or TC above 5 mmol/L and a family history of premature cardiovascular disease were genotyped for variants in *LDLR*, *PCSK9*, *APOB*, and *APOE*. Of the 272 children, 155 (57.0%) carried disease-causing variants for FH, 38.6% in *LDLR*, 18.4% in *APOB*, and none in *PCSK9* (217).

In UK, 10 095 children were screened at 1 to 2 years of age during routine immunization visits. Both cholesterol measurements and genetic testing for FH were performed, testing for pathogenic mutations in *LDLR*, *APOB* and *PCSK9*, including Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) testing in a sequential manner. The overall mutation prevalence was 1 in 273 children (37 in 10 095). Some of the children with a positive mutation did not have elevated cholesterol levels (below the 95<sup>th</sup> percentile) and in some, with an elevated cholesterol level on repeat testing (above the 99<sup>th</sup> percentile), a mutation was not found. Using a combination of a cutoff cholesterol level and a mutation yielded a total of 40 children (1 in 252) and 40 parents diagnosed with FH (218) i.e. some children and parents were diagnosed with FH based on elevated cholesterol levels without the finding of a mutation, and in some with a mutation, but cholesterol levels below a predefined threshold, the mutation was considered not to be pathogenic. In our paper V, as many as 8% of the children with an FH-mutation considered to be pathogenic had pretreatment plasma LDL-C levels  $\leq 3.5$  mmol/L, a level suggested as a cutoff for high probability of FH in offspring of probands. There is great variability in the LDL-C levels, interindividually and intraindividually over time. Also, during growth and puberty, the cholesterol levels are reduced (65). Therefore, these children, although having normal values at a snapshot or during a period in childhood, may develop higher cholesterol later in life, and must be followed with monitoring of their lipid profile.

With the increasing availability of relatively low-cost genetic testing procedures, the objections to universal screening are easier to overcome. In the future, genetic testing at birth, for the most prevalent pathogenic mutations in the population, could identify most of the affected individuals, overcoming many of the limitations of lipid screening or combined lipid and genetic screening later in childhood or in the adult population.

#### 4.4.2 When to start drug treatment?

Atherosclerosis, in the form of increased cIMT thickness, has been demonstrated in FH-children as early as before 8 years of age, as compared with unaffected siblings (160). Statin treatment, both in the shorter (2 years) and longer (10 years) term, has been shown to lessen these deposits (121, 163, 219). It is not known at which age the atherosclerotic changes become irreversible. A prudent approach would be to prevent these changes from developing, by initiating statin treatment in childhood, rather than delaying treatment for many years, anticipating that the arterial wall changes are reversible. The cIMT findings in our rosuvastatin pediatric study (paper III) has been part of the basis for recommending initiation of statin therapy at a young age in children with FH (145).

The concept of LDL-C load or “LDL-C burden”, i.e. the LDL-C level multiplied by the number of years this level has persisted, has been used to illustrate the effects of early versus later initiation of cholesterol lowering treatment. Based on this concept, Vuorio et al. have calculated that initiation of low-dose statin therapy at 10 years of age, compared with no treatment until 18 years of age, and then initiation of high-dose statin therapy, would imply 15% lower LDL-C burden. An FH-patient treated with high-dose statin therapy since 18 years of age would reach an LDL-C burden of 160 mmol, postulated to be sufficient to cause CHD, by the age of 48 years, as compared with 53 years in an FH-patient treated since 10 years of age. The 5 years gained from the early treatment start could be assumed to protect against the development of atherosclerosis and CAD (175). Recently, and somewhat surprisingly, Vuorio et al. have launched a contrary idea; to postpone statin-treatment in young FH-patients until 18 years of age, and then initiating lifelong treatment with PCSK9-inhibiting mAbs (220), a view that has been opposed by the author of this thesis (221).

The impact of LDL-burden is strikingly demonstrated by Mendelian randomization studies published in recent years. Loss-of function mutations in *PCSK9* that leads to moderate, lifelong lowering of LDL-C levels by 28% and 15% was associated with 88 % and 47% reduction in the risk of CHD, respectively over a 15-year period (222).

Similarly, carriers of loss-of-function mutations that disrupt the function of the NPC1L1 protein, which is the intestinal cholesterol transporter, absorb less cholesterol from the intestine. In a cohort study it was shown that mutation carriers vs. non-carriers had 0.31 mmol/L lower LDL-C levels and a relative risk reduction of 53% for CHD (223). Compared

with lowering of LDL-C with statins, a lowering by 30% over 5 years, would only reduce CHD events by 1/3 (30%) (88).

Conversely, the significantly elevated risk of CHD in FH also supports the concept of LDL-burden when trying to understand the effects LDL-C levels over a lifetime.

Developing guidelines for long-term prophylactic treatment of children to avoid disease in adulthood is a difficult task. Recommendations to initiate statin therapy at an early age in children with FH are based on results from shorter term placebo-controlled and open-label studies in children with FH, statin studies and studies on other LLTs in adult populations with and without FH, cohort and register studies in adolescent and adult populations with and without FH, and lastly on expert opinion. The recent information from “Mendelian randomization studies” supports this evidence.

Balancing possible harms and benefits of early initiation of statin treatment, guidelines from the European Atherosclerotic Society (EAS), the NICE-guidelines from the UK and guidelines from the US unanimously recommend that statin treatment in children with FH should be considered from 10 years of age, and from 8 years of age in high-risk cases (143, 145, 146). However, no hard evidence exists to underpin a definite age for initiation of LLT, and when considering initiation of statin therapy, risk factors in the child, cholesterol level, family history, parent preferences and sometimes skepticism must be taken into account.

In our experience, it has been difficult to start statin therapy at the recommended 10 years of age with today’s routines. Among our 302 children seen in the period 2014-2016, mean age (SD) at diagnosis and age at first visit was 8.5 (3.2) and 9.5 (2.9) years, respectively, despite that we recommend to parents that a genetic test be performed during childhood, and that we see the FH child first time between age 6 to 8. At the first visit, the child and parent(s) are informed about the condition, including viewing of age-tailored information videos, and dietary advice is given by a clinical nutritionist. A cholesterol-lowering diet and advice about a healthy lifestyle is considered to be an important part of the treatment. To emphasize this, and to make the children familiar with their condition and the clinic, statin treatment has traditionally not been started at the first visit. Mean age of 12.5 years at initiation of statin therapy in our paper V, reflects this practice. To get the age down to around 10 years would require that the children are diagnosed and seen at our clinic for the first time at a younger age. Still, also there may be reluctance among parents as well as doctors, to initiate lifelong statin therapy at this relatively young age. Age at initiation of statin therapy in our clinic

today compares favorably, however, with our cohort of 67 FH children initiated on LLT 10-15 years earlier, when mean age at start of statin therapy was 14.6 years (paper IV), and way better than their parents and grandparents, who usually were not initiated on treatment until adult age.

Previous guidelines recommended initiation of treatment at a higher age in girls than in boys, due to concerns about impact of treatment on development, growth and hormone synthesis. Data from statin studies and clinical experience indicates, however, that the risk of such impact is low and not higher in girls than in boys. Current guidelines therefore recommend initiating statin treatment at equal ages in girls and boys. An additional reason for starting treatment early in girls is that LLT should be discontinued when trying to conceive, during pregnancy and lactation, which can sum up to several years without treatment. Overall, in our cohort, there was no difference between girls and boys in age at start of statin treatment, but in children below 12 years of age, significantly fewer girls than boys were statin treated, even though there was no significant difference in pretreatment LDL-C levels. Seemingly, a traditional attitude towards later treatment initiation in girls is still lingering.

#### 4.4.3 Treatment goals?

In adults with hypercholesterolemia, a 30% lowering of LDL-C with statins, will reduce CHD events by 1/3 (30%) over 5 years, much less than the 88% lowering of CHD conferred by a comparable 28% lifelong genetically lowering of LDL-C (109, 222). So, what counts is not only how low or high LDL-C levels are, but also how long these levels have persisted (109). Only a moderate reduction of LDL-C maintained over many years may substantially reduce the risk of CHD. LDL-C treatment goals must be viewed in the light of such considerations. As with age of treatment initiation, no hard evidence exists for a specific LDL-C treatment goal in FH. Also, no study in patients with hypercholesterolemia has explored the cardiovascular effects of treating to a specific LDL-C goal. The CVD endpoint trials with LLT show, however, that lower LDL-C is associated with lower incidences of CVD, across all baseline levels of LDL-C. Most recently this was shown in the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial with evolocumab, for LDL-C levels down to under 0.2 mmol/L (224).

In children with FH, an LDL-C treatment goal of  $<3.5$  mmol/L, or an alternative goal of 50% reduction of LDL-C, are arbitrarily chosen (225). The argument for a higher LDL-C goal in children than in adults may be that treatment initiated at an earlier age results in lower LDL-C load when used over many years. Also, an argument is that children should be treated with lower statin doses for safety reasons. Furthermore, it is the initial statin dose that has the greatest LDL-C lowering effect, 5 mg rosuvastatin or 10 mg atorvastatin lowers LDL-C by about 30%, while doubling of the dose only lowers LDL-C by an additional 6%. Hence, the greatest difference in LDL-C load will be between those treated with statins and those not treated, and not between those treated with low doses versus high doses. Therefore, it may be more important to initiate low-dose statins at a young age, rather than to treat to a specific LDL-C goal. However, a strong argument for an LDL-C treatment goal below 3.5 mmol/L in children is that when treated to this level over 2 years, as reported in paper I and II, cIMT was normalized in the children with FH, compared with healthy unaffected siblings.

## **5. Conclusions**

Although the open-label design, relatively short duration, and the limited number of participants in our statin studies (paper I and III) are limiting in drawing firm conclusions, they add important knowledge about efficacy and safety of statin treatment in children and adolescents with FH. The normalization of the elevated baseline cIMT after 2 years of rosuvastatin treatment in children with FH (paper II), support the value of early initiation of statin treatment in these children.

The findings in our cohort studies from the Lipid Clinic (paper IV and V) substantiates that earlier diagnosis and yearly follow-up of children and young adults with FH is warranted to comply with treatment recommendations, to provide health education and to ensure adherence to the lifelong preventive treatment.

Follow-up and monitoring of cohorts of young FH-patients, having initiated LLT at a young age, is necessary to capture and better understand the long-term effects and possible side effects of the therapy, in order to optimize mode of treatment, dosing of drugs and age at initiation.

If FH is diagnosed and treated early in life, CVD, resulting from prolonged elevated LDL-C levels, may be prevented in most individuals. Universal genetic screening in early childhood should therefore be considered to find affected individuals. Treatment with statins and ezetimibe, in addition to diet and lifestyle, is simple and effective. Future treatments, especially PCSK9 inhibiting options, is promising for those not achieving satisfactory LDL-C levels on statins and ezetimibe, and for those unable to tolerate an effective dose of statins.





## 6. References

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## **7. Papers I-V**

















# A 3-year study of atorvastatin in children and adolescents with heterozygous familial hypercholesterolemia



Gisle Langslet, MD\*, Andrei Breazna, PhD,  
Euridiki Drogari, MD, PhD, MSc (Biochem), MSc (Nutr)

Department of Endocrinology, Morbid Obesity and Preventive Medicine, Lipid Clinic, Oslo University Hospital, Oslo, Norway (Dr Langslet); Pfizer Inc, New York, NY, USA (Dr Breazna); and Metabolic Unit, Choremio Goudi Research Institute, 1st Department of Pediatrics, Medical School University of Athens, Aghia Sofia Children's Hospital, Athens, Greece (Dr Drogari)

## KEYWORDS:

Heterozygous familial hypercholesterolemia;  
Atorvastatin;  
Children;  
Adolescents;  
Tanner stage;  
Low-density lipoprotein cholesterol

**BACKGROUND:** The efficacy and safety of atorvastatin in children/adolescents aged 10–17 years with heterozygous familial hypercholesterolemia (HeFH) have been demonstrated in trials of up to 1 year in duration. However, the efficacy/safety of >1 year use of atorvastatin in children/adolescents with HeFH, including children from 6 years of age, has not been assessed.

**OBJECTIVE:** To characterize the efficacy and safety of atorvastatin over 3 years and to assess the impact on growth and development in children aged 6–15 years with HeFH.

**METHODS:** A total of 272 subjects aged 6–15 years with HeFH and low-density lipoprotein cholesterol (LDL-C)  $\geq 4.0$  mmol/L (154 mg/dL) were enrolled in a 3-year study (NCT00827606). Subjects were initiated on atorvastatin (5 mg or 10 mg) with doses increased to up to 80 mg based on LDL-C levels.

**RESULTS:** Mean percentage reductions from baseline in LDL-C at 36 months/early termination were 43.8% for subjects at Tanner stage (TS) 1 and 39.9% for TS  $\geq 2$ . There was no evidence of variations in the lipid-lowering efficacy of atorvastatin between the TS groups analyzed (1 vs  $\geq 2$ ) or in subjects aged <10 vs  $\geq 10$  years, and the treatment had no adverse effect on growth or maturation. Atorvastatin had a favorable safety and tolerability profile, and only 6 (2.2%) subjects discontinued because of adverse events.

**CONCLUSIONS:** Atorvastatin over 3 years was efficacious, had no impact on growth/maturation, and was well tolerated in children and adolescents with HeFH aged 6–15 years.

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\* Corresponding author. Lipid Clinic, Oslo University Hospital, Post box 4950, Nydalen, 0424, Oslo, Norway.

E-mail address: [glangsle@ous-hf.no](mailto:glangsle@ous-hf.no)

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## Introduction

Elevated cholesterol levels in childhood are associated with an increased incidence of atherosclerosis in adulthood.<sup>1,2</sup> The severity of atherosclerosis can be correlated to

the extent and duration of hypercholesterolemia.<sup>3</sup> Familial hypercholesterolemia (FH) is a common inherited autosomal dominant disorder of lipoprotein metabolism characterized by reduced clearance of low-density lipoprotein cholesterol (LDL-C) from the circulation leading to elevations of LDL-C.<sup>4,5</sup> In most cases (85%–90%), FH is caused by defects in the low-density lipoprotein-receptor (LDL-R) gene. Defects in the genes for apolipoprotein B (apoB) and proprotein convertase subtilisin/kexin type 9 account for about 5% and <5% of cases, respectively.<sup>4</sup> Rarely, autosomal recessive hypercholesterolemia can cause FH.<sup>6,7</sup> In most populations, heterozygous familial hypercholesterolemia (HeFH) affects ~1 in 200–250 individuals.<sup>8</sup> FH is associated with increased morbidity of coronary heart disease and with premature death<sup>8–11</sup>, and children with FH have greater carotid intima media thickness than their unaffected siblings.<sup>12,13</sup>

Early intervention with cholesterol-lowering treatment, primarily statins, has been shown to prevent early coronary heart disease.<sup>14–16</sup> The evidence base for the efficacy and safety of statins in children is growing.<sup>17–28</sup> However, gaps in this evidence base remain.<sup>29</sup> For example, studies conducted with atorvastatin in children/adolescents with HeFH were up to 1 year in duration with the impact on growth/development evaluated at 26 weeks.<sup>26</sup> Furthermore, the 1-year study was conducted in children/adolescents of 10–17 years,<sup>26</sup> whereas statins are now considered in children with HeFH <10 years.<sup>5</sup> This 3-year study enrolling ~250 subjects aged 6–15 years with genetically confirmed HeFH was therefore conducted to characterize the long-term efficacy and safety of atorvastatin and to assess the impact of this medication on growth and development. Also, as part of this work, we examined the impact of atorvastatin treatment on endothelial function in the brachial arteries assessed by flow-mediated dilation (FMD).<sup>30</sup> This was an optional exploratory study to assess the potential for a change in the FMD to act as a surrogate biomarker for the efficacy of LDL-C lowering as previously shown in a study with simvastatin therapy in children and adolescents with FH.<sup>31</sup>

## Methods

### Standard protocol approvals, registrations, and patient consents

This open-label, multicenter, prospective study was conducted between March 30, 2009 and October 8, 2013 at 30 centers across 14 counties in compliance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. In addition, all local regulatory requirements were followed. The protocol and the informed consent documents were reviewed and approved by the institutional review boards and/or independent ethics committees at each participating

center. Parents or legal guardians provided informed consent. The subject could also sign a written consent form, if they were able to do so. The exact processes and procedures for attaining assent and consent varied between countries. However, all country-specific guidelines were complied with.

### Study population

Girls and boys aged 6–15 years with genetically confirmed HeFH (those girls and boys who had no prior record of genetically confirmed HeFH underwent genetic testing pre-randomization to confirm whether they had HeFH). DNA was extracted from saliva or from blood, and the 18 exons and flanking regions of the LDL-R gene and exon 26 of the apoB gene were sequenced using SANGER methodology. Samples were also tested for large deletions or insertions in the LDL-R gene, using Multiplex Ligase-dependent Probe Amplification (MLPA) analysis (Details provided in [Supplementary Material](#)).

All those who did not have genetically confirmed HeFH were excluded. All patients also had to have an LDL-C of  $\geq 4.0$  mmol/L (154 mg/dL) for inclusion. Exclusion criteria included a history of active liver disease, hepatic dysfunction, or persistent elevations of serum transaminases  $>3\times$  the upper limit of normal (ULN) or conditions likely to delay puberty. Pregnant or breastfeeding females, and females of childbearing potential not using adequate contraception, were excluded. Subjects with hypersensitivities to statins or receiving statin therapy within 4 weeks of randomization were excluded. However, a 4-week washout of lipid-lowering medication was permitted.

### Study design

The doses of atorvastatin used in this study were based on the results of a study comparing the efficacy and safety of different starting doses of atorvastatin in adults with dyslipidemia<sup>32</sup> and earlier studies conducted in 6–17-year-old subjects.<sup>21,26</sup>

In total, 272 subjects with HeFH were stratified into 2 cohorts according to their Tanner stage (TS) (1 or  $\geq 2$ ) at screening. Subjects aged 6 to <10 years (mostly TS 1) initiated therapy on atorvastatin 5 mg per day (a pediatric chewable formulation), and those aged 10 to 15 years (mostly TS  $\geq 2$ ) initiated treatment with atorvastatin 10 mg per day.

Subjects had their dose titrated based on an LDL-C target of  $<3.35$  mmol/L ( $<130$  mg/dL). Doses were increased from 5 to 10 to 20 mg or 10 to 20 to 40 mg per day. Titrations above 40 mg per day were permitted after discussions with the study sponsor. Subjects initiating treatment on atorvastatin 10 mg per day were permitted to decrease their dose if their LDL-C decreased to  $<2.59$  mmol/L (100 mg/dL). Subjects with LDL-C  $<2.59$  mmol/L on the 5 mg dose were discontinued.



Atorvastatin was dispensed at visit 1 (day 0), and subjects were seen monthly until month 6 at which point they were seen every 6 months. TS assessments were made at screening and every 6 months.

## Outcome assessments

The primary assessments were measures of growth and development (height, weight, body mass index [BMI], TS), efficacy (absolute and percentage change from baseline in LDL-C, total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], apo A-1, apoB), tolerability, and safety. An exploratory secondary efficacy endpoint was change from baseline in FMD in a subset of subjects (see [Supplemental Information](#)). Subjects were required to fast for  $\geq 10$  hours before the collection of samples for lipid assessments. The incidence, severity, and potential causal relationship of treatment-emergent adverse events (AEs) were monitored alongside abnormal laboratory findings. Hematology, blood chemistry, and urinalysis were also evaluated at screening and throughout the study. AEs were classified as mild (does not interfere with subject's usual function), moderate (interferes to some extent with subject's usual function), or severe (interferes significantly with subject's usual function).

## Statistical analysis

The full analysis set, defined as all subjects who received  $\geq 1$  doses of the study drug, was included in all analyses. No imputations were used for missing values. Subjects were categorized by TS 1 or  $\geq 2$  for analyses of lipid endpoints and safety/tolerability, and also by age  $< 10$  years and  $\geq 10$  years for some analyses. Height, weight, BMI and FMD were analyzed by gender. Analyses were performed using descriptive statistics. Demographic and baseline data were summarized as mean  $\pm$  standard deviation (SD) for continuous variables. The mean dose of atorvastatin was calculated as the average dose per subject weighted by duration on each dose and the unweighted average calculated regardless of the duration on each dose and the mean maximum dose. The percentage of subjects receiving 5, 10, 20, 40, or 80 mg atorvastatin as their maximum dose stratified by TS and age was calculated. The percentage of subjects who attained an LDL-C goal of  $< 3.35$  mmol/L ( $< 130$  mg/dL) at each visit was also calculated.

## Results

### Study population

A total of 400 pediatric subjects were screened and 272 with a genetically confirmed diagnosis of HeFH were enrolled (87 subjects without a previously confirmed

genetic diagnosis of HeFH were considered for genetic screening and 66 were screened of which 49 [74.2%] were confirmed positive for HeFH) ([Fig. 1](#)). This study was conducted at 30 centers worldwide, and the methodologies for genetic testing and the manner in which this information was collated differed both between centers and the central laboratory used to screen the patients without confirmed FH. Therefore, unfortunately, it has not been possible to collate this information for patients across the study. One subject was assigned to study treatment, but not treated, due to a protocol violation. The 271 subjects treated comprised 139 children at TS 1 and 132 adolescents at TS  $\geq 2$ .

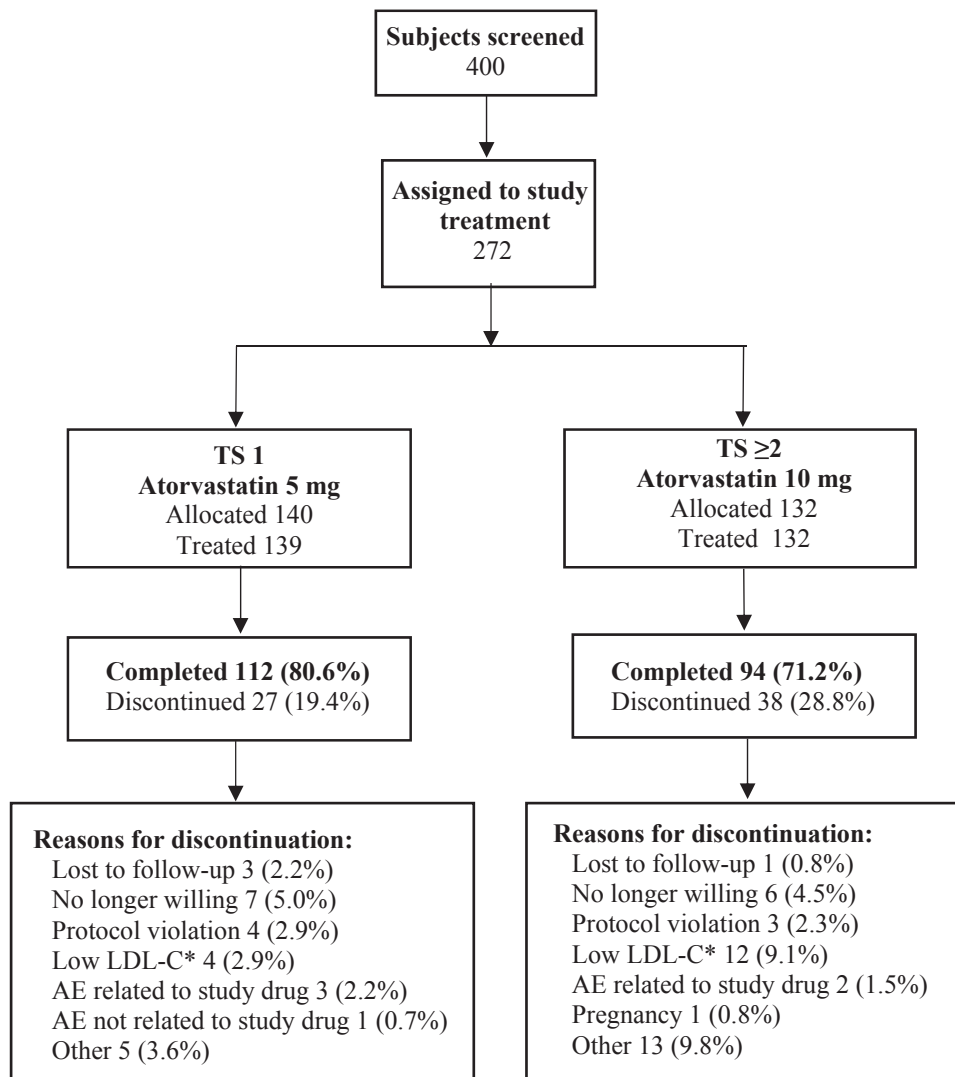
Overall, 206 subjects (76.0%) completed the study with 65 (24%) discontinuing. In subjects at TS1, the most frequent cause of discontinuation was no longer being willing to participate in the study, whereas for subjects at TS  $\geq 2$ , the most common reason for discontinuation was "low LDL-C" (defined as LDL-C  $< 2.59$  mmol/L [100 mg/dL] in subjects receiving atorvastatin 5 mg; 12 subjects). Unfortunately, in some cases, investigators were unaware that subjects initiating treatment on 10 mg per day could be down-titrated to 5 mg per day; this knowledge may have prevented some of these discontinuations.

The mean age of children at TS 1 at baseline was 8.5 years (SD = 1.9), the majority were male (66.9%) and white (96.4%; [Table 1](#)). The adolescents at TS  $\geq 2$  at baseline had a mean age of 12.0 years (SD = 1.7), the majority were female (59.8%) and white (98.5%). Overall, 27.3% of subjects were aged 6–8 years, and 51.7% were aged  $\leq 10$  years at baseline.

The mean weighted and mean maximum doses were similar in the subjects at TS 1 and  $\geq 2$ , whereas mean doses were consistently higher in the subjects aged  $\geq 10$  years vs those aged  $< 10$  years ([Fig. 2A](#)). Few subjects (18, 6.6%) received atorvastatin 80 mg during the study, and 12 subjects (8.6%) at TS 1 received this maximum dose ([Fig. 2B](#)). The median duration of treatment was 1085 days (interquartile range [IQR], 1035.0–1099.0) overall for all subjects, 1084.0 days (IQR, 1060.0–1099) for TS 1 and 1085.0 (IQR, 634.0–1099.35) for TS  $\geq 2$ . Approximately 70% of subjects received concomitant medications, most commonly ibuprofen and paracetamol ([Supplemental Table 1](#)).

### Efficacy

There was very little difference in the mean percentage changes from baseline in LDL-C in all subjects and those at TS 1 or  $\geq 2$  over the study duration ([Fig. 3](#) and [Table 2](#)). Mean LDL-C levels were reduced by  $\sim 35\%$  from month 1 in all subject groups, with a further reduction to  $\sim 45\%$  at month 3. LDL-C levels were then maintained at this level to month 30. At month 36/early termination, the mean reduction in LDL-C in the TS 1 group remained at  $-43.8\%$ , whereas in the TS  $\geq 2$  group, it decreased to  $-39.9\%$ .



**Figure 1** Patient disposition. AE, adverse event; LDL-C, low-density lipoprotein cholesterol; TS, Tanner stage. \*LDL-C <2.59 mmol/L (100 mg/dL).

A total of 52% of subjects aged <10 years attained the LDL-C target of <3.35 mmol/L (130 mg/dL) at month 3, and goal attainment remained above 50% for the duration of the study, peaking at 67.4% at month 18. For the age  $\geq 10$  years' cohort, 52% of subjects achieved this goal at month 2, and goal attainment remained above 50% for the duration of the study, peaking at 78% at month 30 (Fig. 4).

The mean percentage reductions from baseline at month 36/early termination in TC, non-HDL-C, and apoB were comparable for subjects at TS 1 and TS  $\geq 2$  (Fig. 5 and Table 2). HDL-C levels decreased slightly (1.1%) from baseline at month 36/early termination in subjects at TS 1 and increased slightly (1.6%) in subjects at TS  $\geq 2$ . Baseline TG levels were slightly higher in the TS  $\geq 2$  group when compared with the TS 1 group (0.980 vs 0.880 mmol/L [86.8 vs 77.9 mg/dL], respectively). A larger mean percentage reduction in TG level at month 36/early termination was observed in the TS  $\geq 2$  group than in the TS 1 group (−7.76% vs −0.70%). A modest reduction in

apo-A1 levels was reported for both TS groups (−4.80% vs −1.95% for TS 1 and TS  $\geq 2$  at month 36/early termination, respectively).

### Flow-mediated dilation

The FMD profile showed no discernable trends in either male or female subjects (See Supplemental Information).

### Development and growth

At the month 36/early termination visit, male subjects had increased in height from baseline by a mean of 11.0% vs a mean of 8.1% for female subjects. The mean height in female subjects increased from 145 cm (mean age, 10.6 years) at baseline to 157 cm (mean age, 13.1 years) at month 36/early termination. The mean height in male subjects increased from 144 cm (mean age, 9.9 years) to 161 cm (mean age, 12.7 years) at month 36/early

**Table 1** Demographics and baseline characteristics

Characteristic	TS 1, Total (n = 139)	TS $\geq$ 2, Total (n = 132)
Age, y, n (%)		
6–8	69 (49.6)	5 (3.8)
9–10	45 (32.4)	21 (15.9)
11–14	25 (18.0)	104 (78.8)
15–15	0	2 (1.5)
Mean	8.5	12.0
SD	1.9	1.7
Range	6–12	8–15
Gender, Male, n (%)	93 (66.9)	53 (40.2)
Race		
White	134 (96.4)	130 (98.5)
Black	1 (0.7)	1 (0.8)
Asian/other	4 (2.9)	1 (0.8)
Weight, kg		
Mean	33.8	49.4
SD	11.3	12.1
Range	17–75	26–82
BMI, kg/m <sup>2</sup>		
Mean	18.1	20.3
SD	3.7	3.7
Range	12.6–30.7	13.8–33.7
Height, cm		
Mean	135.0	155.1
SD	12.1	10.5
Range	109–162	132–184
Baseline LDL-C, mmol/L (mg/dL)		
Mean	6.30 (243.62)	5.92 (228.93)
SD	1.31 (50.66)	1.16 (44.86)

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.

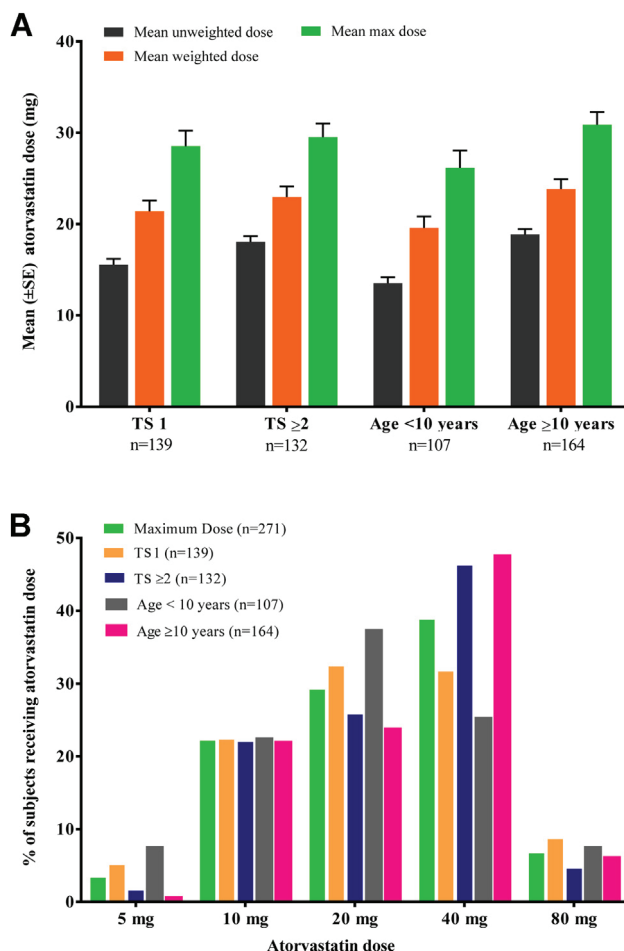
To convert mmol/L to mg/dL multiply by 38.67.

termination. At the month 36/early termination, male subjects had increased in weight from baseline by a mean of 32.5% compared with 27.3% for female subjects.

The shift in TS over this 3-year study is shown in Figure 6. By month 36/early termination visit just 41 of 253 subjects (16.2%) were at TS 1 in comparison with 139 of 271 (51.3%) at baseline. Conversely, the percentage of subjects at TS 5 increased from 6.6% (18 of 271) at baseline to 25.3% (64 of 253) at study end.

## Safety

The overall incidence of all-causality AEs was very similar in the TS 1 (81.3%) and TS  $\geq$ 2 (79.5%) groups (Table 3). Most AEs were of mild or moderate intensity. There were no deaths. Overall, 21 patients (7.7%) reported a serious AE (SAE). Overall, 6 (2.2%) subjects discontinued because of AEs (Table 3). This included 4 subjects at TS 1 (1 subject experienced Ewing's sarcoma, 1 increase in blood bilirubin, 1 intravascular papillary endothelial hyperplasia, and 1 subject experienced abdominal pain,

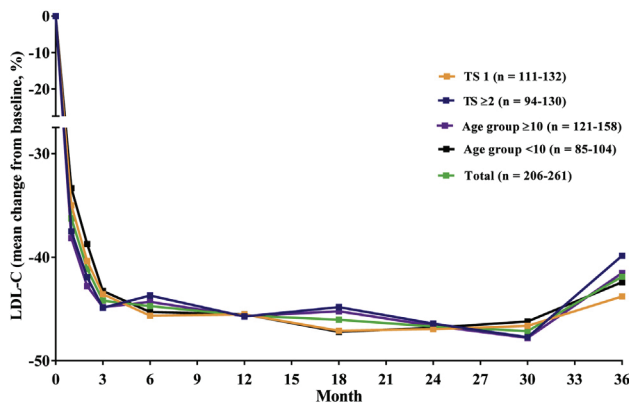


**Figure 2** (A) Mean unweighted, weighted, and maximum doses of atorvastatin received by children and adolescents with HeFH stratified by TS and age. (B) The percentage of subjects receiving atorvastatin 5, 10, 20, 40, or 80 mg as their maximum dose, all subjects, by TS and age. HeFH, heterozygous familial hypercholesterolemia; SE, standard error; TS, Tanner stage.

constipation, fatigue, and headache), and 2 subjects at TS  $\geq$  2 (1 subject experienced myalgia and 1 eosinophilia). A total of 24 (8.9%) subjects had their dose reduced or temporarily discontinued because of treatment-related AEs. All the treatment-related AEs were mild or moderate in intensity. The most commonly occurring all-causality AEs are shown in Table 3.

Of the subjects with a SAE, 14 were from the TS 1 group, and 7 were from the TS  $\geq$ 2 group (see Supplemental Information for full details of these SAEs). A single SAE considered to be treatment-related by the investigator was reported: a 9-year-old male receiving atorvastatin 80 mg experienced an SAE of Ewing's sarcoma on day 704 of the study. The subject was hospitalized as a result of this condition. There was no evidence of dose-related increase in the overall incidence of AEs or discontinuations/dose reduction of study medication (Supplemental Tables 2 and 3).

There were no obvious trends, or dose-related trends, in the incidence of laboratory abnormalities. No subjects had



**Figure 3** Mean percentage change from baseline in LDL-C in all subjects with HeFH and by TS 1 and  $\geq 2$  and aged  $<10$  and  $\geq 10$  years at baseline. HeFH, heterozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; TS, Tanner stage.

aspartate aminotransferase or alanine aminotransferase levels  $>3 \times$  ULN. Overall, 23 subjects (8.6%) had creatine kinase (CK) of  $>2 \times$  ULN. The incidence of this abnormality was much greater in the  $TS \geq 2$  (17; 12.9%) than in the  $TS 1$  group (6; 4.4%). One subject, a 14-year-old male, was reported with increased CK ( $10 \times$  ULN) as a SAE. This was thought to be related to hard physical exercise. Elevations in CK were observed in other male subjects after intense exercise and in both genders after viral infections. All the other AEs related to laboratory abnormalities were of mild or moderate intensity.

**Discussion**

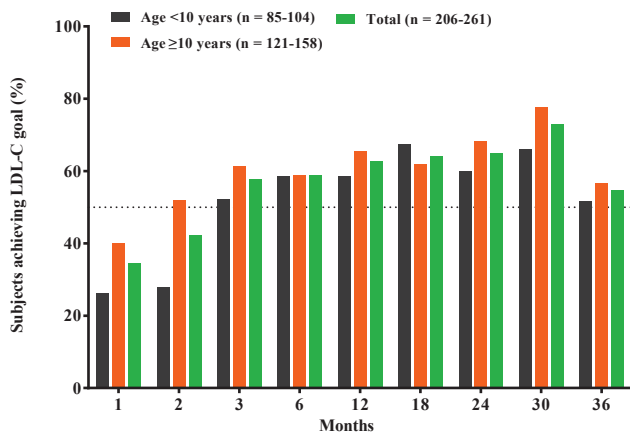
This 3-year, open-label, prospective study enrolling very young subjects with HeFH demonstrated that atorvastatin was well tolerated and efficacious in children and adolescents (6–15 years at study entry). Furthermore, there was no evidence that treatment with atorvastatin had any clinically relevant effect on growth or maturation. Because endogenous steroid hormone production is derived from cholesterol, a potential concern is how statin therapy may affect sexual development in children and adolescents.<sup>33</sup> An earlier study demonstrated that treatment with lovastatin (20–40 mg/day) for 24 weeks had no effect on hormone levels or menstrual cycle length in adolescent girls with HeFH aged 10–17 years.<sup>19</sup>

The TS shift from baseline during our 3-year trial was consistent with the normal trajectory for maturation and development. For example, movement to  $TS 2$  occurs from age 11 years (range, 8–13 years) in females and slightly later in males (range, 9.5–13.5 years), and  $TS 5$  is usually reached at 14–15 years in females (range, 13–18 years) and 15 years in males (range, 13.5–17 years).<sup>34,35</sup> Furthermore, the mean height of both male and female subjects was above the 0 z-score lines on the gender-specific WHO height for age charts.<sup>36,37</sup> The mean height in males

**Table 2** Lipid parameters at baseline and month 36/early termination in all subjects and by TS

Parameter, mg/dL* or g/L**	TS 1 (n = 139)		TS $\geq 2$ (n = 132)		Total (n = 271)	
	Baseline		Baseline		Baseline	
	Mean (SD)	% change from baseline	Mean (SD)	% change from baseline	Mean (SD)	% change from baseline
LDL-C	243.6 (50.7)	-43.79	228.9 (44.9)	-39.86	236.7 (48.7)	-41.87
TC	311.7 (51.8)	-35.06	295.9 (47.6)	-32.01	303.9 (50.3)	-33.58
HDL-C	52.2 (10.4)	-1.13	49.5 (9.7)	1.60	50.7 (10.4)	0.20
Non-HDL-C	259.5 (53.4)	-41.8	246.3 (48.3)	-38.3	252.9 (51.0)	-40.09
Triglycerides	77.9 (38.1)	-0.70	86.8 (44.3)	-7.76	82.4 (41.6)	-4.14
Apo A-1**	1.40 (0.18)	-4.80	1.31 (0.20)	-1.95	1.35 (0.20)	-3.42
Apo B**	1.45 (0.29)	-34.5	1.38 (0.26)	-31.4	1.42 (0.28)	-33.0
			Month 36		Month 36	
			Mean (SD)		Mean (SD)	
			133.4 (28.6)		133.4 (31.3)	
			198.4 (30.5)		198.0 (33.3)	
			51.8 (11.2)		50.7 (10.4)	
			146.9 (30.9)		147.3 (33.6)	
			67.3 (37.2)		69.1 (36.3)	
			1.33 (0.21)		1.30 (0.19)	
			0.93 (0.19)		0.93 (0.20)	

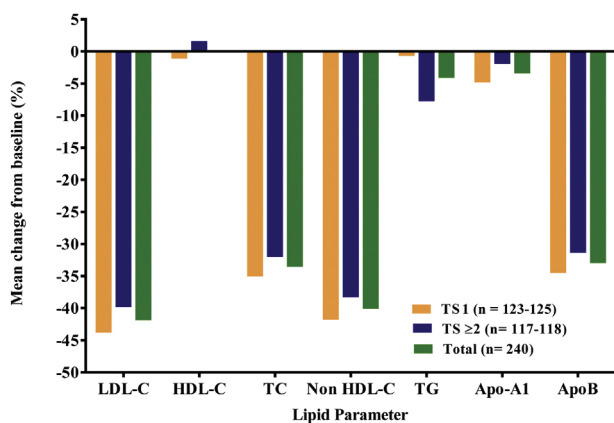
Apo A-1, apolipoprotein A-1; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TS, Tanner stage; TC, total cholesterol. \* Denotes units used.



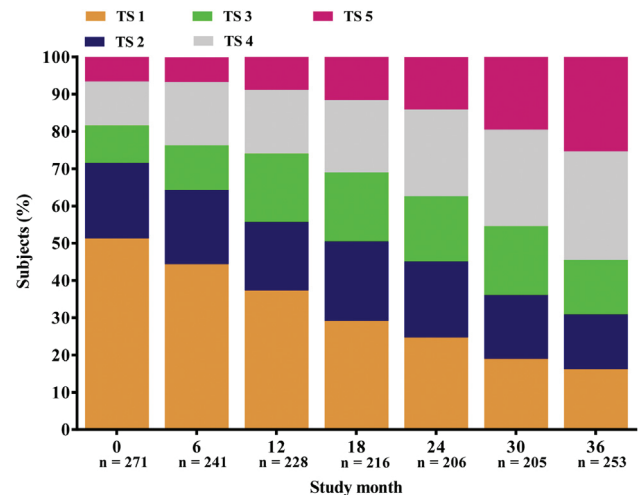
**Figure 4** Percentage of subjects aged <10 years or  $\geq 10$  years at baseline achieving LDL-C goal of <3.35 mmol/L (<130 mg/dL) at each study visit. LDL-C, low-density lipoprotein cholesterol.

followed the 1 z-score line. In both males and females, the mean increase in height over the 3-year study followed the same trajectory as this reference group.

There is increasing evidence that the administration of statins early in life is beneficial in preventing or reducing atherosclerosis in subjects with FH.<sup>8,38</sup> In this study, we observed that atorvastatin administration led to substantial reductions in LDL-C and other atherogenic lipid parameters. The reductions in LDL-C reached maximal levels of  $\sim 45\%$  at months 3 to 6 and then remained at this level throughout the follow-up period. There was a slight increase in LDL-C at month 36/early termination, possibly due to the inclusion of data from subjects who discontinued study medication early in the trial. Substantial reductions at month 36/early termination were also observed in apo B and non-HDL-C. There appeared to be no evidence of variations in the lipid-lowering efficacy of atorvastatin



**Figure 5** Mean percentage change from baseline in lipid parameters at month 36/early termination in all subjects with HeFH and by TS 1 and  $\geq 2$  at baseline. Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; HeFH, heterozygous familial hypercholesterolemia; TC, total cholesterol; TG, triglyceride; TS, Tanner stage.



**Figure 6** Changes in the percentage of subjects in each TS over 36 months/early termination among children and adolescents with HeFH receiving atorvastatin therapy. HeFH, heterozygous familial hypercholesterolemia; TS, Tanner stage.

between the TS groups analyzed (1 vs  $\geq 2$ ) or in subjects aged <10 vs  $\geq 10$  years.

The reductions of LDL-C observed are in accordance with the findings from other clinical studies conducted in pediatric and adolescent populations in which atorvastatin and other statins have been administered.<sup>17,19–26,28</sup> Moreover, the early efficacy of atorvastatin in this study is consistent with that observed in a similar but smaller ( $n = 39$ ) short-term (8-week) atorvastatin study in which doses of 5–20 mg per day were administered to children and adolescents with HeFH. In the small 8-week study, mean LDL-C reductions from baseline of 40.7% and 39.7% were observed at week 8 in subjects at TS 1 and  $\geq 2$ , respectively.<sup>21</sup> Similar reductions in LDL-C (40%) were observed at week 26 in a larger study ( $n = 187$ ) in which atorvastatin (10–20 mg per day) was administered to children and adolescents with FH or severe hypercholesterolemia.<sup>26</sup> The slightly greater reductions in LDL-C observed in this ( $\sim 45\%$ ) vs the earlier studies ( $\sim 40\%$ ) might be related to the higher doses of atorvastatin permitted in this study (up to 80 mg per day).

Atorvastatin was shown in this 3-year study to have a favorable safety and tolerability profile. Only 6 (2.2%) patients discontinued because of AEs. Regarding the SAE of Ewing's sarcoma in a 9-year-old male receiving atorvastatin 80 mg, evidence that this was not associated with statin use comes from a meta-analysis of 26 clinical trials of statin therapy, containing over 170,000 participants, which demonstrated no increase in cancer incidence among subjects receiving statins.<sup>39</sup> Also, the etiology of Ewing's sarcoma suggests that it is very unlikely that the atorvastatin treatment was associated with this cancer.

No unexpected or new safety findings were observed despite 27.3% of the children enrolled being 6–8 years and 51.7% <10 years at the start of the study. The differences in AEs and treatment-related AEs were not considered to be

**Table 3** The percentage of subjects\* reporting all-causality and treatment-related AEs, discontinuations because of AEs, and the most commonly reported AEs by TS and overall

Type or category of AE	TS 1 (n = 139)	TS ≥2 (n = 132)	Total (n = 271)
Subjects with AEs	81.3, <i>40.3</i>	79.5, <i>37.1</i>	80.4, <i>38.7</i>
Serious AEs	10.1, <i>0.7</i>	5.3, <i>0</i>	7.7, <i>0.4</i>
Discontinued because of AE	2.9, <i>2.2</i>	1.5, <i>1.5</i>	2.2, <i>1.8</i>
Dose of study medication reduced or temporarily discontinued	18.7, <i>7.9</i>	15.2, <i>9.8</i>	17.0, <i>8.9</i>
Most frequently occurring AEs (≥10% all causality in any group)			
Nasopharyngitis	18.7, <i>3.6</i>	19.7, <i>5.3</i>	19.2, <i>4.4</i>
Headache	18.0, <i>7.9</i>	18.9, <i>6.8</i>	18.5, <i>7.4</i>
Abdominal pain	15.1, <i>9.4</i>	7.6, <i>4.5</i>	11.4, <i>7.0</i>
Upper respiratory tract infection	14.4, <i>3.6</i>	7.6, <i>1.5</i>	11.1, <i>2.6</i>
Gastroenteritis	12.2, <i>2.2</i>	9.1, <i>0.8</i>	10.7, <i>1.5</i>
Pyrexia	10.8, <i>1.4</i>	9.8, <i>3.0</i>	10.3, <i>2.2</i>
Influenza	9.4, <i>0</i>	10.6, <i>0</i>	10.0, <i>0</i>
Cough	10.1, <i>2.9</i>	8.3, <i>3.0</i>	9.2, <i>3.0</i>
Vomiting	10.8, <i>2.9</i>	6.1, <i>4.5</i>	8.5, <i>3.7</i>
Pain in extremity	10.1, <i>4.3</i>	3.8, <i>3.0</i>	7.0, <i>3.7</i>

AE, adverse event; TS, Tanner stage.

\*Treatment-related AEs are shown in italics.

clinically meaningful, irrespective of TS of the studied subjects. In addition, no clinically meaningful potential safety trends were noted. Moreover, the safety and tolerability profile observed in this study was qualitatively and quantitatively similar to that observed previously in both adult and pediatric populations.<sup>21,26,40</sup>

The 2013 and 2015 European Atherosclerosis Society consensus statements on FH and the clinical guidance from the National Lipid Association Expert Panel<sup>41</sup> recommend that lipid-lowering therapies should be strongly considered alongside lifestyle changes in children, starting at 8–10 years.<sup>5,8</sup> This recommendation differs from the 2011 US Integrated Guidelines for the CV Health and Risk Reduction in Children and Adolescents, which notes that children <10 years should not be treated with lipid-lowering medication unless they have LDL-C levels of ≥400 mg/dL (10.36 mmol/L).<sup>42</sup>

This study is limited by its open-label design, lack of an active comparator and by the limited number of subjects who received the 80-mg dose of atorvastatin, which mean our safety data are too limited to draw any conclusions regarding this dose. An additional limitation is the lack of genetic information collated across this cohort of children with HeFH. However, the study has some notable strengths such as children as young as 6 years old being enrolled, a duration of 3 years and the utilization of measures of growth and maturation.

The results of this study suggest that atorvastatin in doses of 5–40 mg is effective and can be used safely in children with HeFH aged as young as 6 years. These findings together with observations from other studies, which have demonstrated that atherosclerotic changes are apparent in children with HeFH before the age of 8 years,<sup>12,13</sup> and that statins slow this progression,<sup>27,43</sup> highlight the importance of starting statin therapy early in

children with HeFH.<sup>8,42–44</sup> Indeed, atorvastatin is now approved for use from 6 years in Australia.<sup>8</sup>

In conclusion, our study demonstrates the favorable efficacy, safety, and tolerability profile of atorvastatin in both children as young as 6 years and adolescent subjects, treated for a period of 3 years.

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Author contributions: Dr Breazna was involved in the design of the study, the analysis of the data, interpreted the findings, participated in drafting of the manuscript, and approved the final version of the article for submission. Dr Langslet recruited and treated patients enrolled in the study, interpreted the findings, participated in drafting of the manuscript, and approved the final version of the article for submission. Dr Drogari is the top recruiter and treated patients enrolled in the study, interpreted the findings, participated in drafting of the manuscript, and approved the final version of the article for submission.

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Dr Breazna is an employee of Pfizer Inc. Dr Langslet has received Advisory Board and lecture fees from Amgen, Sanofi, Boehringer-Ingelheim, and Janssen. Dr Drogari's University Department has received fees from Sanofi for Dr Drogari's attending Advisory Boards.

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## Appendix

### Methods

This open-label, multicenter, prospective study was conducted at 30 centers in Belgium, Canada, Germany, Greece, Hungary, Italy, Norway, Poland, Russian Federation, Slovakia, Spain, Switzerland, Turkey, and the United States.

HeFH genetic testing was conducted using the following methodologies: DNA was extracted from saliva (collected in Oragene DNA sample collection kit, DNA Genotek) or from blood using the QiAmp Blood DNA Mini Kit (Qiagen, Germany), according to manufacturer instructions. The promoter region and all the 18 exons and flanking regions of the LDL-R gene were amplified by polymerase chain reaction, and exon 26 of the apoB gene was sequenced in an ABI 3730 DNA analyzer using SANGER methodology after polymerase chain reaction amplification of the DNA samples. Sequence analysis was carried out using SeqScape software, v2.6. Reference sequences for the genes analyzed were based on the hg19-GRCh37 genome assembly database (LDLR: Ref Seq NM\_000527.5 and APOB: Ref Eeq NM\_000384.2). Samples were also tested for large deletions or insertions, using the MLPA—Multiplex Ligase dependent Probe Amplification kit (MRC, Holland) and following manufacturer's instructions.

Flow-mediated dilatation (FMD) measurements were made using each center's validated peer-review and published FMD protocol. FMD measurements were made at baseline and at months 6, 12, 18, 24, 30, and 36. The lipid panel was assessed at screening and at months 1, 2, 3, 6, 12, 18, 24, 30, and 36. FMD data were based on collected measures (hyperemic and resting diameter) and calculated as follows:  $\text{FMD (\%)} = (\text{hyperemic diameter} - \text{resting diameter}) / \text{resting diameter} \times 100$ .

## Results

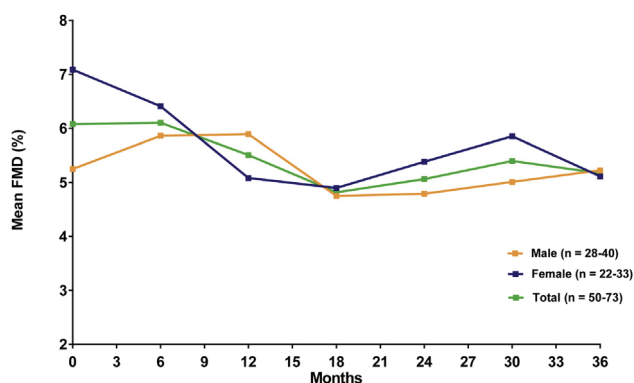
### Flow-mediated dilation

In an effort to measure the effects of low-density lipoprotein cholesterol (LDL-C) lowering on endothelial function, we applied the established method of ultrasonically measured FMD in a part of our study population. A total of 73 subjects (37 at Tanner stage [TS] 1 and 36 at TS stage  $\geq 2$ ) from four centers were included in the FMD substudy. The FMD profile showed no discernable trends in either male or female subjects with the mean percentage dilation exhibiting little change over the duration of this substudy (Supplemental Fig. 1), possibly due to issues both in the methodology and the study design. For example, only a small number of patients took part in this substudy. Also, it is possible that the methodology used varied between the four centers leading to inconsistent results. Finally, the blood vessel walls of the children and adolescents examined may not have been sufficiently thickened by atherosclerosis

for a beneficial effect of statins to be observed. Additional studies may be required to evaluate whether treatment with statins can have an effect on FMD in children and adolescents with familial hypercholesterolemia (FH) and whether this technique is useful for measuring endothelial dysfunction and atherosclerosis in children.

### Supplemental safety information

The serious adverse events reported by the subjects in the TS 1 group were myositis (this was not associated with abnormal CK values and the subject continued taking study medication), feeling abnormal/syncope, intravascular papillary endothelial hyperplasia, testicular appendage torsion, hemorrhoids, viral infection, appendicitis (2 subjects), bipolar disorder, appendix disorder, concussion, abdominal pain, ulna fracture, and Ewing's sarcoma. The serious adverse events reported by the subjects in the TS  $\geq 2$  group were syncope, limb injury, abdominal pain and vomiting, suicide attempt, lumbar and thoracic vertebral fracture, obesity, and type 1 diabetes mellitus.



**Supplemental Figure 1** Mean FMD in a subset of subjects over 36 months among children and adolescents with HeFH receiving atorvastatin therapy. FMD, flow-mediated dilation; HeFH, heterozygous familial hypercholesterolemia.

**Supplemental Table 1** Concomitant medications used in subjects by TS ( $\geq 10$  total subjects)

	TS 1	TS $\geq 2$	Total
Concomitant medication	139	132	271
Number (%) of subjects with any concomitant drug treatment	97 (69.8)	95 (72.0)	192 (70.8)
Paracetamol	46 (33.1)	42 (31.8)	88 (32.5)
Ibuprofen	28 (20.1)	30 (22.7)	58 (21.4)
Amoxicillin	14 (10.1)	9 (6.8)	23 (8.5)
Clavulin	9 (6.5)	7 (5.3)	16 (5.9)
A/H1N1 influenza pandemic vaccine	9 (6.5)	4 (3.0)	13 (4.8)
Methylphenidate hydrochloride	7 (5.0)	4 (3.0)	11 (4.1)
Azithromycin	4 (2.8)	7 (5.3)	11 (4.1)
Cetirizine hydrochloride	7 (5.0)	3 (2.3)	10 (3.7)
Clarithromycin	8 (5.8)	2 (1.5)	10 (3.7)
Fluticasone propionate	7 (5.0)	3 (2.3)	10 (3.7)
Salbutamol	7 (5.0)	3 (2.3)	10 (3.7)

TS, Tanner stage.

**Supplemental Table 2** The incidence of AEs (all causality) and the most commonly occurring AEs by the dose of maximum duration in patients at TS 1

Type or category of AE	Dose of maximum duration, n (%) of subjects at TS 1					
	5 mg (n = 15)	10 mg (n = 35)	20 mg (n = 54)	40 mg (n = 30)	80 mg (n = 5)	All (n = 139)
Adverse event						
Subjects with any AEs	12 (80.0)	23 (65.7)	48 (88.9)	25 (83.3)	5 (100)	113 (81.3)
Subjects with serious AEs	2 (13.3)	4 (11.4)	4 (7.4)	3 (10.0)	1 (20.0)	14 (10.1)
Subjects with severe AEs	2 (13.3)	3 (8.6)	2 (3.7)	2 (6.7)	0	9 (6.5)
Subjects who discontinued due to AEs, n (%)	1 (6.7)	1 (2.9)	0	1 (3.3)	1 (20.0)	4 (2.9)
Subjects who had their dose reduced or temporarily discontinued due to AEs	1 (6.7)	7 (20.0)	12 (12.2)	6 (20.0)	0	26 (18.7)
Most commonly occurring AEs*, n (%)						
Nasopharyngitis	2 (13.3)	3 (8.6)	14 (25.9)	6 (20.0)	1 (20.0)	26 (18.7)
Headache	1 (6.7)	6 (17.4)	11 (20.4)	7 (23.3)	0	25 (18.0)
Abdominal pain	1 (6.7)	4 (11.4)	11 (20.4)	4 (13.3)	1 (20.0)	21 (15.1)
Upper respiratory tract infection	2 (13.3)	5 (14.3)	8 (14.8)	5 (16.7)	0	20 (14.4)
Gastroenteritis	0	3 (8.6)	8 (14.8)	6 (20.0)	0	17 (12.2)
Pyrexia	0	3 (8.6)	6 (11.1)	5 (16.7)	1 (20.0)	15 (10.8)
Vomiting	0	2 (5.7)	8 (14.8)	4 (13.3)	1 (20.0)	15 (10.8)
Cough	1 (6.7)	4 (11.4)	6 (11.1)	3 (10.0)	0	14 (10.1)
Pain in extremity	1 (6.7)	1 (2.9)	6 (11.1)	5 (16.7)	1 (20.0)	14 (10.1)
Influenza	1 (6.7)	3 (8.6)	5 (9.3)	4 (13.3)	0	13 (9.4)
Rhinitis	1 (6.7)	1 (2.9)	7 (13.0)	3 (10.0)	1 (20.0)	13 (9.4)
Viral upper respiratory tract infection	0	4 (11.4)	7 (13.0)	1 (3.3)	1 (20.0)	13 (9.4)
Arthralgia	1 (6.7)	2 (5.7)	4 (7.4)	3 (10.0)	0	10 (7.2)
Tonsillitis	0	3 (8.6)	3 (5.6)	3 (10.0)	1 (20.0)	10 (7.2)
Bronchitis	1 (6.7)	2 (5.7)	4 (7.4)	2 (6.7)	0	9 (6.5)
Ear infection	2 (13.3)	2 (5.7)	4 (7.4)	0	1 (20.0)	9 (6.5)
Pharyngitis	0	1 (2.9)	4 (7.4)	4 (13.3)	0	9 (6.5)
Diarrhea	0	2 (5.7)	5 (9.3)	1 (3.3)	0	8 (5.8)
Abdominal pain upper	0	1 (2.9)	2 (3.7)	3 (10.0)	1 (20.0)	7 (5.0)
Respiratory tract infection†	1 (6.7)	3 (8.6)	1 (1.9)	2 (6.7)	0	7 (5.0)

AE, adverse event; TS, Tanner stage.

Severe AEs are those that interfere significantly with subject's usual function.

\*AEs observed in  $\geq 5\%$  of all subjects.

**Supplemental Table 3** The incidence of AEs (all causality) and the most commonly occurring AEs by the dose of maximum duration in patients at TS  $\geq 2$ 

Type or category of AE	Dose of maximum duration					
	5 mg (n = 8)	10 mg (n = 44)	20 mg (n = 29)	40 mg (n = 48)	80 mg (n = 3)	All (n = 132)
<b>Adverse event</b>						
Subjects with any AEs, n (%)	6 (75.0)	29 (65.9)	25 (86.21)	42 (87.5)	3 (100.0)	105 (79.6)
Subjects with serious AEs, n (%)	1 (12.5)	2 (4.6)	1 (3.5)	3 (6.3)	0	7 (5.3)
Subjects with severe AEs, n (%)	1 (12.5)	4 (9.1)	2 (6.9)	4 (8.3)	0	11 (8.3)
Subjects who discontinued due to AEs, n (%)	0	2 (4.6)	0	0	0	2 (1.5)
Subjects who had their dose reduced or temporarily discontinued due to AEs, n (%)	0	6 (13.6)	6 (20.7)	8 (16.7)	0	
<b>Most commonly occurring AEs*, n (%)</b>						
Nasopharyngitis	0	8 (18.2)	8 (27.6)	10 (20.8)	0	26 (19.7)
Headache	0	7 (15.9)	10 (34.5)	8 (16.7)	0	25 (18.9)
Influenza	0	2 (4.6)	3 (10.3)	9 (18.8)	0	14 (10.6)
Pyrexia	1 (12.5)	3 (6.8)	2 (6.9)	7 (14.6)	0	13 (9.9)
Gastroenteritis	0	4 (9.1)	3 (10.3)	5 (10.4)	0	12 (9.1)
Cough	0	4 (9.1)	2 (6.9)	5 (10.4)	0	11 (8.3)
Abdominal pain	1 (12.5)	4 (9.1)	4 (13.8)	1 (2.1)	0	10 (7.6)
Myalgia	0	3 (6.8)	3 (10.3)	4 (8.3)	0	10 (7.6)
Upper respiratory tract infection	0	3 (6.8)	3 (10.3)	4 (8.3)	0	10 (7.6)
Blood creatine phosphokinase increase	0	2 (4.6)	3 (10.3)	3 (6.3)	1 (33.3)	9 (6.8)
Diarrhea	0	2 (4.6)	2 (6.9)	5 (10.4)	0	9 (6.8)
Rhinitis	0	4 (9.1)	1 (3.5)	4 (8.3)	0	9 (6.8)
Nausea	0	4 (9.1)	2 (6.9)	2 (4.2)	0	8 (6.1)
Pharyngitis	0	1 (2.3)	4 (13.8)	2 (4.2)	1 (33.3)	8 (6.1)
Vomiting	0	6 (13.6)	0	2 (4.2)	0	8 (6.1)

AE, adverse event; TS, Tanner stage.

Severe AEs are those that interfere significantly with subject's usual function.

\*AEs observed in  $\geq 5\%$  of all subjects.









