Sex differences in cholesterol levels from birth to 19 years of age may lead to increased cholesterol burden in females with FH

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Running title: Lipid profile and sex differences in FH children

Abstract

Background The increased risk of cardiovascular disease in familial hypercholesterolemia (FH) is caused by increased cholesterol burden from birth. Even small elevation in cholesterol level accumulates over time and aggravates atherosclerosis.

Objectives: The aim of the present study was to describe the lipid profile across sex and age in a large cohort of non-statin treated children and adolescents with FH, as this have not clearly been described.

Methods: FH children (438 girls, 452 boys) not receiving lipid-lowering therapy, aged 0-19 years were included, and divided into four age groups (<5, 5-9, 10-14 and 15-19 years). Information was retrieved from the medical records. Total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C) and non-HDL cholesterol (non-HDL-C) and were studied in relation to sex and age by multiple linear regression analysis.

Results: Girls with FH as compared to boys had significantly higher TC, LDL-C and non-HDL-C ($P<0.001$ for all) levels with mean (95% confidence interval) differences of 0.48 mmol/L (0.28, 0.68) (18.6 g/dl), 0.39 mmol/L (0.19, 0.59) (15.08 mg/dl) and 0.42 mmol/L (0.22, 0.63) (16.24 mg/dl), respectively. These estimates did not change after adjustment for age. We also observed sex differences for HDL-C; girls had higher HDL-C in the youngest (< 5 years, $P=0.05$) and oldest age group (15-19 years, $P<0.001$).

Conclusions: FH girls have higher levels of TC, LDL-C, and non-HDL-C levels than boys from birth up to 19 years of age. This may contribute significantly to the total life-long cholesterol-burden in FH women.

Keywords: Familial hypercholesterolemia; children, sex, age, cholesterol

Introduction

Familial hypercholesterolemia (FH) is an inherited, autosomal dominant disorder characterized by reduced capacity to clear low-density lipoprotein (LDL) from the circulation [1]. LDL-cholesterol (LDL-C) levels are high from birth in individuals with FH resulting in high lifelong cholesterol exposure and increased risk of premature cardiovascular disease (CVD) [2-4]. The atherosclerotic process starts early in life, and higher intima media thickness (IMT) of carotid arteries has been reported in children with FH compared to healthy children [5, 6] and IMT has been reported to be higher in boys than girls with FH [7, 8].

Recently, we showed that mean age of first hospitalization for CVD was 45 years in cohort of Norwegian patients with FH [4]. Further, mean age of death was 60 years which is 15 (men) to 21 years (women) earlier than the normal population [3, 9]. An interesting finding was that both age at first CVD event and age at death were similar among FH men and women [3, 9] in contrast to the general population where there is an almost 10 year gap in age at first CVD event between the sexes [10]. The concept of a total lifelong cholesterol burden defining the risk of disease has developed over the last years [2]. In women, pre-pregnancy and pregnancy periods without lipid-lowering therapy (LLT) increase the cholesterol burden, as compared to men. However, small differences in cholesterol levels through lifespan, in men and women, could also play a role for CVD risk. The aim of the present study was therefore to study the lipid profile in a large cohort of untreated FH children in relation to sex and age.

Materials and Methods

Subjects

Data in this analysis was previously collected retrospectively from medical charts as a cross-sectional review. Children with FH followed at the Lipid Clinic, Oslo University Hospital, Oslo, Norway and the Cardiovascular Genetics Center and the Sophia Children's Hospital of the Erasmus MC Rotterdam, the Netherlands, were included in the study. The study sample has been described previously [11]. In brief, children between the age of 0-19 years visiting the Lipid Clinic in Norway between 1990 and September 2010, and children visiting the two Dutch lipid clinics between April 1992 and April 2014 were included. Inclusion criteria were that the children should not be on current lipid-lowering therapy. Nonetheless, fifty-three children were excluded from the analysis due to cholesterol-lowering treatment and therefore data on untreated lipid values were not available for these children. Seventeen of these were female (mean age 11.8 ± 4.2 years) and 36 were male (mean age 12.2 ± 4.5 years). However, also among this subgroup, the females had significant higher TC and LDL-C levels (8.0 mmol/L (309.4 mg/dL) and 6.1 mmol/L (235.9 mg/dL), respectively) compared to the boys (6.9 mmol/L (266.8 mg/dL) and 4.6 mmol/L (177.9 mg/dL), respectively), $P=0.045$ and $P=0.005$, respectively. In addition, in case of siblings, only one sibling was randomly included if the siblings were of same sex; and one of each sex were randomly included in case of multiple siblings. All children had a definite FH diagnosis based on genetic testing or clinically based on the Dutch Lipid Clinic Network classification [2]. The Dutch Lipid Clinic Network classification assign different points for family history of heart disease or hypercholesterolemia, clinical history (e.g. family history of premature coronary heart disease or cerebral or peripheral vascular disease), tendinous xanthomas or arcus cornea, elevated LDL-C levels and/or an identified mutation in the LDL-R gene, apoB100 gene or positive for gain of function PCSK9 mutation. A diagnosis is based upon the total number of points

obtained. Definite FH diagnosis requires more than 8 points, probable FH between 6-8 points, possible FH between 3-5 points and one is classified as unlikely FH if the score is less than 3 points. Demographic characteristics, mutation type, and pretreatment information of weight, height, and levels of blood lipids were collected from the medical records, primarily from the first visit if data were available. Friedewald's equation [12] was used to calculate missing LDL-C values where TC and HDL-C and triglycerides were available (n=116). Blood biochemistry parameters including lipids were measured by standard methods at the Oslo University Hospital, Rikshospitalet, Oslo, Norway (NS-EN ISO 15189:2007 accredited) and at the Erasmus MC, Rotterdam, the Netherlands. Children with FH are usually followed up yearly, but it can be shorter or longer depending on individual patient needs or capacity. In a recent register study, we have shown that children with FH seen at the Lipid Clinic in Oslo in the period 2014-2016 had a mean age at diagnosis of 8.5 years, age at first visit to the clinic were 9.5 years, and age at initiation of lipid lowering therapy (statins) were 12.5 years. Mean time to next planned visit 1.4 years [13]. At the Cardiovascular Genetics Center and Sophia Children's hospital in Rotterdam, once statin therapy is initiated, FH children treated initially have a follow-up visit every 3 to 4 months until they are treated to target (LDLC<3.5 mmol/l) or are using maximum statin therapy available for their age. When they are using stable lipid-lowering therapy they have a yearly follow-up visit. All the pediatric FH children in the Lipid Clinic in Norway and Netherlands included in the present study have been identified through cascade screening as children normally do not have the cholesterol levels measured. More than half of the children diagnosed with FH in Norway are followed in specialized lipid clinics. A smaller number of children are followed in pediatric outpatient clinics at regional hospitals.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Regional Committee of Medical and Health Research

Ethics, south-east region of Norway (no. 2012/2008) where permission to perform the study with passive consent (subjects were contacted by mail and given an opportunity to withdraw consent) was approved. The Medical Ethical Review Committee of the Erasmus MC, the Netherlands considered the protocol non WMO (Wet Medisch Onderzoek) research and therefore it did not have to be reviewed.

Statistics

Age was categorized as <5, 5-9, 10-14 and 15-19 years. Descriptive results are presented as means (95% confidence intervals [CI], medians (25th – 75th percentile) or frequencies (%). Differences between boys and girls were tested by two sample t-test or Mann Whitney U test for continuous variables and Chi-squared test for categorical variables. TC, LDL-C, non-HDL cholesterol and HDL-C were studied in relation to sex and age by multiple linear regression analysis. Results are presented as unadjusted and adjusted regression coefficients with 95% CIs. In the case of interaction between sex and age, we present the results of sex stratified by age groups. The residuals were examined to check model assumptions. P-values ≤ 0.05 were considered significant. The statistical analyses were performed with SPSS version 24.0.

Results

Characteristics

We included 890 children and adolescents with FH aged 0-19 years, none of which were treated with lipid-lowering medication. Of these, 438 were girls (49.2%). Selected characteristics stratified by sex and age are shown in Table 1. There was no significant difference in age ($P=0.16$), body mass index ($P=0.18$) and presence of genetic diagnose ($P=0.98$) between the girls and boys.

Total cholesterol

Table 2 shows the results from the regression analyses. Mean TC was 0.48 mmol/L [95% CI (0.28, 0.68)] (18.56 mg/dL) higher in girls than boys ($P<0.001$) and was almost unchanged after adjustment for age [mean 0.50 mmol/L (19.34 mg/dL), 95% CI (0.30, 0.70)]. Mean TC were consistently higher for girls than boys in all age groups and significantly higher in all age groups except the youngest ($P=0.08$ for age <5 years, $P=0.03$ for age 5-9 years, $P=0.004$ for age 10-14 years and $P=0.002$ for age ≥ 15 years, Supplementary Table 1).

Age was significantly associated with TC ($P<0.001$), and the regression coefficients for age were similar after adjustment for sex (Table 2). Children <5 years and 5-9 years had significantly higher TC than those ≥ 15 years [sex adjusted mean differences (95% CIs) were 0.92 mmol/L (0.50, 1.34) (35.58 mg/dL) and 0.44 (0.15, 0.74) (17.01 mg/dL), respectively]. There was no significant difference in TC between the two oldest age groups [sex adjusted mean differences [(95% CI) for 10-14 versus ≥ 15 years was 0.18 mmol/L (-0.12, 0.47)] (6.96 mg/dL) (Table 2). We found no significant interaction between sex and age (i.e. the effect of sex was similar in all age groups) ($P_{\text{interaction}}=0.55$).

LDL cholesterol

The results of regression analyses for LDL-C showed similar pattern as for TC (Table 2) with a significant effect of sex ($P<0.001$) and age ($P<0.001$), and no significant interaction between sex and age ($P_{\text{interaction}}=0.92$). Mean LDL-C was 0.39 mmol/L [95% CI (0.19, 0.59)] (15.08 mg/dL) higher in girls than boys, ($P<0.00$), and was almost unchanged after adjustment for age [mean 0.41 mmol/L (15.85 mg/dL), 95% CI (0.21, 0.61)]. When comparing the age groups separately, mean LDL-C was only significantly higher for the age group 10-14 years, though borderline significant for the age groups 5-9 years and >15 years ($P=0.17$ for age <5 years, $P=0.06$ for age 5-9 years, $P=0.005$ for age 10-14 years and $P=0.06$ for age ≥ 15 years, (Supplementary Table 1).

Non-HDL cholesterol

The results of regression analyses for non-HDL-C showed similar pattern as for TC and LDL-C (Table 2) with significant effect of sex ($P<0.001$) and age ($P<0.001$), and no significant interaction between sex and age ($P_{\text{interaction}}=0.83$) (Table 2). Mean non-HDL-C was 0.42 mmol/L (16.24 mg/dL) [95% CI 0.22, 0.63] higher in girls than boys, ($P<0.001$), and was almost unchanged after adjustment for age [mean 0.44 mmol/L (17.01 mg/dL), 95% CI (0.24, 0.65)]. Mean non-HDL-C was consistently higher for girls than boys in all age groups except the youngest ($P=0.19$ for age <5 years, $P=0.05$ for age 5-9 years, $P=0.004$ for age 10-14 years and $P=0.002$ for age ≥ 15 years, Supplementary Table 1).

HDL cholesterol

The results of regression analyses for HDL-C revealed a significant interaction between sex and age ($P_{\text{interaction}}<0.001$), i.e. showing different effects of sex by age (Table 2). Mean HDL-C was higher for girls for the youngest (<5 years, $P=0.05$) and oldest age group (15-19 years,

$P<0.001$), whereas HDL-C were almost similar for girls and boys aged 5-9 years ($P=0.70$) and only slightly higher for boys than girls in the age group 10-14 years ($P=0.29$) (Table 2).

Figure 1A-D presents means and 95% CIs for TC, LDL-C, HDL-C and non-HDL-C, stratified by sex and age (see Supplementary Table 1 for the values), illustrating the results from the regression analyses.

Other lipids

Plasma triglycerides, apoB, apoA1, lipoprotein (a) [Lp(a)] and CRP levels did not differ significantly between the sexes in the total cohort or within each age group ($0.08\leq P\leq 1.00$) except for apoA1 in 15-19 year olds, where girls had higher levels than boys ($P<0.001$) (Supplementary Table 2).

Mutation dependency

We investigated the sex difference among the children with a LDL-receptor negative mutation. In total, 261 girls and 263 boys had LDL-receptor negative mutations. The same pattern with increased levels of TC, LDL-C and non-HDL-C levels in girls compared to boys was observed for both children with and without LDL-receptor negative mutation (Supplementary Table 3).

Discussion

In the present study, we show that girls with FH have higher TC, LDL-C and non-HDL-C levels than boys with FH. The average difference in TC and LDL-C between girls and boys with FH was 0.4-0.5 mmol/L (15.47-19.34 mg/dL) leading to an overall increased life-long cholesterol burden. The same sex difference pattern was shown for children with and without LDL-receptor negative mutation. The increased cholesterol burden in female FH patients may further increase risk of premature coronary heart disease (CHD). In fact, recent studies have shown that female FH patients present with premature cardiovascular disease and death at similar age as male FH patients in contrast to the general population where females experience CHD 10 years later than men [3, 4, 14]. However, not all studies have found a difference in age of CHD debut between women and men with FH [15]. Similar levels of TC and LDL-C in children with FH have been reported previously, however without direct comparisons between the sex. Tonstad and coworkers reported higher levels of TC and LDL-C levels in girls [n=29 and mean age 14 years; 8.8 and 7.0 mmol/L, (340.0 and 270.7 mg/dL) respectively] compared to boys [n=61 and mean age 13.9 years; 8.0 and 6.3 mmol/L, (309.4 and 243.6 mg/dL), respectively]. Starr and coworkers developed age- and sex specific LDL-C cutoffs for universal diagnosis of first-degree relatives with FH [16]. The proposed cut-off for LDL-C is higher for girls than boys in the age-groups 0-14 years and 15-24 years. However, relatively few children were included in these age-groups in that study. Nicholls and coworkers investigated cholesterol levels of 115 children (boys and girls) between the age of 3 to 16 years of which 69 had FH [17]. Among the genotyped FH children, they found that FH girls had higher TC levels [8.0 (309,4 mg/dL) and 7.2 mmol/L (278.4 mg/dL), girls and boys, respectively], with no difference in LDL-C but girls had higher HDL-C levels. These data are in line with our results for total cholesterol. However, Nicholls and coworkers explained that the increase in TC may be due to the increased HDL-C levels in girls. In the

present study, we found that not only TC, but also LDL-C and non-HDL-C are higher among girls than boys, thus the increased HDL-C levels found among the youngest and oldest children cannot explain this sex difference alone.

In the present study, the average difference in TC and LDL-C between the sex was 0.4-0.5 mmol/L (15.47-19.34 mg/dL). This is similar to the effect of some LDL-C lowering single-nucleotide polymorphisms (SNPs) which has been associated with a significant lifetime reduced risk of coronary heart disease (CHD) of more than 50% [18-20]. This sex difference in cholesterol levels during the first 20 years of life may increase the total cholesterol burden of 6 to 10 extra cholesterol years. Pregnancy related off-statin periods may further increase the number of cholesterol years. Together with our present observation this may partly explain the previous observation that the gender-gap, with respect to first CVD event and at time of death, in FH subjects is shorter or lacking [3, 4]. Recently, it was shown in a Japanese population of 271 FH patients (mean age 44 and 43 years, women and men, respectively) who did not use lipid-lowering medication that LDL-C was higher among women than men (5.9 and 5.5 mmol/L (228.2 and 212.7mg/dL); women and men, respectively) [21]. Kwiterovich et al observed a non-significant higher LDL-C in FH women compared to men (mean age 37 for the total FH group; 402.2 and 375.3 mg/dL; (10.4 mmol/L and 9.7 mmol/L) respectively) [22]. Taken together, the latter findings suggest that a sex difference in cholesterol levels may also exist in adult FH subjects and underscores the importance of early treatment, particular among girls.

The same pattern with increased TC levels in girls has also been consistently observed in numerous publications among healthy children through large age-spectra and different countries and ethnicities [23-25]. Abe and coworkers identified age and sex-specific reference ranges for non-HDL lipoprotein levels. They included more than 421.000 children from 9 to 16 years of age. Non-HDL-C levels were significant higher in girls than boys [23]. In a

recent Dutch study [26], involving more than 130.000 subjects, it was shown that at 20 years of age men had significantly lower LDL-C levels compared with women (85 vs 97 mg/dL; 2.2 vs 2.5 mmol/L (85.1 vs, 96.7 mg/dL), respectively) and with prominent gender- and age-related differences in all main plasma lipids and lipoproteins. In both genders the LDL-C level increased with age. Porkka et al. showed in 1994, in The Young Finns Study cohort, a difference in plasma lipid levels between male and female children and adolescents (n=2370; ages 9, 12, 15, 18, 21, and 24 years) [24]. Approximately 79%, 33% and 7% of the boys had TC values > 4.0 mmol/L (154.7 mg/dL), > 5.0 mmol/L (193.4 mg/dL) and >6.0 mmol/L (232.0 mg/dL), respectively, whereas the corresponding percentages for females were 87%, 43% and 10%. In a recent German study, lipid levels in healthy children (n=2571) ranging from 6 months to 16 years were analyzed. Consistent with the previous studies, they found that serum concentrations of TC and LDL-C as well as apoB levels were higher in girls than in boys [27]. A systematic literature review from Andersen et al [28] from 1990, concluded that “a distinct sex difference was observed in the majority of the age groups; in the age groups below 25 years and above 50 years, women had higher TC values whereas in the age group 25-50 years, men had higher TC values”, thus in line with the results in the present study. The consistent finding of a small increase in cholesterol levels in healthy girls as compared to healthy boys thus seems to be a general feature in humans [23-25].

Men and women are characterized by different body composition and this seems to be evident even in young pre-puberty children [29]. Girls (age 3-8 years) have higher fat mass (approximately 50%) than boys at same age, despite no difference in age, height and weight [29]. This may lead to increased cholesterol levels in girls as a correlation between total body fat and plasma lipids previously has been shown [30]. Plasma levels of leptin are associated with fat mass and are higher among girls than boys in all age groups [31] and leptin has

previously been shown to be associated with increased TC and LDL-C in boys, but not in girls [32].

Differences in diet, physical activity or lifestyle related factors may play a role. However, the observed difference is consistent across a large age-span and across different countries and ethnicities, thus including different lifestyle and diets according to age. Particularly in the very young age group (0-4 years of age), diet and lifestyle are less likely to cause any difference between sexes. Previously, we observed no major difference in dietary behavior among boys and girls with FH of about 10-14 years of age [33], suggesting dietary difference between girls and boys is not important for the observed difference in LDL-C.

A limitation of the study is the lack of data of fat mass distribution in the children. Moreover despite the large number of FH children included in the present study the youngest and oldest age group had fewer subjects, thereby limiting the power to detect differences in these age groups. A major strength of the study is the large cohort of mainly genetically verified FH children with good characteristics and the consistent finding in the linear regression model with similar values when adjusted for sex or age.

In conclusion, untreated FH girls are characterized by increased levels of TC and LDL-C levels from birth and across all age-groups from birth to 19 years of age compared to FH boys. This may suggest that the total life-long cholesterol-burden for FH girls has been underestimated and underscores the importance of early statin treatment in children with FH and girls in particular.

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Author contribution

Conceived and designed research: KBH, IN, JRvL, MPB; Conducted research: KBH, IN, JRvL, JV, LKLØ, MBV, MPB; Performed statistical analyses: KBH, IN, LKLØ, MBV; Interpreted results: KBH, IN, JRvL, JV, LKLØ, ABG, GL, SMU, KR, MBV, MPB; Responsible for drafting the manuscript: KBH, IN, JRvL, MBV, MPB; Responsibility for final content: KBH, IN, JRvL, JV, LKLØ, ABG, GL, SMU, KR, MBV, MPB; All authors read, critically revised and approved the final manuscript.

References

- 1 Goldstein JL, Brown MS. Familial hypercholesterolemia: pathogenesis of a receptor disease. *Johns Hopkins Med J* 1978; **143**: 8-16.
- 2 Nordestgaard BG, Chapman MJ, Humphries SE, *et al.* 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. *Eur Heart J* 2013; **34**: 3478-90a.
- 3 Krogh HW, Mundal L, Holven KB, Retterstol K. Patients with familial hypercholesterolaemia are characterized by presence of cardiovascular disease at the time of death. *Eur Heart J* 2016; **37**: 1398-405.
- 4 Mundal L, Veierod MB, Halvorsen T, *et al.* Cardiovascular disease in patients with genotyped familial hypercholesterolemia in Norway during 1994-2009, a registry study. *Eur J Prev Cardiol* 2016; **23**: 1962-69.
- 5 Narverud I, Retterstol K, Iversen PO, *et al.* Markers of atherosclerotic development in children with familial hypercholesterolemia: A literature review. *Atherosclerosis* 2014; **235**: 299-309.
- 6 Kusters DM, Wiegman A, Kastelein JJ, Hutten BA. Carotid intima-media thickness in children with familial hypercholesterolemia. *Circ Res* 2014; **114**: 307-10.
- 7 Wiegman A, de GE, Hutten BA, *et al.* Arterial intima-media thickness in children heterozygous for familial hypercholesterolaemia. *Lancet* 2004; **363**: 369-70.
- 8 Tonstad S, Joakimsen O, Stensland-Bugge E, *et al.* Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Biol* 1996; **16**: 984-91.
- 9 Mundal L, Sarancic M, Ose L, *et al.* Mortality among patients with familial hypercholesterolemia: a registry-based study in Norway, 1992-2010. *J Am Heart Assoc* 2014; **3**: e001236.
- 10 Albrektsen G, Heuch I, Lochen ML, *et al.* Lifelong Gender Gap in Risk of Incident Myocardial Infarction: The Tromso Study. *JAMA Intern Med* 2016; **176**: 1673-79.
- 11 Narverud I, van Lennep JR, Christensen JJ, *et al.* Maternal inheritance does not predict cholesterol levels in children with familial hypercholesterolemia. *Atherosclerosis* 2015; **243**: 155-60.
- 12 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499-502.
- 13 Bogsrud MP, Langslet G, Wium C, *et al.* Treatment goal attainment in children with familial hypercholesterolemia: A cohort study of 302 children in Norway. *J Clin Lipidol* 2017. Dec 6. pii: S1933-2874(17)30534-2. doi: 10.1016/j.jacl.2017.11.009. [Epub ahead of print]
- 14 Mundal L, Igland J, Ose L, *et al.* Cardiovascular disease mortality in patients with genetically verified familial hypercholesterolemia in Norway during 1992-2013. *Eur J Prev Cardiol* 2017; **24**: 137-44.
- 15 Jansen AC, van Aalst-Cohen ES, Tanck MW, *et al.* The contribution of classical risk factors to cardiovascular disease in familial hypercholesterolaemia: data in 2400 patients. *J Intern Med* 2004; **256**: 482-90.
- 16 Starr B, Hadfield SG, Hutten BA, *et al.* Development of sensitive and specific age- and gender-specific low-density lipoprotein cholesterol cutoffs for diagnosis of first-degree relatives with familial hypercholesterolaemia in cascade testing. *Clin Chem Lab Med* 2008; **46**: 791-803.
- 17 Nicholls DP, Cather M, Byrne C, Graham CA, Young IS. Diagnosis of heterozygous familial hypercholesterolaemia in children. *Int J Clin Pract* 2008; **62**: 990-4.
- 18 Willer CJ, Sanna S, Jackson AU, *et al.* Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008; **40**: 161-9.
- 19 Kofler BM, Miles EA, Curtis P, *et al.* Apolipoprotein E genotype and the cardiovascular disease risk phenotype: impact of sex and adiposity (the FINGEN study). *Atherosclerosis* 2012; **221**: 467-70.
- 20 Myocardial Infarction Genetics Consortium I, Stitzel NO, Won HH, *et al.* Inactivating mutations in NPC1L1 and protection from coronary heart disease. *N Engl J Med* 2014; **371**: 2072-82.

- 21 Doi T, Kataoka Y, Asaumi Y, *et al.* P631 Sex-related differences in clinical characteristics, low-density lipoprotein cholesterol control and cardiovascular outcomes in familial hypercholesterolemia. *European Heart Journal* 2017; **38**: ehx501.P631-ehx501.P631.
- 22 Kwiterovich PO, Jr., Fredrickson DS, Levy RI. Familial hypercholesterolemia (one form of familial type II hyperlipoproteinemia). A study of its biochemical, genetic and clinical presentation in childhood. *J Clin Invest* 1974; **53**: 1237-49.
- 23 Abe Y, Okada T, Sugiura R, Yamauchi K, Murata M. Reference Ranges for the Non-High-Density Lipoprotein Cholesterol Levels in Japanese Children and Adolescents. *J Atheroscler Thromb* 2015; **22**: 669-75.
- 24 Porkka KV, Viikari JS, Ronnema T, Marniemi J, Akerblom HK. Age and gender specific serum lipid and apolipoprotein fractiles of Finnish children and young adults. The Cardiovascular Risk in Young Finns Study. *Acta Paediatr* 1994; **83**: 838-48.
- 25 Aasvee K, Kurvinen E, Jordania R, Jauhiainen M, Sundvall J. Lipoprotein parameters in relation to other risk factors of atherosclerosis in adults and newborns: Tallinn Young Family Study. *Scand J Clin Lab Invest* 2004; **64**: 245-53.
- 26 Balder JW, de Vries JK, Nolte IM, *et al.* Lipid and lipoprotein reference values from 133,450 Dutch Lifelines participants: Age- and gender-specific baseline lipid values and percentiles. *J Clin Lipidol* 2017; **11**: 1055-64 e6.
- 27 Dathan-Stumpf A, Vogel M, Hiemisch A, *et al.* Pediatric reference data of serum lipids and prevalence of dyslipidemia: Results from a population-based cohort in Germany. *Clin Biochem* 2016; **49**: 740-9.
- 28 Anderson P, Voss A, Horder M. [Distribution of serum total cholesterol in a population with varying risks of cardiovascular disease]. *Ugeskr Laeger* 1990; **152**: 523-6.
- 29 Taylor RW, Gold E, Manning P, Goulding A. Gender differences in body fat content are present well before puberty. *Int J Obes Relat Metab Disord* 1997; **21**: 1082-4.
- 30 Choi JW, Pai SH, Kim SK. Associations between total body fat and serum lipid concentrations in obese human adolescents. *Ann Clin Lab Sci* 2002; **32**: 271-8.
- 31 Lausten-Thomsen U, Christiansen M, Louise Hedley P, *et al.* Reference values for serum leptin in healthy non-obese children and adolescents. *Scand J Clin Lab Invest* 2016; **76**: 561-67.
- 32 Wu YK, Chu NF, Huang YH, Syu JT, Chang JB. BMI, body fat mass and plasma leptin level in relation to cardiovascular diseases risk factors among adolescents in Taitung. *Obes Res Clin Pract* 2016; **10**: 432-41.
- 33 Torvik K, Narverud I, Ottestad I, *et al.* Dietary counseling is associated with an improved lipid profile in children with familial hypercholesterolemia. *Atherosclerosis* 2016; **252**: 21-7.

Figure legends

Figure 1. Mean (95% confidence interval) levels of total cholesterol (A), LDL cholesterol (B), HDL cholesterol (C) and non-HDL cholesterol (D) in FH children stratified by sex and age. LDL, low density lipoprotein; HDL, high density lipoprotein; FH, familial hypercholesterolemia.

Figure 1

A)

B)

C)

D)

Table 1: Characteristics of the FH children

	n	Total	n	FH girls	n	FH boys	P
All							
Total number of patients, n (%)	890		438	438 (49.2)	452	452 (50.8)	
Age, y	890	10.5 (10.3, 10.8)	438	10.7 (10.4, 11.1)	452	10.4 (10.0, 10.7)	0.16
BMI, kg/m ²	725	17.8 (16.0-20.5)	365	18.1 (15.9-20.9)	360	17.7 (16.0-20.3)	0.33
FH diagnosis	890		438		452		0.98
<i>Clinical, n (%)</i>	890	32 (3.5)	438	16 (3.7)	452	16 (3.5)	
<i>Genetic, n (%)</i>	890	858 (96.4)	438	422 (96.3)	452	436 (96.5)	
< 5 years							
Total number of patients, n (%)	78		30	30 (38.5)	48	48 (61.5)	
Age, y	78	3.5 (3.3, 3.8)	30	3.8 (3.4, 4.2)	48	3.3 (3.0, 6.7)	0.06
BMI, kg/m ²	54	15.9 (14.9-16.4)	19	15.5 (14.9-16.2)	35	16.0 (15.1-16.9)	0.30
5-9 years							
Total number of patients, n (%)	336		174	174 (51.8)	162	162 (48.2)	
Age, y	336	7.7 (7.6, 7.9)	174	7.8 (7.6, 8.0)	162	7.7 (7.5, 7.9)	0.68
BMI, kg/m ²	279	16.3 (15.3-18.0)	145	16.4 (15.2-18.4)	134	16.2 (15.3 (17.3)	0.44
10-14 years							
Total number of patients, n (%)	327		153	153 (46.8)	174	174 (53.2)	
Age, y	327	12.3 (12.2, 12.5)	153	12.3 (12.1, 12.6)	174	12.3 (12.1, 12.5)	1.00
BMI, kg/m ²	280	18.8 (16.8-21.0)	136	18.7 (16.7-21.0)	144	18.9 (16.8-21.2)	0.65
15-19 years							
Total number of patients, n (%)	148		80	80 (54.0)	68	68 (45.9)	

Age, y	148	16.7 (16.5, 16.9)	80	16.8 (16.6, 17.1)	68	16.6 (16.3, 16.8)	0.18
BMI, kg/m ²	111	21.6 (20.1-24.2)	64	21.6 (20.3-24.5)	47	21.4 (20.1-23.5)	0.45

Data are shown as mean (95% confidence interval), median (25th – 75th percentile), or n (%).

P-values from independent samples t-test (age) or Mann Whitney U test (BMI) for continuous variables and chi-squared test for categorical variables (FH diagnosis).

FH, Familial hypercholesterolemia; BMI, body mass index.

To Convert From mmol/L to mg/dL. For total, HDL, and LDL cholesterol multiply mmol/L by 38.67. For triglycerides multiply mmol/L by 88.57.

Table 2. Regression analysis of total, LDL-C, HDL-C and non-HDL-cholesterol in relation to sex and age.

	Unadjusted		Adjusted§	
	Regression coefficient (95% CI)	P-value	Regression coefficient (95% CI)	P-value
Total cholesterol				
Sex*	0.48 (0.28, 0.68)	<0.001	0.50 (0.30, 0.70)	<0.001
Age		<0.001		<0.001
<5 years	0.84 (0.42, 1.26)		0.92 (0.50, 1.34)	
5-9 years	0.43 (0.13, 0.73)		0.44 (0.15, 0.74)	
10-14 years	0.14 (-0.16, 0.44)		0.18 (-0.12, 0.47)	
15-19 years	Reference		Reference	
LDL-C				
Sex*	0.39 (0.19, 0.59)	<0.001	0.41 (0.21, 0.61)	<0.001
Age		<0.001		<0.001
<5 years	0.99 (0.57, 1.41)		1.04 (0.63, 1.46)	
5-9 years	0.41 (0.12, 0.71)		0.42 (0.13, 0.71)	
10-14 years	0.10 (-0.20, 0.39)		0.13 (-0.17, 0.42)	
15-19 years	Reference		Reference	
HDL-C†				
<5 years				
Sex*			0.15 (0.001, 0.29)	0.05
5-9 years				
Sex*			0.01 (-0.05, 0.08)	0.70
10-14 years				
Sex*			-0.04 (-0.11, 0.03)	0.29

15-19 years				
Sex*			0.25 (0.15, 0.34)	<0.001
Non-HDL-C				
Sex*	0.42 (0.22, 0.63)	<0.001	0.44 (0.24, 0.65)	<0.001
Age		<0.001		<0.001
<5 years	0.92 (0.49, 1.35)		0.98 (0.56, 1.41)	
5-9 years	0.32 (0.02, 0.62)		0.33 (0.04, 0.63)	
10-14 years	0.05 (-0.25, 0.35)		0.09 (-0.21, 0.38)	
15-19 years	Reference		Reference	

§Mutual adjustment. *Girls vs. boys. †It was a significant interaction between age and sex for HDL-C ($P_{\text{interaction}} < 0.001$), thus the results are presented stratified by sex. CI, confidence interval; LDL-C, low-density cholesterol; HDL-C, high-density cholesterol.

To Convert From mmol/L to mg/dL. For total, HDL, and LDL cholesterol multiply mmol/L by 38.67. For triglycerides multiply mmol/L by 88.57.

Supplementary Table 1: Total, LDL-C, HDL-C and non-HDL cholesterol in FH children

	n	Total	n	FH girls	n	FH boys	P
< 5 years							
Total number of patients, n (%)	78			30 (38.5)		48 (61.5)	
Total cholesterol, mmol/L	78	8.4 (8.0, 8.7)	30	8.8 (8.1, 9.4)	48	8.1 (7.7, 8.6)	0.08
LDL cholesterol, mmol/L	75	6.8 (6.4, 7.1)	30	7.1 (6.4, 7.8)	45	6.5 (6.1, 7.0)	0.17
HDL cholesterol, mmol/L	74	1.16 (1.09, 1.24)	30	1.25 (1.13, 1.38)	44	1.10 (1.02, 1.19)	0.05
Non-HDL cholesterol, mmol/L	74	7.2 (6.8, 7.6)	30	7.5 (6.8, 8.2)	44	7.0 (6.5, 7.5)	0.19
5-9 years							
Total number of patients, n (%)	336			174 (51.8)		162 (48.2)	
Total cholesterol, mmol/L	336	8.0 (7.8, 8.1)	174	8.1 (7.9, 8.4)	162	7.8 (7.5, 8.0)	0.03
LDL cholesterol, mmol/L	332	6.2 (6.0, 6.4)	172	6.3 (6.1, 6.6)	160	6.0 (5.8, 6.3)	0.06
HDL cholesterol, mmol/L	327	1.33 (1.30, 1.36)	171	1.34 (1.29, 1.38)	156	1.33 (1.28, 1.37)	0.70
Non-HDL cholesterol, mmol/L	327	6.6 (6.4, 6.8)	171	6.8 (6.5, 7.0)	156	6.4 (6.2, 6.7)	0.05
10-14 years							
Total number of patients, n (%)	327			153 (46.8)		174 (53.2)	
Total cholesterol, mmol/L	327	7.7 (7.5, 7.8)	153	7.9 (7.7, 8.2)	174	7.4 (7.2, 7.6)	0.004
LDL cholesterol, mmol/L	327	5.9 (5.7, 6.0)	153	6.1 (5.9, 6.4)	174	5.7 (5.5, 5.9)	0.005
HDL cholesterol, mmol/L	320	1.32 (1.29, 1.36)	147	1.30 (1.25, 1.35)	173	1.34 (1.29, 1.39)	0.29
Non-HDL cholesterol, mmol/L	320	6.3 (6.2, 6.5)	147	6.6 (6.3, 6.8)	173	6.1 (5.9, 6.3)	0.004
15-19 years							
Total number of patients, n (%)	148			80 (54.0)		68 (45.9)	
Total cholesterol, mmol/L	148	7.5 (7.3, 7.8)	80	7.9 (7.5, 8.2)	68	7.1 (6.7, 7.5)	0.002
LDL cholesterol, mmol/L	148	5.8 (5.3, 6.0)	80	6.0 (5.6, 6.3)	68	5.5 (5.2, 5.9)	0.06

HDL cholesterol, mmol/L	146	1.23 (1.18, 1.28)	79	1.34 (1.27, 1.41)	67	1.09 (1.02, 1.16)	<0.001
Non-HDL cholesterol, mmol/L	146	6.3 (6.0, 6.5)	79	6.6 (6.2, 6.9)	67	6.0 (5.6, 6.3)	0.02

Data are shown as mean (95% CI) or n (%).

P-values from independent samples t-test. LDL: Low-density lipoprotein. HDL: High-density lipoprotein.

FH, Familial hypercholesterolemia.

To Convert From mmol/L to mg/dL. For total, HDL, and LDL cholesterol multiply mmol/L by 38.67. For triglycerides multiply mmol/L by 88.57.

Supplementary Table 2: Characteristics of the FH children

	n	Total	n	FH girls	n	FH boys	P
< 5 years							
Triglycerides, mmol/L	77	0.8 (0.7-1.0)	30	0.8 (0.7-1.0)	47	0.9 (0.7-1.2)	0.31
apoB, g/L	33	1.9 (1.8, 2.0)	14	2.0 (1.8, 2.2)	19	1.9 (1.6, 2.1)	0.46
apoA1, g/L	31	1.2 (1.1, 1.2)	13	1.2 (1.1, 1.2)	18	1.2 (1.1, 1.3)	0.44
CRP, mg/L	7	0.6 (0.4-1.0)	4	0.5 (0.3-0.9)	3	1.0 (0.4-1.0)	0.46
Lp(a), mg/L	25	150 (105-340)	10	279 (210-554)	15	113 (105-276)	0.10
5-9 years							
Triglycerides, mmol/L	323	0.7 (0.5-0.9)	168	0.7 (0.6-0.9)	155	0.7 (0.5-1.0)	0.46
apoB, g/L	166	1.6 (1.5, 1.7)	80	1.7 (1.6, 1.8)	86	1.6 (1.5, 1.6)	0.10
apoA1, g/L	164	1.3 (1.2, 1.3)	79	1.3 (1.2, 1.3)	85	1.3 (1.2, 1.3)	0.80
CRP, mg/L	77	1.0 (0.8-1.0)	39	1.0 (0.6-1.0)	38	1.0 (0.9-1.0)	0.66
Lp(a), mg/L	162	258 (118-526)	77	230 (105-543)	85	263 (119-521)	0.84
10-14 years							
Triglycerides, mmol/L	316	0.9 (0.6-1.2)	147	0.9 (0.6-1.2)	169	0.8 (0.6-1.2)	0.13
apoB, g/L	162	1.5 (1.4, 1.5)	73	1.5 (1.4, 1.6)	89	1.4 (1.4, 1.5)	0.24
apoA1, g/L	149	1.3 (1.2, 1.3)	68	1.3 (1.2, 1.3)	81	1.3 (1.3, 1.4)	0.19
CRP, mg/L	79	1.0 (0.9-1.5)	29	1.0 (1.0-2.7)	50	1.0 (0.8-1.0)	0.08
Lp(a), mg/L	166	241 (107-540)	77	267 (108-673)	89	226 (105-448)	0.40
15-19 years							
Triglycerides, mmol/L	145	0.9 (0.6-1.3)	79	0.9 (0.6-1.4)	66	0.9 (0.6-1.2)	1.00
apoB, g/L	83	1.5 (1.4, 1.6)	50	1.5 (1.4, 1.6)	33	1.4 (1.3, 1.6)	0.65
apoA1, g/L	81	1.3 (1.2, 1.3)	49	1.3 (1.3, 1.4)	32	1.1 (1.1, 1.2)	<0.001
CRP, mg/L	45	1.0 (1.0-2.2)	28	1.0 (1.0-2.2)	17	1.0 (1.0-1.9)	0.75

Lp(a), mg/L	88	245 (105-579)	54	331 (114-389)	34	138 (88-519)	0.10
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Data are shown as mean (95% confidence interval) or median (25th – 75th percentile).

P-values from independent samples t-test (apoA1 and apoB) or Mann Whitney U test (triglycerides, CRP and Lp(a)).

FH, familial hypercholesterolemia; apo, apolipoprotein, CRP, C-reactive protein; Lp(a), lipoprotein(a).

To Convert From mmol/L to mg/dL. For total, HDL, and LDL cholesterol multiply mmol/L by 38.67. For triglycerides multiply mmol/L by 88.57.

Supplementary Table 3:
Characteristics in relation to LDL-receptor (LDLR) mutation type in FH children

	LDLR negative mutation types					Other LDLR mutation types				
	n	FH girls	n	FH boys	P ¹	n	FH girls	n	FH boys	P ²
Total number of patients, n (%)	524	261 (49.8)		263 (50.2)		297	145 (48.8)		152 (51.2)	
Age, y	261	10.5 (10.0, 11.0)	263	9.8 (9.2, 10.3)	0.041	145	11.2 (10.6, 11.9)	152	11.3 (10.7, 11.9)	0.86
Total cholesterol, mmol/L	261	8.3 (8.1, 8.5)	263	7.9 (7.7, 8.1)	0.001	145	7.7 (7.5, 7.9)	152	7.2 (6.9, 7.4)	0.002
LDL cholesterol, mmol/L	260	6.6 (6.4, 6.7)	261	6.2 (6.0, 6.4)	0.005	144	5.8 (5.6, 6.1)	151	5.4 (5.2, 5.6)	0.02
HDL cholesterol, mmol/L	255	1.27 (1.24, 1.31)	256	1.23 (1.19, 1.27)	0.10	142	1.38 (1.33, 1.43)	148	1.33 (1.28, 1.38)	0.13
Non-HDL cholesterol, mmol/L	255	7.0 (6.8, 7.2)	256	6.6 (6.5, 6.8)	0.004	142	3.3 (6.1, 6.5)	148	5.8 (5.6, 6.1)	0.006
Triglycerides, mmol/L	253	0.8 (0.6-1.1)	258	0.8 (0.6-1.1)	0.94	140	0.9 (0.6-1.2)	146	0.8 (0.6-1.1)	0.19
apoB, g/L	127	1.7 (1.7, 1.8)	130	1.6 (1.6, 1.7)	0.09	73	1.4 (1.3, 1.5)	79	1.3 (1.2, 1.4)	0.14
apoA1, g/L	119	1.2 (1.2, 1.3)	123	1.2 (1.2, 1.3)	0.60	72	1.3 (1.3, 1.4)	75	1.3 (1.3, 1.4)	0.84
CRP, mg/L	52	1.0 (0.6-1.9)	50	1.0 (0.8-1.0)	0.35	38	1.0 (1.0-2.0)	47	1.0 (1.0-1.0)	0.33
Lp(a), mg/L	118	337 (142-657)	116	180 (105-390)	0.002	81	211 (105-441)	90	254 (119-451)	0.32

Data are shown as mean (95% confidence interval) or median (25th – 75th percentile) or n (%).

¹ LDL-R negative; Girls vs boys; ² other LDL-R; Girls vs boys.

P-values from independent samples t-test or Mann Whitney U test (only triglycerides, CRP and Lp (a)).

FH, Familial hypercholesterolemia; LDLR, low-density lipoprotein receptor; HDL, high-density lipoprotein; Apo, apolipoprotein, CRP, C-reactive protein; Lp(a), lipoprotein(a).
 To Convert From mmol/L to mg/dL. For total, HDL, and LDL cholesterol multiply mmol/L by 38.67. For triglycerides multiply mmol/L by 88.57.