Maternal inheritance does not predict cholesterol levels in children with familial hypercholesterolemia

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ABSTRACT

Background and aims: Pregnancy exerts metabolic changes with increasing levels of total cholesterol and triglycerides as prominent features. Maternal hypercholesterolemia may thus contribute to an unfavorable in utero environment potentially influencing the susceptibility of adult cardiovascular disease in the offspring. We investigated the impact of maternal familial hypercholesterolemia (FH) on pre-treatment plasma lipids and C-reactive protein (CRP) levels in non-statin treated FH children.
Methods: Children with FH (n = 1063) aged between 0 and 19 years were included. Of these, 500 had inherited FH maternally, 563 paternally and 97.6% had a verified FH mutation. Information about inheritance, mutation type and pretreatment levels of blood lipids and CRP was retrieved from the medical records.
Results: There were no significant differences in the plasma levels of lipids and C-reactive protein (CRP) in children with maternal FH compared with children with paternal FH, ($0.12 \leq P \leq 0.90$). Independent of which parent transmitted FH, children with LDL receptor negative mutations had significantly higher levels of total and LDL cholesterol and Apolipoprotein (Apo) B, and lower levels of HDL cholesterol and ApoA1, compared with children with other LDL receptor mutations ($P < 0.001$).
Conclusion: Maternal inheritance of FH was not associated with detectable long-term effects in the offspring's phenotype measured by adverse lipid profiles and increased CRP levels, whereas a LDL receptor negative mutation was associated with an unfavorably phenotype in FH offspring. Our findings do not support the fetal origin of adulthood disease hypothesis, while at the same time not excluding the hypothesis since other pathways leading to atherosclerosis may be involved.

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1. Introduction

Atherosclerosis is the underlying cause of most cardiovascular diseases (CVDs), and is driven by an interrelated network of

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abnormalities in lipids, inflammatory and hemostatic pathways, including immune cells and a variety of mediators prompting this progressive process [1]. The atherogenesis seems to start early in life, and interestingly, a pregnancy itself exerts metabolic changes with increasing levels of total cholesterol and triglyceride from first to third trimester as a prominent feature [2]. Maternal hypercholesterolemia may thus contribute to an unfavorable *in utero* environment which could lead to an increased susceptibility of CVD in the offspring later in life [3–5]. Indeed, oxidation of low density lipoprotein (LDL) and fatty streak formation occurs during fetal development and maternal hypercholesterolemia may potentiate these processes [6]. Additionally, maternal hypercholesterolemia during pregnancy exacerbates atherogenesis in children aged 1–13 years as shown by autopsy in deceased children [7,8]. Taken together, existing data suggest that maternal hypercholesterolemia can adversely influence long-term health in the offspring, but the mechanisms are far from clear.

Hypercholesterolemia is highly prevalent, therefore, any association between maternal cholesterol level and disease susceptibility in the offspring is important to understand. However, maternal cholesterol level is strongly influenced by factors such as diet and lifestyle, making it challenging to study the isolated effect of maternal hypercholesterolemia on cardiovascular risk factors in their offspring. Familial hypercholesterolemia (FH) is an inherited disorder with a major locus effect caused by mutations in the LDL receptor-(*LDLR*), apolipoprotein B- (apoB) (*APOB*) or proprotein convertase subtilisin/kexin type 9 (*PCSK9*) -gene [9–11]. FH may therefore serve as a model disease to study the isolated effect of maternal hypercholesterolemia on CVD development and risk factors in their children.

Pregnant women with FH have higher plasma lipid levels and are more pro-coagulant compared with healthy pregnant women [3,4]. FH subjects with maternal inheritance have higher all-cause mortality than FH subjects with paternal inheritance [12], but findings on plasma lipids are inconsistent [13,14]. At adult age, heterozygous FH shows large phenotypic variation related to environmental and genetic factors, whereas children with FH show a more homogenous phenotype. Therefore the effect of maternal inheritance should be assessed before exposure to these additional factors [15]. Notably, children with FH have raised plasma cholesterol and increased intima-media thickness (IMT) of their carotid arteries compared with healthy non-FH children [16]. Based on this accelerated atherosclerotic process, children with FH represent a unique model system to investigate the effect of maternal hypercholesterolemia on CVD and related risk factors such as lipids and inflammatory markers.

The aim of the present study was to determine the impact of maternal and paternal FH on pre-treatment plasma lipids and C-reactive protein (CRP) in children with FH. To validate our study we also assessed the known effect of LDL receptor mutation type on the same markers.

2. Materials and methods

2.1. Subjects

Subjects with FH from 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 recruited to the study. All Norwegian children had age below 20 years and all Dutch children were below 19 years. From the Norwegian database, we included all subjects with an International Classification of Diseases (ICD)-10 primary diagnosis of Familial Hypercholesterolemia between 1990 and September 2010. The Dutch database consisted of children with FH, who

visited the outpatient clinics between April 1992 and April 2014. Exclusion criteria were 1) no definite FH diagnosis, 2) unknown inheritance status, 3) no medical record at the outpatient clinics, 4) above 18 or 19 years at first visit at the respective outpatient clinic, 5) deceased patients, 6) homozygous or compound heterozygous FH diagnosis, 7) growth hormone replacement therapy, 8) unknown lipid profile and 9) currently on cholesterol-lowering medication. In addition, one patient was excluded due to long-term case history of osteogenesis imperfecta (Fig. 1). All participants had a definite FH diagnosis based on genetic testing or clinically defined by the Dutch Lipid Clinic Network classification (World Health Organization publication no WHO/HGN/FH/CONS/99.2) where definite (certain) FH is defined with a score of 8 or more. Patients were categorized into those in whom FH was transmitted by the mother (maternal FH) or by the father (paternal FH). For three patients, FH inheritance was unknown since both parents had heterozygous FH and the mutation type was either similar in both parents (n = 1) or unknown in the child (n = 2). Nevertheless, as these patients had been exposed to a familial hypercholesterolemic intrauterine environment, they were categorized as maternal FH. From the medical records, demographic characteristics, mutation type, family history of early CVD, and pre-treatment information of weight, height, Achilles tendon thickening and levels of blood lipids and CRP was recorded. For lipoprotein (a) (Lp[a]), samples below the different detection limits were set to the detection limit of 105 mg/l. The method for CRP measurements had changed during the period in which the children had visited the out-patient clinic. The old CRP methods had cut-off values for CRP measurements <5 mg/l or <8 mg/l. In the current study, these measurements were excluded as there was no knowledge as to whether the actual value was <1 mg/l or closer to 5 or 8 mg/l. Furthermore, when there was no absolute value measured and only cut-off values were registered (as <1 mg/l or <0.6 mg/l), these values were both set to 1 mg/l in the analysis. Finally, samples with values > 20 mg/l were set to 20 mg/l. FH children with a mutation in the LDL receptor were categorized into two groups: LDL receptor negative mutations including class 1 and 2A mutations comprising nonsense, splice site mutations, or large rearrangements [17,18] and all remaining LDL receptor mutations (defective and unclassified), respectively. Blood biochemistry parameters including lipids and CRP 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.

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 where permission to perform the study with passive consent (where the subjects were 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.

2.2. Statistics

To account for familial dependency, differences in lipids and CRP between children with maternal and paternal inherited FH were tested using a random intercept linear mixed model, adjusting for between-family variation, age, gender and body mass index (BMI) in addition to an indicator for paternal or maternal FH inheritance. In secondary analyses, indicators for LDL receptor mutation type were included. All linear and logistic mixed models were fitted using the package *lme4*, while P-values for the fixed effects were calculated using the package *lmerTest*, both in the open-source
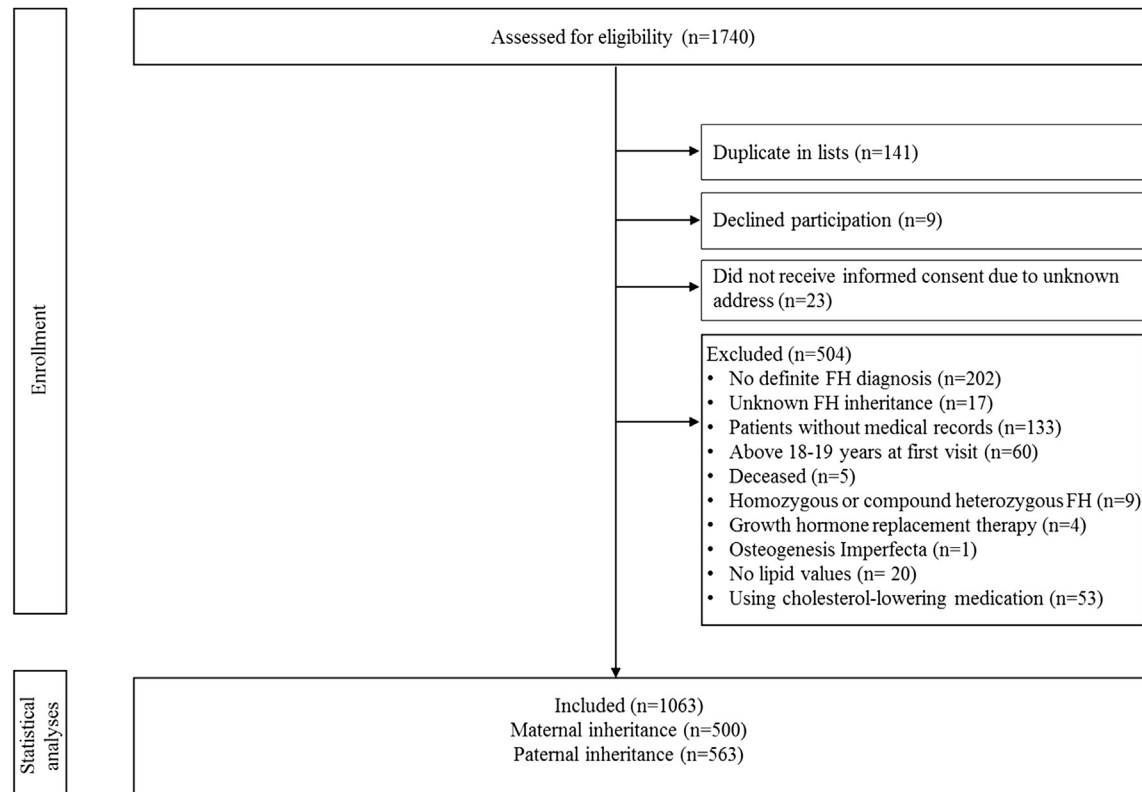
Fig. 1. Flow-chart of the included study population.

statistical software R, version 3.1.0 [19]. *P*-values <0.05 were considered statistically significant. As adjusted and unadjusted effect estimates were not very different; unadjusted *P*-values are given in all tables.

The analyses were also performed by an independent sample t-test comparing the two groups (FH children with maternal vs paternal inheritance) and using unique parent—child pairs from the study sample (n = 777), where the youngest child (among the siblings) in each family was included in the analysis, resulting in similar findings (data not shown).

Data are described by mean and standard deviation (SD) if normally distributed, or median and 25th—75th percentile for variables without normal distribution.

3. Results

3.1. Subject characteristics

In total 1063 children with FH aged 10.7 years (mean) between 0 and 19 years (min—max) were eligible for comparison (Fig. 1), whereof 500 (47%) had inherited FH maternally and 563 (53%) paternally. Subject characteristics are given in Table 1. Of the study sample, 97.6% had a verified FH mutation in the family, whereas the remaining 2.4% had a clinical diagnosis. In total, 125 different LDL receptor mutations were found. Additionally, one mutation for *PCSK9* and three for *APOB* were present. Totally, 624 (63.6%) children with genetically verified FH were categorized to have a LDL receptor negative mutation, which is similar to previous reported data [20]. Baseline characteristics were similar in the Norwegian (n = 808) and Dutch (n = 255) study samples (Table 1). There was a positive significant correlation between BMI and CRP in the FH subjects (n = 241) (r_{sp} = 0.213; *P* = 0.001).

3.2. Effect of FH inheritance on blood lipids and CRP

Children with maternal FH did not have significantly different pre-treatment levels of total, LDL or high density lipoprotein (HDL) cholesterol, triglycerides, ApoA1, ApoB, Lp(a) LDL/HDL and ApoB/ApoA1 than the children with paternal FH ($0.12 \leq P \leq 0.90$) (Table 2). To investigate the effect of the type of FH transmission on markers of systemic inflammation with relevance to atherosclerosis, CRP was available from a subgroup of the Norwegian FH children (n = 248). No significant differences were found in pre-treatment levels of CRP between children with maternal and paternal FH (*P* = 0.18). Gender, age and distribution of LDL receptor-negative mutations were similar among the children with maternal and paternal inheritance (Table 2). Stratified by age (below or above 12 years), there were no significant differences in plasma total, LDL or HDL cholesterol related to inheritance of FH in children below (n = 658) or above (n = 405) 12 years of age ($0.18 \leq P \leq 0.99$) (data not shown). However, a small but significantly higher plasma triglyceride level was detected in children above the age of 12 years with maternal inheritance of FH (n = 183) [1.0 (0.7—1.3) mmol/L] compared with children with paternal inheritance (n = 209) [0.9 (0.6—1.2) mmol/L] (*P* = 0.02).

3.3. Effect of LDL receptor negative mutations on blood lipids and CRP

We next investigated differences in lipoproteins and CRP in FH children with LDL receptor negative mutations (n = 624) compared to FH children with other mutations (n = 357), excluding children with mutations in *APOB* and *PCSK9*. Children with LDL receptor negative mutations had significantly higher levels of total and LDL cholesterol in addition to ApoB, and lower levels of HDL cholesterol and ApoA1 compared with children with other LDL receptor

Table 1
Characteristics of study sample.

	Total study sample	n	Norwegian sample	n	Dutch sample	n
Age, years	10.7 (4.0)	1063	10.7 (4.0)	808	10.7 (4.1)	255
Females, n (%)	528 (49.7)	1063	394 (48.8)	808	134 (52.5)	255
Weight, kg	39.0 (26.5–53.6)	896	38.0 (26.3–53.0)	675	41.5 (27.1–54.8)	221
Height, cm	147 (130–162)	881	146 (129–161)	662	151 (131–165)	219
BMI, kg/m^2	17.9 (16.0–20.6)	872	17.9 (16.0–20.6)	654	18.1 (16.0–20.6)	218
Achilles tendon thickening, n (%)	36 (4.5)	804	30 (5.1)	589	6 (2.8)	215
Mutation-verified FH within family, n (%)	1038 (97.6)	1063	786 (97.3)	808	252 (98.8)	255
Number of different LDL receptor mutations	125	981	76	752	61	229
LDL receptor negative mutations, n (%)	624 (63.6)	981	468 (62.2)	752	156 (68.6)	229

Data are presented as mean (SD) or median (25th–75th percentile) for continuous variables, and as frequencies (%) for categorical variables.
BMI: body mass index, FH: familial hypercholesterolemia, LDL: low-density lipoprotein, CVD: cardiovascular disease.

Table 2
Biochemical and clinical parameters of all children and subdivided by inheritance.

	Total population	n	Maternal inheritance	n	Paternal inheritance	n	P value
Descriptives:							
Females, n (%)	528 (49.7)	1063	241 (48.2)	500	287 (51.0)	563	
Age, years	10.7 (4.0)	1063	10.7 (4.0)	500	10.7 (4.0)	563	
LDL receptor negative mutations, n (%)	624 (63.6)	981	293 (63.0)	465	331 (64.1)	516	
Lipids:							
Total cholesterol, mmol/l	7.8 (1.5)	1063	7.9 (1.6)	500	7.7 (1.5)	563	0.12
LDL cholesterol, mmol/l	5.9 (1.5)	918	6.0 (1.6)	434	5.8 (1.4)	484	0.15
HDL cholesterol, mmol/l	1.3 (0.3)	1035	1.3 (0.3)	485	1.3 (0.3)	550	0.74
Triglycerides, mmol/l	0.8 (0.6–1.1)	1028	0.8 (0.6–1.1)	484	0.8 (0.6–1.1)	544	0.24
ApoA1, g/l	1.3 (0.2)	509	1.3 (0.2)	237	1.2 (1.1–1.4)	272	0.63
ApoB, g/l	1.6 (0.5)	528	1.5 (0.4)	245	1.5 (1.3–1.8)	283	0.77
Lp (a), mg/l	242 (105–514)	521	220 (105–502)	247	256 (105–531)	274	0.90
LDL/HDL ratio	4.5 (3.5–5.7)	897	4.6 (3.5–5.8)	423	4.4 (3.4–5.5)	474	0.13
ApoB/ApoA1 ratio	1.2 (0.9–1.6)	508	1.2 (0.9–1.6)	237	1.2 (0.9–1.5)	271	0.53
Inflammation markers:							
CRP, mg/l	1.0 (0.9–1.2)	248	1.0 (0.8–1.4)	137	1.0 (0.9–1.0)	111	0.18

Data are presented as mean (SD) or median (25th–75th percentile) for continuous variables and as frequencies (%) for categorical variables.
LDL: low-density lipoprotein, FH: familial hypercholesterolemia, CVD: cardiovascular disease, HDL: high-density lipoprotein.
Apo B/A1: apolipoprotein B/A1, Lp (a): lipoprotein (a), CRP: C-Reactive protein.

mutations (P < 0.001) (Table 3). Moreover, both the LDL/HDL and the ApoB/ApoA1 ratios were significantly different between the groups (P < 0.001). In contrast, no significant differences were found in plasma levels of triglycerides, Lp(a) and CRP levels between the two groups (0.11 ≤ P ≤ 0.68) (Table 3). Finally, no significant difference in any of the plasma lipids or CRP was observed in children with LDL receptor negative mutation with maternal versus paternal transmission of FH (0.25 ≤ P ≤ 0.90) (data not shown).

Table 3
Biochemical and clinical parameters of children with LDL receptor negative and non-LDL receptor negative mutations.

	LDL receptor negative mutations	n	Other mutations	n	P value
Descriptives:					
Females, n (%)	312 (50.0)	624	178 (49.9)	357	
Age, years	10.2 (7.6–13.1)	624	11.4 (8.8–13.9)	357	
Lipids:					
Total cholesterol, mmol/l	8.1 (1.5)	624	7.4 (1.5)	357	<0.001
LDL cholesterol, mmol/l	6.2 (1.5)	516	5.5 (1.4)	328	<0.001
HDL cholesterol, mmol/l	1.2 (1.0–1.4)	609	1.3 (1.1–1.5)	347	<0.001
Triglycerides, mmol/l	0.8 (0.6–1.1)	611	0.8 (0.6–1.1)	340	0.68
ApoA1, g/l	1.2 (1.1–1.4)	286	1.3 (1.2–1.4)	181	0.003
ApoB, g/l	1.6 (1.4–2.0)	301	1.3 (1.1–1.6)	186	<0.001
Lp (a), mg/l	257 (105–531)	274	219 (105–433)	206	0.60
LDL/HDL ratio	4.9 (3.8–6.0)	505	4.0 (3.1–5.1)	320	<0.001
ApoB/ApoA1 ratio	1.4 (1.0–1.7)	286	1.0 (0.8–1.3)	181	<0.001
Inflammation markers:					
CRP, mg/l	1.0 (0.6–1.1)	121	1.0 (1.0–1.2)	104	0.11

Data are presented as mean (SD) or median (25th–75th percentile) for continuous variables and as frequencies (%) for categorical variables.
LDL: low-density lipoprotein, FH: familial hypercholesterolemia, CVD: cardiovascular disease, HDL: high-density lipoprotein.
Apo B/A1: apolipoprotein B/A1, Lp (a): lipoprotein (a), CRP: C-reactive protein.

4. Discussion

In the present study, non-statin treated children with maternal FH inheritance did not have significantly different plasma levels of lipids or CRP than children with paternal FH inheritance. However, FH children with LDL receptor negative mutations, regardless of maternal or paternal FH origin, had a more unfavorable lipid profile than children with other LDL receptor mutations. Moreover, the median CRP levels were similar in these two groups with different types of mutations.

Cholesterol-lowering drugs are contraindicated during pregnancy and lactation, and LDL-cholesterol may increase 2–3-fold in FH women due to the lack of medication combined with the normal physiological increase during pregnancy [3]. The consequence of this potentially unfavorably "in utero" environment is unknown. It has been hypothesized that offspring born of FH women could carry an increased risk for disease later in life i.e. in adolescence and adulthood, compared to offspring with paternal FH. Despite these suggestions of an unfavorable "in utero" effect, studies have produced inconsistent results so far, confirming the effects of the type of FH transmission on cardiovascular risk in the offspring [12–14,21]. Tonstad and co-workers found no significant difference in the carotid IMT and prevalence of atherosclerotic plaques in children who had inherited FH maternally or paternally [21], and recently Kusters et al. found no difference in cardiovascular risk markers between offspring from FH mothers and FH fathers [13]. In the latter study, a pooled analysis of LDL cholesterol, triglycerides or IMT was performed from three different FH cohorts with age span 0–18 years, 8–30 years and 18–60 years, respectively. In contrast, van Graf et al. [14] found that maternal (compared to paternal) hereditary hypercholesterolemia led to increase in total cholesterol, LDL cholesterol and ApoB levels, i.e. established risk markers for CVD, in their adult offspring. We have previously observed, in a small study, that healthy siblings and FH children, born from mothers with FH had increased levels of certain haemostatic markers compared to healthy control children [22]. Moreover, a Dutch study which was performed in a pedigree before statins were used, showed higher all-cause mortality rates, the most indisputable endpoint, when FH is transmitted by the mother, supporting the fetal origin of adulthood disease hypothesis [12]. In the present study, examining a large number of FH children, we show that the unfavorably maternal environment, does not seem to induce a long-term unfavorably phenotype with regard to plasma lipid and CRP levels. Our data extend the findings in a recent large Norwegian-based registry study comprising about 2000 births, where it was demonstrated that women with FH did not have a higher risk of preterm delivery or of having infants with low birth weight or congenital malformations than women in general [23], indicating no detrimental effect of hypercholesterolemia on birth outcomes.

Our findings confirm that FH children with LDL receptor negative mutations, as with adult FH subjects, are characterized with higher total and LDL cholesterol as previously described in a smaller study and among adult FH subjects [20,24]. Our data extend these previous observations by showing that these children are characterized by a more overall unfavorably lipid profile including not only higher total and LDL cholesterol plasma levels, but concomitantly reduced HDL cholesterol and ApoA1 plasma levels. However, we did not find increased CRP levels among the carriers of LDL receptor negative mutations. Earlier studies have shown that clinical symptoms of FH and the risk of CVD are characterized by a low-grade systemic inflammation [25,26]. Our findings underscore that the FH genotype strongly influences the phenotypic expression, but the early stages of atherogenesis in young FH children are not reflected in CRP levels. Previously, we have observed significantly increased carotid IMT's in children with heterozygous FH

from age 12 years onwards [27]. Therefore, the children in our present study might have been too young in order for us to achieve differences in the markers of atherosclerosis. However, although CRP is a reliable marker of inflammation in adults, we cannot rule out the possibility that other relevant inflammatory markers may have shown differences. Indeed, in an earlier study we found that children with FH have an inflammatory imbalance [28] between tumor necrosis factor-α and interleukin-10 compared with controls [29], despite similar plasma levels of CRP. Thus, although our present findings do not support that maternal hypercholesterolemia may influence atherosclerosis in offspring, this possibility should not be excluded.

The major strength of the present study is the large cohort of non-statin treated FH children. Hypercholesterolemia in adults is markedly influenced by lifestyle factors. These factors represent an important source of confounders for studies on the pure role of LDL in inflammation and atherosclerosis. A limitation of the study is the lack of end-points, surrogate end-points such as measurement of IMT and measurement of other markers of the atherosclerotic process such as endothelial dysfunction markers. Moreover, a limitation when classifying the LDL-receptor negative mutations is that functional data on LDL receptor activity does not exists for all mutation-types. We have divided the mutations according to previous published data [17] on classification, but we cannot rule out the possibility of misclassification.

In conclusion, maternal transmission of LDL receptor mutations did not contribute to deteriorated lipid profiles in the FH offspring. Although children with LDL receptor negative mutations had a more unfavorable lipid profile than those with mutations with residual function, we did not find differences in CRP levels between these two groups. In children with FH, lifelong exposure to hypercholesterolemia (cholesterol year score) is probably the main driver of atherosclerosis rather than maternal/paternal inheritance of the condition. Thus, our findings do not support the fetal origin of adulthood disease hypothesis, while at the same time not excluding the hypothesis since other pathways leading to atherosclerosis may be involved such as endothelial dysfunction. Future research is required including measurement of markers of endothelial dysfunction, a wider spectrum of inflammatory markers and other more specific markers of the atherosclerotic process after extended follow-up.

Conflict of interest

During the past five years Retterstøl has received honoraria for lectures or expert meetings from Merck, Pfizer, Mills DA, Melk.no, Apotek1, Pronova, Amgen, Genzyme, and Sanofi; none of which are related to the contents of this manuscript. During the past five years Holven has received research grants or honoraria from Tine, Mills, and Olympic Seafood, and Amgen; none of which are related to the contents of this manuscript. The rest of the authors declare no conflict of interest.

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