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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 pretreatment 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.

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