Maternal familial hypercholesterolaemia (FH) confers altered haemostatic profile in offspring with and without FH

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A b s t r a c t

Introduction: Patients with familial hypercholesterolaemia (FH) are characterized by high total and LDL cholesterol. Pregnant women with FH have higher absolute levels of total and LDL cholesterol, and a more pro-coagulant pattern compared with healthy pregnant women. Maternal hypercholesterolaemia has been shown to affect early atherosclerosis formation in the offspring. The aim of the present study was to investigate whether maternal FH leads to differences in plasma or serum levels of haemostatic and fibrinolytic markers in children with and without FH born of mothers with FH compared to control children born of non-FH mothers.

Methods and results: Children with (n = 9) and without (n = 7) FH born of mothers with FH, as well as control children (n = 16) born of non-FH mothers were included in the study. The concentrations of tissue plasminogen activator, plasminogen activator inhibitor (PAI-1), tissue factor (TF), TF pathway inhibitor (TFPI), thrombomodulin, fibrinogen, prothrombin fragment 1 + 2 and von Willebrand Factor were measured. Our findings show i) higher levels of PAI-1 and TFPI in children with and without FH born of mothers with FH compared with control children, ii) lower levels of thrombomodulin in children with FH compared with control children, and iii) significant correlations between maternal PAI-1 levels during pregnancy and PAI-1 levels in the offspring. Conclusions: We found that maternal FH may confer an unfavourable phenotype by affecting haemostatic and fibrinolytic markers in offspring independent of the children’s FH status. However, the association between maternal hypercholesterolaemia and haemostatic risk markers in the offspring needs to be further elucidated.

Abbreviations: Apo, apolipoprotein; BMI, body mass index; CV, coefficient of variation; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; F1 + 2, prothrombin fragment 1 + 2; FH, familial hypercholesterolaemia; LDL, low density lipoprotein; PAI-1, plasminogen activator inhibitor-1; TF, tissue factor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator; vWF, von Willebrand factor.

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Introduction

Atherosclerosis is the underlying mechanism for most cardiovascular disease (CVD) [1], where lipids, inflammation and haemostatic mediators together with various inflammatory cells such as monocytes/macrophages and T cells orchestrate the formation of the atherosclerotic lesion [2]. Haemostasis can be described as a complicated network of processes that include the balance between pro- and anti-thrombotic mediators as well as endothelial cell activation as major features. While the role of haemostatic mediators in thrombus formation following plaque destabilization is well established, their role in the chronic atherosclerotic process is less clear [3–6]. Consistent findings have been reported regarding increased levels of plasminogen activator inhibitor (PAI)-1 and tissue factor (TF), the main initiator of coagulation, in CVD patients [3,7–9]. In contrast, discrepant results have been found for levels of TF pathway inhibitor (TFPI) and the anti-thrombotic mediator thrombomodulin that binds thrombin with subsequent activation of the coagulation inhibitor protein C [3–6].

The autosome dominant disease familial hypercholesterolaemia (FH) is mainly caused by mutations in the low density lipoprotein (LDL) receptor gene, and is characterized by elevated plasma concentrations of total and LDL cholesterol from birth. Patients with FH have accelerated atherosclerosis and an increased risk of developing premature CVD [10–12]. In addition to hyperlipidaemia, these patients also display low-grade systemic inflammation [13,14], whereas the role of
pro-thrombotic mediators in the enhanced atherogenesis in these individuals is less clear.

A normal pregnancy exerts enhancing effects on total and LDL cholesterol [15]. Lipid-lowering drugs like statins are contraindicated in pregnant women with FH, and previously we have shown that women with FH have higher absolute levels of total and LDL cholesterol as well as triglycerides in the trimester third compared with healthy pregnant women [16]. Concomitantly, women with FH develop a pro-thrombotic and pro-inflammatory phenotype during pregnancy due to increased concentrations of several pro-thrombotic and inflammatory mediators compared to healthy pregnant women [17].

Some studies show that maternal hypercholesterolaemia, irrespectively of the FH status, leads to fatty streak formation and atherosclerotic lesions in a larger extent in offspring compared to offspring from normocholesterolaemic mothers [18,19]. Also, adult offspring with maternal FH inheritance have higher lipid levels and excess mortality compared to offspring with paternal FH inheritance [20,21]. Thus, maternal hypercholesterolaemia caused by FH or not may potentially increase the risk for atherosclerosis in the offspring later in life, i.e. in adolescence/adulthood. However, it is unclear whether maternal hypercholesterolaemia results in a pro-thrombotic phenotype in their offspring. In general, maternal hypercholesterolaemia may have other causes than FH, for example consumption of an unhealthy diet and/or a sedentary life style that in itself may promote a pro-thrombotic phenotype. FH patients represent a unique model to study the pure effect of elevated maternal total and LDL cholesterol on the offspring.

The aim of the present study was therefore to investigate whether maternal FH leads to differences in plasma or serum levels of haemostatic and fibrinolytic markers in children with and without FH born of mothers with FH compared to healthy children born of non-FH mothers.

Methods

Subjects

Women (n = 22) with heterozygous FH participating in our previous study conducted in 2001-2003 [16] received information and were asked to participate together with their children aged ≥6 years (with and without FH) by postal mail in October 2009. Eleven women responded, and nine women with definite FH as diagnosed by genetic testing [11] and with offspring willing to participate were included in the study. For eight of the women blood samples were previously collected during pregnancy in gestational weeks 30 and 36. All the pregnancies were single. None of the women were related to each other in any way. Among the sixteen children included, nine had a genetic verified FH diagnosis inherited from their mothers, whereas seven were healthy siblings without a FH diagnosis (Table 1). The healthy siblings represented five families. For comparison, blood samples were also collected from 16 healthy sex- and age-matched control children without FH born of non-FH mothers (Table 1). The controls were recruited among children of colleagues, friends and employees at the University of Oslo and Oslo University Hospital in the same time frame as the patients and were from the same part of Norway (eastern part). All the participants were clinically healthy without diagnosis of CVD or any other concomitant diseases such as infection, autoimmune disorders, diabetes mellitus, endocrine disorders or thromboembolic disease. None of the children were using anti-thrombotic drugs or statins at the time of blood sampling. The study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway Regional Health Authority, and it complied with the Declaration of Helsinki. Written informed consent was obtained from all participants or from one of their parents if the child was <16 years of age.

Venous, non-fasting serum and citrate plasma (platelet-poor) samples were collected from all the participants and prepared and stored as previously described [22]. Not all analyses were performed in all the subjects due to the limited amount of blood sample available from each individual.

Enzyme Linked Immunosorbant Assay (ELISA)

Plasma levels of TF and TFPI were measured by ELISA from R&D Systems (Minneapolis, MN, USA), whereas the concentrations of PAI-1 antigen and prothrombin fragment 1 + 2 (F1+2) were measured by ELISA from Technoclone GmbH (Vienna, Austria) and Siemens (Erlangen, Germany), respectively. Primarily, the TFPI ELISA kit measures free TFPI in addition to a very small percentage of LDL- and high density lipoprotein (HDL)-bound TFPI. The serum concentrations of tissue plasminogen activator (tPA) and thrombomodulin were measured by ELISA from ebioscience (Vienna, Austria) and R&D Systems, respectively. Intra- and inter-assay coefficients of variation (CV) were <10% for all assays, except for F1 + 2 where the CVs were <11.2%.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the participants: Children with FH, their healthy siblings and healthy control children.</th>
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</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
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<tr>
<td></td>
<td>FH</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>7 (7-10)</td>
<td>8 (8-13)</td>
</tr>
<tr>
<td>Male, %</td>
<td>33</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>16.0 (14.8-17.2)</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.5 (0.5-0.6)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>8.0 (7.9-8.6)</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>5.7 (5.4-5.9)</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.4 (1.3-1.6)</td>
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<tr>
<td>Lp(a), mmol/l</td>
<td>317 (144-485)</td>
</tr>
<tr>
<td>Apo B, g/l</td>
<td>1.3 (1.2-1.4)</td>
</tr>
<tr>
<td>Apo A-1, g/l</td>
<td>1.3 (1.2-1.3)</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>0.6 (0.6-0.6)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.9 (4.5-5.0)</td>
</tr>
<tr>
<td>AST</td>
<td>33 (25-35)</td>
</tr>
<tr>
<td>ALT</td>
<td>19 (12-21)</td>
</tr>
</tbody>
</table>

Data are presented as median (25 th -75 th percentile). *FH n = 7, Healthy siblings n = 6, Controls n = 10. * Controls n = 13, * Controls n = 11. **FH n = 8, Healthy siblings n = 6, Controls n = 11. FH: familial hypercholesterolemia; BMI: body mass index; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; Lp(a): lipoprotein (a), Apo (A-1/B): apolipoprotein (A-1/B); hsCRP: high sensitive C-reactive protein, ASAT/ALAT: aspartate/alanin aminotransferase.

1 FH versus Healthy siblings.
2 FH versus Controls.
3 Healthy siblings versus Controls.
Miscellaneous

Standard blood chemistry, lipid parameters, von Willebrand Factor (vWF) antigen and fibrinogen were measured in plasma at the Department of Medical Biochemistry, Oslo University Hospital. A wall-mounted stadiometer was used to measure height. Weight was measured without shoes by a digital body weight. Body mass index (BMI) was calculated manually by the formula (weight, kg/height, m²).

Statistics

Data were given as median (25th–75th percentiles) if not otherwise stated. Differences between the three children groups (FH, healthy siblings and controls) were assessed in an explorative manner by the non-parametric Kruskal-Wallis test. If significant, the difference between each pair of groups was assessed by Mann-Whitney U test. Fisher’s exact test was used for categorical data. Coefficients of correlation were calculated by the non-parametric Spearman’s rank test. The correlations between laboratory results in gestational weeks 30 and 36 were calculated by the non-parametric Spearman’s rank test. The results between any of the groups.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FH (n=9)</th>
<th>Healthy Siblings (n=8)</th>
<th>Controls (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
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<td></td>
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<tr>
<td>HDL cholesterol (mg/dL)</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
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<tr>
<td>ApoA1 (mg/dL)</td>
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<tr>
<td>ApoB (mg/dL)</td>
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<td>HDL to LDL ratio</td>
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</tbody>
</table>

Results

Characterization of the Subjects

Children with FH (n=9) had significantly higher plasma level of total and LDL cholesterol, and apolipoprotein (Apo) B compared with both their healthy siblings without FH (n=7) and with age- and sex-matched control children (n=16) (Table 1). A significant difference in aspartate aminotransferase levels was also observed between the healthy siblings and the controls, but the levels were within the reference range for both groups. We observed no significant differences in the other baseline parameters that are outlined in Table 1 between any of the groups.

Plasma Levels of Haemostatic and Fibrinolytic Markers

While there was no difference in the level of the fibrinolytic activator tPA between the 3 groups (Fig. 1A), children with FH had significantly higher plasma levels of total and LDL cholesterol, and apolipoprotein (Apo) B compared with both their healthy siblings without FH (n=7) and with age- and sex-matched control children (n=16) (Table 1). A significant difference in aspartate aminotransferase levels was also observed between the healthy siblings and the controls, but the levels were within the reference range for both groups. We observed no significant differences in the other baseline parameters that are outlined in Table 1 between any of the groups.

Discussion

In the present study we demonstrate higher plasma levels of PAI-1 and TFPI in children with and without FH born of mothers with FH compared to control children born of non-FH mothers, suggesting that the genotype of the mother, rather than the genotype of the child, may mediate enhancing effect of these haemostatic markers. Thus, although children with FH had markedly higher cholesterol levels than their healthy siblings, both groups had higher levels of PAI-1 and TFPI than control children. Moreover, PAI-1 levels in the mother were positively correlated with PAI-1 levels in their offspring, independent of the child’s FH status, further suggesting a link between the mother’s genotype and the haemostatic phenotype of the offspring. Finally, there were some associations between the mother’s lipid status and the haemostatic markers in the children, potentially suggesting an interaction between lipid metabolism and mediators of coagulation in FH.

Circulating levels of PAI-1 have previously been shown to be positively associated with total and LDL cholesterol, and triglycerides [7], and children with FH have previously been shown to have increased levels of PAI-1 compared to healthy controls [23]. Furthermore, Sébestyen and coworkers found that the FH subjects with premature myocardial infarction had higher levels of PAI-1 than those without this complication [24]. In the present study we report higher PAI-1 levels in children of mothers with FH compared to control children, independent of the children’s FH status, indicating that the genotype of the mother may potentiate a more pro-thrombotic phenotype also in children without the FH genotype. The positive correlation between PAI-1 levels in the mother during gestation and the PAI-1 levels in their offspring, independent of their FH status, further support such a notion. TFPI is the endogenous inhibitor of TF which both are the main regulators of the initiation of the coagulation process [3]. Plasma levels of TFPI have been found to be positively associated with intima-media thickness in healthy subjects [4,25], and increased in CVD patients [26]. Thus, as TFPI have anti-thrombotic effects, the increased levels in patients with CVD suggest that this may represent a counteracting mechanism in these patients. In relation to this, in the present study we found higher levels of TFPI in children with and without FH born of mothers with FH compared to control children. This finding may further suggest that increased TFPI could reflect an adaptive response to increased “haemodynamic” stress. In contrast to TFPI, thrombomodulin as a potent anti-thrombotic mediator was lower in FH children, suggesting that these anti-thrombotic mediators are differently regulated in FH.

We have previously demonstrated that pregnant women with FH have higher absolute levels of total and LDL cholesterol and triglycerides accompanied by an increased pro-coagulant activity compared to healthy, pregnant women [16,17]. In the present study, we report differences in plasma levels of PAI-1 and TFPI in children with and without FH born of women with FH compared to control children. To our knowledge, this is the first report indicating that maternal FH may confer an unfavourable phenotype, not only to their children with FH, but also to their apparently healthy children. Hypothetically, it is possible that maternal hypercholesterolaemia per se may trigger reactions such as haemostatic and inflammatory pathways, and together these factors

The Associations Between Haemostatic Markers in Offspring With and Without FH, and Lipids and Haemostatic Markers in Their Mothers During Pregnancy

For eight of the children with and without FH (age 7–8 years), we have previously examined the plasma levels of lipids and haemostatic markers in their mothers during the pregnancy [16,17]. We therefore investigated whether the plasma levels of haemostatic markers in the offspring were associated with the plasma levels of lipids and haemostatic markers in their mothers during their pregnancies. The plasma levels of PAI-1 in the mothers at gestational weeks 30 and 36 were significantly, positively correlated to PAI-1 plasma levels in their offspring (r = 0.893, P = 0.007 [n=7] and r = 0.821, P = 0.023 [n=7]; 30 and 36 weeks, respectively). The plasma levels of ApoA1 in the mothers at gestational week 30 correlated inversely with the plasma levels of PAI-1 (r = -0.873, P = 0.01, n=8), whereas ApoB in gestational week 36 positively correlated with the plasma levels of TFPI (r = -0.811, P = 0.027, n=7) in the children. None of the other haemostatic and lipid parameters in the mothers correlated significantly with the haemostatic parameters in their offspring (data not shown).
may further affect the phenotype of the child by indirect (i.e., through impairing the placental function) and direct (transplacental) pathways [27]. Firstly, maternal hypercholesterolaemia may together with haemostatic and inflammatory mediators contribute to endothelial dysfunction and enhanced atherogenesis which may impair the placental function. In pregnant women with FH, a higher resistance to blood flow in the uterine arteries potentially caused by atherosclerotic plaque, has been shown [28]. Thus, the differences observed between children from mothers with FH and those from healthy mothers, regardless of the children’s genotype, could be caused by an impaired placental function in pregnant women with FH. Secondly, there is also a possibility that haemostatic and inflammatory mediators potentially induced by maternal hypercholesterolaemia, may cross the placental barrier [27]. Taken together, these mechanisms may affect the development of the fetus through i.e. fetal programming, hypothetically including the immune and haemostatic system, and both children with and without FH born of mothers with FH may be influenced by this potential in utero effect. Thus, although children with FH clearly have more characteristics of atherosclerosis than their healthy siblings [29,30], our data may indicate a potential unfavourable effect of in utero exposure to hypercholesterolaemia even in healthy siblings as recently hypothesized by others [21,31].
The present study has some limitations. In particular, although FH is a relatively rare disorder, the study group was relatively small. Also, because of multiple testing in such a relatively small group of subjects, some of the findings may be by chance, and our findings in this pilot study need to be confirmed in larger studies. Forthcoming studies should also include more long-time follow-up. Furthermore, we were not able to measure haemostatic and lipid parameters in the fathers since the blood samples were collected from the mothers during the pregnancy 6–7 years ago. Nonetheless, such data should also be included in forthcoming studies. Our findings in the present study suggest that maternal FH may confer an unfavourable phenotype by affecting haemostatic and fibrinolytic markers in the offspring independent of the children’s FH status. However, the association between maternal hypercholesterolaemia and haemostatic risk markers in the offspring needs to be further elucidated. Future studies should investigate if healthy children born of mothers with FH are at any potential increased risk for disease later in life.

Conflict of Interest Statement

None declared.

Acknowledgement

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