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# Original papers

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# Factor V Leiden, pregnancy complications and adverse outcomes: the Hordaland Homocysteine Study

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## Summary

**Background:** The factor V Leiden (FVL) mutation is the most common cause of inherited thrombophilia in Caucasian populations, and women with this variant allele are at increased risk for pregnancy complications.
**Aim:** To examine whether the FVL allele is associated with pregnancy complications and adverse outcomes in a population-based study, and to identify potential factors that interact with the FVL genotype.
**Design:** Retrospective cohort study in a geographically-defined area.
**Methods:** Polymorphisms of factor V 1691G→A, methylenetetrahydrofolate reductase (MTHFR) 677C → T and 1298A → C and plasma levels of total homocysteine, folate and vitamin $B_{12}$ were determined in blood samples collected in 1992–1993 from 5874 women aged 40–42 years, and linked with 14 474 pregnancies in the same women, recorded in the Medical Birth Registry of Norway, 1967–1996.
**Results:** The allelic frequency of FVL was 3.7% (6.9% heterozygotes, 0.3% homozygotes). Maternal FVL mutation was associated with significantly higher risks of pre-eclampsia (OR 1.63, 95%CI 1.15–2.30), pre-eclampsia at <37 weeks (OR 2.76, 1.34–5.70), low birth weight (OR 1.34, 95%CI 1.03–1.74) and stillbirth (OR 2.20, 95%CI 1.45–3.36). The presence of a variant allele for the 677C → T MTHFR polymorphism strengthened the association between FVL and stillbirth (OR 3.34, 95%CI 1.95–5.73) ($p_{\text{interaction}} = 0.034$).
**Discussion:** FVL mutation is a significant risk factor for pregnancy complications and adverse outcomes, and MTHFR 677CT/TT genotype can further enhance the risk of stillbirth.

## Introduction

Both inherited and acquired thrombophilias predispose to thromboembolism. Carriage of the Factor V Leiden (FVL) mutation is the most common genetic predisposition to thrombosis.[1] The G to A substitution at nucleotide 1691 of the factor V gene results in resistance to activation by protein C, causing a prothrombotic state in FVL carriers.[1–3] The FVL mutation is a risk factor for 20–30% of venous thromboembolic events,[4] and people who are heterozygous for FVL are at 3–7-fold higher risk for venous thromboembolism than those with the wild-type genotype.[1,5] Among homozygotes for the FVL mutation, the risk may be as much as 80-fold higher.[5,6] However, some forms of thromboembolic disease,

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such as pulmonary embolism[7] and upper-extremity deep vein thrombosis,[8] appear not to be associated with the FVL mutation.

Carriage of the FVL mutation is relatively common among Caucasians in Europe and the US, varying from 2% to 8% in different geographical regions.[1,5,6,9] The mutation is uncommon in ethnic groups from African countries, the Middle East, Asia, Australasia and the Americas.[6,9]

Pregnant women have 2–5-fold higher risk for venous thromboembolism compared to non-pregnant women.[3,6] The FVL variant allele increases the risk for venous thromboembolism during pregnancy 8–10 times, compared to non-pregnant women with the wild-type genotype.[6]

Endothelial dysfunction, vasoconstriction, placental ischaemia and enhanced coagulation are associated with abnormal placental development, and disturbances of haemostasis may lead to inadequate fetomaternal circulation and decreased placental perfusion.[10,11] The subsequent vasculopathy and secondary thrombosis from hypercoagulability may result in inadequate perfusion of the intervillous space, pre-eclampsia, placental infarcts, intrauterine growth restriction (IUGR), placental abruption, stillbirth and probably premature delivery.[4,11]

The thrombotic nature of the placental vascular lesions and the increased thrombotic risk associated with thrombophilias strongly suggest a cause-and-effect relationship between acquired and inherited thrombophilias (among others, the FVL carrier state) and the pregnancy complications and adverse pregnancy outcomes listed above.[1–4,12–15]

We examined whether the FVL mutation is associated with pregnancy complications and adverse outcomes in a large population, and investigated possible interactions between the FVL variant allele and other known risk factors for such outcomes, including age, smoking, and plasma levels of total homocysteine (tHcy), folate and vitamin B12 and methylenetetrahydrofolate reductase (MTHFR) polymorphisms (677C → T and 1298A → C).

# Methods

## Study population

The Hordaland Homocysteine Study[16] was a collaboration between the University of Bergen, local health services and the National Health Screening Service. In 1992–1993, about 18 000 individuals born in 1925–1952 and living in Hordaland county in Western Norway participated in the study.[17] Using the national identification number, data from this cohort were linked with data obtained from the Medical Birth Registry of Norway,[18] which includes compulsory notification of all pregnancies in the country from 16 weeks of gestation onwards. There is record linkage between the birth registry, population registry and the cause of death registry, ensuring complete ascertainment of all births, as well as perinatal and infant deaths. Because of births by foreigners, and Norwegian citizens giving birth abroad, about 500–1000 births (~1%) annually do not match between the birth registry and civil registration of births.[19] Because the birth registry was established in 1967, only data from 6348 women born in 1950–1952 were considered. Of these women, ~93% were registered with one or more pregnancies in the birth registry from 1967 to 1996. Thus, the study population in the present report consists of 5874 women, who from 1967 to 1996 had a total of 14 474 pregnancies. The Regional Committee for Medical Research Ethics of Western Norway had approved the study protocol.

## Data collection

Details of the data collection in the Hordaland Homocysteine Study and the Medical Birth Registry of Norway notifications are reported elsewhere.[17,18] Briefly, information about life-style factors was obtained from self-administered questionnaires, and the standard cardiovascular examinations were performed by the National Health Screening Service[20] from April 1992 to April 1993. Non-fasting EDTA-plasma samples were drawn for tHcy, folate and vitamin $B_{12}$ analyses. Plasma tHcy, which includes both the free and protein-bound fractions of homocysteine, was determined using a fully automated HPLC assay.[21] The plasma concentrations of folate and vitamin $B_{12}$ were determined by microbiological assays in microtitre plates using a chloramphenicol-resistant strain of *Lactobacillus casei* and a colistin-sulphate-resistant strain of *Lactobacillus leichmannii*, respectively.[22,23] The factor V 1691G → A, MTHFR 677C → T and 1298A → C polymorphisms were determined in the packed blood cell fraction. The procedures for detection of these polymorphisms were based on real-time PCR[24] or mutagenically separated PCR and multiple-injection capillary electrophoresis.[25]

Pregnancy complications and adverse outcomes used in this paper include: pre-eclampsia (at any time during pregnancy, mean gestation age 39.0 weeks, 95%CI 38.7–39.3; or before week 37 of pregnancy, mean gestation age 33.9 weeks, 95%CI 33.0–34.7); placental abruption (premature partial or complete separation of the placenta, occurring in the latter

half of pregnancy); premature delivery (<37 weeks); low birth weight (<2500 g); intra-uterine growth retardation (IUGR) (<10th percentile for gestational age); and stillbirth (mean gestation age 29.7 weeks, 95%CI 28.5–30.9). The number of pregnancies complicated by pre-eclampsia is lower in the present report than in the previous report[18] using the same data set, because of a change in the Medical Birth Registry's pre-eclampsia definition. The criteria for pre-eclampsia[26] were hypertension (an increase in blood pressure to ≥140/90 mmHg after the 20th week of gestation, an increase in diastolic blood pressure of ≥15 mmHg from the level measured before the 20th week, or an increase in systolic blood pressure of ≥30 mmHg from the level measured before the 20th week) and proteinuria (protein excretion ≥0.3 g/24 h). A diagnosis of pre-eclampsia in the medical record is routinely entered on the medical registration form as a specified diagnosis by the midwife or obstetrician. But in some cases, the registration form may also hold information about specific symptoms of pre-eclampsia, such as hypertension, proteinuria, or oedema during pregnancy. In this study, our case definition included all pregnancies with a specified diagnosis of pre-eclampsia, and pregnancies with a combination of pregnancy-related hypertension and proteinuria with or without oedema. In our previous report, the presence of hypertension and oedema without proteinuria was also defined as pre-eclampsia.

### Statistical analyses

Multiple logistic regression was used to study relations between FVL genotypes, alone or combined with other factors, and pregnancy complications and adverse outcomes. Odds ratios (OR) with 95%CIs are presented after adjustment for parity, age of mother at delivery, and history of diabetes and smoking habits reported in 1992–1993. Smoking habits were coded as: never smoker, former smoker, or 1–9, 10–19, or ≥20 cigarettes/day. Stratification was used to examine combined effects between FVL mutation, smoking, age and factors related to tHcy. Potential effect modification was assessed by multiple logistic regression analyses including an interaction term. To study possible interaction between smoking and the FVL variant allele on pregnancy complications and adverse outcomes we considered those who reported current (39.1%) or previous (22.8%) smoking in 1992–1993 as smokers and those who reported never smoking as non-smokers. SPSS v. 12.0 for Windows was used for statistical analyses. Two-sided $p$ values <0.05 were considered statistically significant.

## Results

### Subject characteristics

Characteristics of the 5874 women aged 40–42 years in 1992–1993 who participated in the study, and who were recorded with one or more pregnancies in the Medical Birth Registry of Norway, are presented in Table 1.

### FVL, pregnancy complications and adverse outcomes

About 7% of the women had the FVL variant allele (6.9% heterozygotes, 0.3% homozygotes). Of 34 pregnancies with a homozygous mother, four (11.8%) had adverse pregnancy outcomes (three IUGR, one premature delivery), all four in different women. Since there were so few homozygous women, we have included them with the heterozygous women in the analyses below. Exclusion of homozygous women from the analyses did not change the results.

The risks (ORs) and prevalence of pregnancy complications and adverse outcomes by maternal FVL genotype are presented in Table 2. The FVL in one or both alleles was associated with increased risk of pre-eclampsia by 63%, compared to those with wild-type genotype, while the risk was almost three times higher for pre-eclampsia at <37 weeks. The risk of stillbirth was more than doubled in pregnancies with one or two maternal FVL variant alleles and 34% increased for low birth weight. The risk of IUGR was of borderline significance.

### FVL, recurrent pregnancy complications and adverse outcomes

The increased risk for recurrence of low birth weight when the woman had one or two FVL alleles was of borderline significance (OR 1.78, 95%CI 1.00–3.21, $p=0.054$). There was a tendency for recurrence of pre-eclampsia and stillbirth among women with one or two FVL variant alleles (OR 2.11, 95%CI 0.89–5.03, $p=0.09$ and OR 3.00, 95%CI 0.85–10.65, $p=0.09$, respectively). Recurrence of other pregnancy complications and adverse outcomes was not associated with FVL status.

### Smoking and pregnancy complications and adverse pregnancy outcome

Associations between maternal smoking status in 1992–1993 and pregnancy complications and adverse pregnancy outcomes are presented in Table 3. Women who were smokers had lower prevalence of pre-eclampsia than non-smoking women. In contrast, pregnancies with low birth

**Table 1** Patient characteristics by Factor V 1691G → A genotype

| Characteristic | GG ($n=5451$)[a] | GA ($n=407$)[b] | AA ($n=16$) | $p$ |
|---|---|---|---|---|
| Total pregnancies | 13 420 | 1020 | 34 | |
| Mean (SD) age at first birth (years) | 23.3 (4.3) | 23.2 (3.8) | 22.2 (3.8) | 0.49[c] |
| Mean (SD) pregnancies | 2.5 (0.9) | 2.5 (0.9) | 2.1 (0.7) | 0.21[c] |
| Ever smokers | 61.5% | 62.4% | 75.0% | 0.51[d] |
| Mean (SD) years ever smoked | 16.3 (7.2) | 16.8 (6.6) | 15.1 (7.0) | 0.49[c] |
| History of diabetes | 0.4% | 1.0% | 0% | 0.17[d] |
| *MTHFR 677C → T genotype* | | | | |
| CC | 49.0% | 55.5% | 50.0% | 0.08[d] |
| CT | 42.2% | 36.1% | 50.0% | |
| TT | 8.8% | 8.4% | 0% | |
| *MTHFR 1298A → C genotype* | | | | |
| AA | 45.6% | 42.0% | 37.5% | 0.24[d] |
| AC | 43.7% | 45.7% | 37.5% | |
| CC | 10.8% | 12.3% | 25.0% | |
| Total homocysteine (μmol/l)[e] | 9.1 (9.1–9.2) | 9.2 (8.9–9.4) | 10.4 (8.9–12.1) | 0.24[c] |
| Folate (nmol/l)[e] | 4.6 (4.6–4.7) | 4.5 (4.3–4.6) | 3.9 (2.8–5.4) | 0.09[c] |
| Vitamin $B_{12}$ (pmol/l)[e] | 309 (305–312) | 320 (308–332) | 294 (238–363) | 0.21[c] |

[a]Smoking status, history of diabetes, folate and vitamin $B_{12}$ based on 5445, 5450, 5447, and 5446 women, respectively. [b]Vitamin $B_{12}$ concentration based on 405 women. [c]One-way ANOVA. [d]$\chi^2$ test, 2×3 table for smoking and history of diabetes, and 3×3 table for MTHFR polymorphisms. [e]Geometric mean (95%CI).

**Table 2** Pregnancy complications and adverse pregnancy outcomes by maternal Factor V 1691G → A genotype

| Outcome | GG ($n=13\,344$) | GA/AA ($n=1049$) | GA/AA risk[a] |
|---|---|---|---|
| Pre-eclampsia | 304 (2.3%) | 38 (3.6%) | 1.63 (1.15–2.30)* |
| Pre-eclampsia at <37 weeks | 42 (0.3%) | 9 (0.9%) | 2.76 (1.34–5.70)* |
| Placental abruption | 65 (0.5%) | 8 (0.8%) | 1.53 (0.73–3.21) |
| Premature delivery at <37 weeks | 711 (5.5%) | 58 (5.7%) | 1.02 (0.78–1.35) |
| Low birth weight (<2500 g) | 641 (4.8%) | 67 (6.4%) | 1.34 (1.03–1.74)* |
| Intrauterine growth restriction | 1579 (12.3%) | 146 (14.5%) | 1.19 (0.99–1.43) |
| Stillbirth | 151 (1.1%) | 26 (2.5%) | 2.20 (1.45–3.36)** |
| Any pregnancy complication or adverse pregnancy outcome[b] | 2409 (18.7%) | 211 (20.9%) | 1.13 (0.96–1.32) |

Data are numbers (%) or odds ratios (95%CI). Multiple logistic regression analysis of 14 393 pregnancies with complete data; odds ratios were adjusted for mother's age, parity, history of diabetes in 1992–1993 and smoking habits in 1992–1993. [a]Versus GG genotype as a reference group. [b]Some pregnancies had multiple outcomes, and thus they may be presented in different groups of adverse pregnancy outcome, whereas for groups of any adverse pregnancy outcome they are counted only once. *$p<0.05$; **$p<0.001$.

weight and IUGR were more common among smokers than among non-smokers. When we combined all pregnancy complications and adverse pregnancy outcomes, smokers had a higher prevalence of affected pregnancies than non-smokers.

## Age and pregnancy complications and adverse pregnancy outcome

Delivery at advanced fertility age (35–45 years) was a significant risk factor for most of the observed outcomes except placental abruption and IUGR, with risk ratios varying from 1.66 to 2.91 (Table 4). The risk of pre-eclampsia was 44% increased and the risk of premature delivery was 55% increased in pregnancies at a young maternal age (16–19 years). For all pregnancy complications and adverse pregnancy outcomes combined, the risk was slightly increased in pregnancies among young mothers.

## Combined effects

We have previously reported the association between tHcy, B vitamins and MTHFR

**Table 3** Previous adverse pregnancy outcomes by smoking status in 1992–1993

| Outcomes | Non-smoking ($n=5814$) | Ever smoking ($n=8597$) | Ever smoking risk[a] |
|---|---|---|---|
| Pre-eclampsia | 164 (2.8%) | 178 (2.1%) | 0.76 (0.61–0.94)* |
| Pre-eclampsia at <37 weeks | 17 (0.3%) | 34 (0.4%) | 1.53 (0.85–2.76) |
| Placental abruption | 27 (0.5%) | 46 (0.5%) | 1.22 (0.75–1.97) |
| Premature delivery at <37 weeks | 293 (5.2%) | 476 (5.7%) | 1.12 (0.97–1.31) |
| Low birth weight (<2500 g) | 238 (4.1%) | 470 (5.5%) | 1.37 (1.17–1.61)** |
| Intrauterine growth retardation | 495 (8.9%) | 1232 (14.9%) | 1.75 (1.57–1.96)** |
| Stillbirth | 72 (1.2%) | 105 (1.2%) | 1.00 (0.74–1.36) |
| Any pregnancy complication or adverse pregnancy outcome[b] | 863 (15.4%) | 1759 (21.2%) | 1.46 (1.34–1.60)** |

Data are numbers (%) or odds ratios (95%CI). Multiple logistic regression analyses of 14 411 pregnancies with complete data; odds ratios were adjusted for mother's age, history of diabetes in 1992–1993 and parity. [a]Versus non-smoking as a reference group. [b]Some pregnancies had multiple outcomes, and thus they may be presented in different groups of adverse pregnancy outcome, whereas for groups of any adverse pregnancy outcome they are counted only once. *$p<0.05$; **$p<0.001$.

**Table 4** Adverse pregnancy outcomes by age at delivery

| Outcomes | 16–19 years ($n=1117$) | 20–34 years ($n=12\,187$) | 35–45 years ($n=1107$) | 16–19 years risk[a] | 35–45 years risk[a] |
|---|---|---|---|---|---|
| Pre-eclampsia | 38 (3.4) | 259 (2.1) | 45 (4.1) | 1.44 (1.00–2.06)* | 2.50 (1.77–3.53)** |
| Pre-eclampsia at <37 weeks | 4 (0.4) | 37 (0.3) | 10 (0.9) | 1.31 (0.44–3.89) | 2.91 (1.35–6.28)* |
| Placental abruption | 5 (0.4) | 63 (0.5) | 5 (0.5) | 0.89 (0.35–2.30) | 0.83 (0.32–2.17) |
| Premature delivery at <37 weeks | 88 (8.1) | 593 (5.0) | 89 (8.7) | 1.55 (1.21–1.99)** | 2.00 (1.56–2.57)** |
| Low birth weight (<2500 g) | 73 (6.5) | 569 (4.6) | 69 (6.2) | 1.20 (0.92–1.56) | 1.66 (1.26–2.18)** |
| Intrauterine growth retardation | 180 (16.8) | 1450 (12.3) | 97 (9.5) | 1.01 (0.85–1.21) | 1.06 (0.85–1.33) |
| Stillbirth | 18 (1.6) | 136 (1.1) | 23 (2.1) | 1.41 (0.84–2.38) | 1.85 (1.14–2.99)* |
| Any pregnancy complication or adverse pregnancy outcome[b] | 286 (26.4) | 2131 (18.0) | 205 (19.9) | 1.24 (1.07–1.44)* | 1.51 (1.27–1.79)** |

Data are numbers (%) or odds ratios (95%CI). Multiple logistic regression analyses of 14 411 pregnancies with complete data; odds ratios were adjusted for mother's age, history of diabetes in 1992–1993 and parity. [a]Versus 20–34 years as a reference group. [b]Some pregnancies had multiple outcomes, and thus they may be presented in different groups of adverse pregnancy outcome, whereas for groups of any adverse pregnancy outcome they are counted only once. *$p<0.05$; ** $p\leqslant 0.001$.

polymorphisms and adverse pregnancy outcome.[18,27] Combined effects of the FVL variant allele and the MTHFR 677C → T polymorphism or smoking are presented in Tables 5 and 6. Compared to pregnancies with wild-type maternal FVL and MTHFR 677CT/TT genotype, we found a 3.3-fold increase in risk of stillbirth in pregnancies with maternal FVL mutation and MTHFR 677CT/TT genotype (Table 5). The test of interaction showed that the MTHFR 677C → T polymorphism modifies the effect of FVL on stillbirth.

Similarly, there was an interaction between the FVL mutation and smoking on stillbirth ($p_{\text{interaction}}=0.047$), (Table 6). Maternal smoking combined with the FVL variant allele was associated with a three-fold risk of stillbirth when compared with pregnancies from smoking mothers with wild-type factor V genotype. The risk of low birth weight was also 50% increased in pregnancies where the mother had the FVL variant allele and smoked, compared to pregnancies in smoking mothers with wild-type factor V genotype. However, the interaction was not statistically significant.

We also looked for possible interactions between FVL genotypes and the MTHFR 1298A → C polymorphism, elevated plasma tHcy in 1992–1993, low folate and vitamin $B_{12}$ status in 1992–1993, young age at delivery and advanced age at delivery. None of these factors significantly increased risk

**Table 5** Pregnancy complications and adverse pregnancy outcomes: interactions between maternal Factor V 1691G → A and methylenetetrahydrofolate reductase (MTHFR) 677C → T polymorphisms

| Outcome | MTHFR 677CC | | | MTHFR 677CT/TT | | | $p_{interaction}$ |
|---|---|---|---|---|---|---|---|
| | GG[a] ($n=6543$) | GA/AA ($n=576$) | OR (95%CI)[b] | GG[a] ($n=6801$) | GA/AA ($n=473$) | OR (95%CI)[b] | |
| Pre-eclampsia | 137 (2.1%) | 24 (4.2%) | 2.04 (1.31–3.18) | 167 (2.5%) | 14 (3.0%) | 1.24 (0.71–2.17) | 0.17 |
| Pre-eclampsia at <37 weeks | 18 (0.3%) | 5 (0.9%) | 3.15 (1.16–8.52) | 24 (0.4%) | 4 (0.8%) | 2.49 (0.85–7.25) | 0.78 |
| Placental abruption | 28 (0.4%) | 4 (0.7%) | 1.60 (0.56–4.57) | 37 (0.5%) | 4 (0.8%) | 1.49 (0.53–4.21) | 0.96 |
| Premature delivery | 364 (5.8%) | 37 (6.6%) | 1.14 (0.81–1.62) | 347 (5.3%) | 21 (4.6%) | 0.86 (0.54–1.34) | 0.32 |
| Low birth weight | 314 (4.8%) | 37 (6.4%) | 1.35 (0.95–1.92) | 327 (4.8%) | 30 (6.4%) | 1.34 (0.91–1.98) | 0.96 |
| Intrauterine growth retardation | 739 (11.7%) | 78 (14.0%) | 1.20 (0.93–1.55) | 840 (12.9%) | 68 (15.1%) | 1.19 (0.91–1.56) | 0.99 |
| Stillbirth | 77 (1.2%) | 9 (1.6%) | 1.36 (0.68–2.72) | 74 (1.1%) | 17 (3.6%) | 3.34 (1.95–5.73) | 0.034 |
| Any pregnancy complication or adverse pregnancy outcome[c] | 1154 (18.2%) | 118 (21.1%) | 1.18 (0.95–1.46) | 1255 (19.1%) | 93 (20.6%) | 1.08 (0.85–1.37) | 0.62 |

Blood samples were collected from 5874 women, aged 40–42 years, in 1992–1993. Multiple logistic regression analysis of 14 393 pregnancies with complete data. [a]Reference group. [b]Odds ratios adjusted for mother's age, parity, history of diabetes in 1992–1993 and smoking habits in 1992–1993. [c]Some pregnancies had multiple outcomes, and thus they may be presented in different groups of adverse pregnancy outcome, whereas for groups of any adverse pregnancy outcome they are counted only once.

**Table 6** Pregnancy complications and adverse pregnancy outcomes: interactions between maternal Factor V 1691G → A and smoking habits in 1992–1993

| Outcome | Non-smoking | | | Ever smoking | | | $p_{interaction}$ |
|---|---|---|---|---|---|---|---|
| | GG[a] ($n=5394$) | GA/AA ($n=417$) | OR (95%CI)[b] | GG[a] ($n=7950$) | GA/AA ($n=632$) | OR (95%CI)[b] | |
| Pre-eclampsia | 145 (2.7) | 19 (4.6) | 1.75 (1.07–2.86) | 159 (2.0) | 19 (3.0) | 1.49 (0.92–2.42) | 0.65 |
| Pre-eclampsia at <37 weeks | 13 (0.2) | 4 (1.0) | 3.94 (1.28–12.15) | 29 (0.4) | 5 (0.8) | 2.21 (0.85–5.73) | 0.41 |
| Placental abruption | 24 (0.4) | 3 (0.7) | 1.62 (0.48–5.39) | 41 (0.5) | 5 (0.8) | 1.49 (0.59–3.80) | 0.93 |
| Premature delivery | 277 (5.3) | 16 (4.0) | 0.74 (0.44–1.24) | 434 (5.7) | 42 (6.9) | 1.21 (0.87–1.68) | 0.11 |
| Low birth weight | 220 (4.1) | 18 (4.3) | 1.07 (0.65–1.74) | 421 (5.3) | 49 (7.8) | 1.50 (1.10–2.04) | 0.24 |
| Intrauterine growth restriction | 456 (8.8) | 39 (9.8) | 1.15 (0.81–1.62) | 1123 (14.7) | 107 (17.5) | 1.22 (0.98–1.52) | 0.74 |
| Stillbirth | 66 (1.2) | 6 (1.4) | 1.18 (0.51–2.74) | 85 (1.1) | 20 (3.2) | 2.96 (1.80–4.86) | 0.047 |
| Any pregnancy complication or adverse pregnancy outcome[c] | 802 (15.4) | 61 (15.3) | 1.00 (0.75–1.32) | 1607 (20.9) | 150 (24.5) | 1.21 (1.00–1.47) | 0.26 |

Blood samples were collected from 5874 women, aged 40–42 years, in 1992–1993. Multiple logistic regression analysis of 14 393 pregnancies with complete data. [a]Reference group. [b]Odds ratios were adjusted for mother's age, parity, and history of diabetes in 1992–1993. [c]Some pregnancies had multiple outcomes, and thus they may be presented in different groups of adverse pregnancy outcome, whereas for groups of any adverse pregnancy outcome they are counted only once.

associated with FVL for the observed pregnancy complications or adverse outcomes (data not shown).

# Discussion

## Main findings

By combining results from a population-based study of women 40–42 years old with notifications from the Medical Birth Registry of Norway, we have shown that the maternal FVL variant allele was significantly associated with pre-eclampsia at any time during pregnancy, pre-eclampsia at <37 weeks, low birth weight and stillbirth. We also found significant interactions between the maternal FVL mutation and the MTHFR 677C → T polymorphism or cigarette smoking on stillbirth.

## Pre-eclampsia

In stark contrast to the early publications, which demonstrated increased risk of pre-eclampsia in FVL carriers,[1,10,12,28–32] many recent studies found no association between the FVL variant allele and pre-eclampsia.[32–36] A meta-analysis by Kosmas *et al.*,[32] including 2742 hypertensive women and 2403 controls suggested that the associations observed in early and small studies may be due to time-lag bias and publication bias. However, two more recent meta-analyses found that the FVL mutation was associated with significantly increased risk of pre-eclampsia, and specially of severe pre-eclampsia.[37,38] Although our study cannot match in size with these recent meta-analyses, it has advantage of including a relatively large number of cases ($n = 342$) and controls recruited from the same population-based cohort. We found that FVL mutation conferred increased risk of pre-eclampsia; the risk was highest for pre-eclampsia at <37 weeks (OR 2.76) and somewhat lower when all pre-eclampsia cases were included (OR 1.63). Thus our results support the FVL mutation being a risk factor of pre-eclampsia.

Patients with prior history of adverse pregnancy outcome and thrombophilia are at increased risk for occurrence of the same or another adverse pregnancy outcome in a subsequent pregnancy, with recurrence rates of 66–83%.[39] For severe pre-eclampsia among thrombophilic women who have experienced a prior pre-eclampsia a somewhat lower (but still high) recurrence rate of 52% has been reported,[40] but specific data on thrombophilia of FVL type and recurrence of pre-eclampsia are lacking. We found a two-fold risk of recurrence of pre-eclampsia among women with one or two FVL variant alleles, but this association was of borderline statistical significance.

Associations between smoking and pregnancy complications and adverse pregnancy outcomes have been widely examined.[41] Earlier studies have demonstrated that smoking is associated with lower risk of pre-eclampsia. Similarly, in the present study, smoking was negatively associated with all pre-eclampsia combined, but no significant association was found for pre-eclampsia at <37 weeks.

## Low birth weight and IUGR

The risk of low birth weight was 34% higher in pregnancies with maternal FVL variant allele vs. those with maternal wild-type factor V gene. Moreover, women with one or two FVL variant alleles had a 78% increased risk of having another baby with low birth weight. In a 2002 study, mothers who were carriers of inherited thrombophilia (defined as having either the FVL or the factor II A20210 mutations) had a significantly higher risk of delivering a baby with low birth weight compared to those without inherited thrombophilia (adjusted OR 2.0, 95%CI 1.1–3.6).[14] The larger effect in that study than in ours may be explained by the inclusion of factor II A20210 in addition to FVL.

A common cause of IUGR is deficient nutritional supply to the fetus via placenta.[42] A positive association between the FVL variant allele and vascular placental insufficiency has been reported earlier.[2] Thus, placental dysfunction related to the FVL mutation may also lead to IUGR or low birth weight. Overall, the studies on the relation between FVL and IUGR show contradictory results: some suggesting an association,[37,43] others not.[35,44,45] In our study, there was a tendency to increased risk (about 20%) of IUGR in pregnancies with maternal FVL genotype, but the relation was of only borderline significance. Thus, the effect of the FVL mutation on risk of low birth weight and IUGR is modest and may be modified by other factors. Notably, pre-eclampsia, associated with FVL, may result in pre-term delivery and thereby low birth weight. In the present study, the prevalence of low birth weight was about five times higher in pregnancies with pre-eclampsia (20.8%) vs. those without (4.5%).

Smoking is a well-known risk factor for IUGR and low birth weight.[41] Consistent with previous data, we found that the risks of IUGR and low birth weight were 75% and 37% higher, respectively, in pregnancies among smoking vs. non-smoking mothers. We also studied the possible interaction between smoking and FVL. Although the risk of

low birth weight was significantly enhanced by the combination of both smoking and the FVL variant allele, the interaction test was not statistically significant.

### Stillbirth

Identifiable causes of stillbirth include infections, placental dysfunction, umbilical cord defects, birth defects, and various maternal illnesses or conditions that may affect pregnancy.[46] Up to 50% of stillbirth babies die of undetermined causes.[46] In their systematic review, Alfirevic *et al*. reported that women with unexplained stillbirth were more often FVL carriers than women with normal pregnancies (OR 6.1, 95%CI 2.8–13.2).[30] More recently, however, a population-based study in North East Germany suggested that there was no association between the FVL mutation and the risk of stillbirth,[47] confirming earlier results from the European Prospective Cohort on Thrombophilia.[48] In our study, we found a more than two-fold increase in risk of stillbirths among pregnancies with the maternal FVL variant allele compared to those with the wild-type factor V genotype. We also observed a borderline significant three-fold risk for recurrence of stillbirth among women with affected FVL genotype.

Although the MTHFR variant that is considered to be a risk factor for thrombosis or adverse pregnancy outcomes is homozygosity for the 677T allele, we have combined hetero- and homozygosity in the present study, due to the low number of women with the MTHFR 677TT genotype. We found that the risk of stillbirth was more than three-fold increased when a pregnancy had both maternal FVL and MTHFR 677CT/TT genotypes. This may indicate that both these MTHFR genotypes enhance the risk of stillbirth in the presence of FVL mutation. The absence of interaction between FVL and elevated plasma tHcy or low folate concentrations also suggests that the MTHFR 677C → T polymorphism is itself a strong effect modifier for stillbirth.

Maternal smoking was another significant effect modifier of the risk for stillbirth conferred by the FVL mutation. The latter finding should be considered with caution, as data on smoking status were usually obtained several years after outcome, and it is not known whether the woman actually smoked during the pregnancy. However, in earlier Norwegian studies, more than one third (35–39%) of women were daily smokers during their pregnancies in the 1980s, and only 15–20% quit smoking during pregnancy at that time.[49,50] Since about half (52%) of the smokers in the present study had smoked for >20 years, and 30% for 10–19 years (i.e. including most of their fertile age), the simultaneous effect of smoking and FVL may in fact be underestimated.

### Weaknesses of the study

The strength of our study is the population-based design of a large cohort recruited within a geographically-defined area. However, it also has definite limitations. Although the Medical Birth Registry of Norway comprises close to 100% of live births and stillbirths in the country, it is possible that we missed some subjects (e.g. women who gave birth from 1967 to 1996, but who did not survive until the time of enrollment in this study or who did not participate in the Hordaland Homocysteine Study). As already mentioned, the data on smoking may be inadequate. Another limitation is that we did not have data on medication use during pregnancy. Anticoagulants in particular have been related to worse outcomes in pregnancy, and these drugs might have been given to carriers of the FVL variant to prevent thrombosis. However because the FVL carrier status is generally not known, this seems unlikely. Last but not least, because the pregnancy complications and adverse outcomes are strongly interrelated, there is always the possibility that one outcome might have biased the associations with another outcome and FVL status.

## Conclusions

In this population-based study of 5874 women with 14 474 pregnancies, maternal FVL mutation was a significant risk factor for pre-eclampsia, low birth weight and stillbirth. Maternal MTHFR 677C → T polymorphism was a significant effect modifier, further increasing the risk of stillbirth. Our data are consistent with previous studies suggesting that the FVL mutation is a risk factor for pregnancy complications and adverse outcomes.

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