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Genetic risk factor for unexplained recurrent early pregnancy loss

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Raised concentrations of plasma homocysteine may result from genetic or nutrient-related disturbances in homocysteine metabolism. Methylene tetrahydrofolate reductase (MTHFR) is responsible for the synthesis of 5-methyltetrahydrofolate, the primary methyl donor in the conversion of homocysteine to methionine. Homozygosity for the 677 C→T mutation in the MTHFR-gene causes increased thermolability of MTHFR, redistribution of folate-derivatives, elevated plasma homocysteine concentrations,¹ and has been reported to be a risk factor for neural tube defects,² and coronary artery disease.³ We determined the prevalence of the 677 C→T mutation in women with recurrent early pregnancy loss (REPL) because raised plasma homocysteine concentrations are a risk factor for REPL.

We studied 185 white women with REPL (two or more spontaneous consecutive miscarriages before 17 weeks of gestation from the same partner) for which no cause was found.⁴ They were compared with 113 unrelated controls, acquaintances of women with REPL, matched for age, sex, district, and social class. Controls had had at least one uncomplicated pregnancy and no spontaneous abortions. All participants were screened for the 677 C→T mutation by PCR and subsequent restriction enzyme analysis with *HinFI*.⁵ Written informed consent was obtained from all participants.

There was a significant OR (χ^2) of 3.3 (95% CI 1.3–10.1) in women with REPL comparing the prevalence of the homozygous genotype versus the other two genotypes (table). Comparing the same women with REPL with a large Dutch population-based control group³ a significant OR of 2.0 with a 95% CI from 1.2 to 3.2 was found.

Homozygosity for the 677 C→T mutation in the MTHFR-gene is associated with a two to three-fold risk of REPL. Improving folate metabolism in these women by folic acid supplements may reduce pregnancy loss.

- 1 Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; **93**: 7–9.
- 2 Van Der Put NM, Eskes TK, Blom HJ. Is the common 677 C→T

mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. *Q J Med* 1997; **90**: 111–15.

- 3 Kluijtmans LAJ, Kastelein JJP, Lindemans J, et al. Thermolabile methylenetetrahydrofolate reductase in coronary artery disease. *Circulation* (in press).
- 4 Wouters MGAJ, Boers GHJ, Blom HJ, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. *Fertil Steril* 1993; **60**: 820–25.
- 5 Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**: 111–13.

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Fetuin protects the fetus from TNF

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Pregnancy has been termed "nature's transplant" because the fetus is protected from rejection by mother.¹ Rejection of a transplanted allograft in an immunocompetent host is normally mediated by the macrophage-derived cytokine, tumour necrosis factor (TNF);² excessive production of TNF during pregnancy causes spontaneous abortion.^{3,4} We recently found that spermine, a ubiquitous biogenic amine present in large amounts in the amnion, counter-regulates the immune response by inhibiting the production of TNF and other proinflammatory cytokines by human mononuclear cells.⁵ We have now discovered that a fetal plasma glycoprotein, fetuin, is required for the inhibition of TNF production by spermine. Although fetuin was first described more than 50 years ago in fetal bovine serum, and subsequently found to share high homology to human fetuin (α 2-HS-glycoprotein), its role in pregnancy and fetal development is unknown.

While investigating the mechanism underlying spermine-mediated suppression of TNF production in the murine macrophage-like cell line RAW 264.7, we accidentally discovered that macrophages lost their responsivity to spermine when cultured under low serum conditions. That is, despite the addition of cytokine-suppressing concentrations of spermine to these cells, the production of TNF was uninhibited by spermine after stimulation with bacterial endotoxin (lipopolysaccharide, LPS). Reasoning that these cells had become deprived of a protein that was required to inhibit the production of TNF, we added fractionated proteins from normal cells and assayed their ability to restore spermine-dependent inhibition of TNF production under serum-free culture conditions. After anion-exchange chromatography and SDS-PAGE gel elution, we isolated a single protein that mediated the responsivity of macrophage cultures to spermine. Protein-

MTHFR genotypes	REPL (n=185)	Matched controls (n=113)	Dutch population-based controls (n=1250)
--/--	77 (42%)	48 (42%)	617 (49%)
+/--	79 (43%)	59 (52%)	527 (42%)
+/+	29 (16%)	6 (5%)	106 (9%)
OR (95% CI)		3.3 (1.3–10.1)	2.0 (1.2–3.2)

--/--=wild type, +/--=heterozygous genotype, and +/+ =homozygous genotype. OR (95% CI): odds ratio and 95% confidence interval calculated for the +/+ genotype versus the other two genotypes in cases versus controls.

Distribution of the MTHFR 677 C→T mutation in patients with recurrent early pregnancy loss (REPL) and controls