FERTILITY AND STERILITY®
VOL. 74, NO. 6, DECEMBER 2000

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis

Willianne L. D. M. Nelen, Ph.D.,[a] *Henk J. Blom, Ph.D.,*[b] *Eric A. P. Steegers, Ph.D.,*[a] *Martin den Heijer, Ph.D.,*[c] *and Tom K. A. B. Eskes, Ph.D.*[a]

University Hospital Nijmegen St. Radboud, Nijmegen, The Netherlands

Objective: To quantify the risk of recurrent early pregnancy loss in the presence of elevated fasting or afterload homocysteine concentrations or homozygosity for the 677C→T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene (T/T genotype).

Design: Case-control studies published between January 1992 and November 1999 were identified with a MEDLINE-search. These studies were combined with a recent case-control study performed by our own research group.

Setting: Academic research environment.

Patient(s): Studies published in the English language, concerning two or more pregnancy losses before 16 weeks' menstrual age were included.

Intervention(s): Meta-analysis of all of the studies included.

Main Outcome Measure(s): The number of subjects with and without hyperhomocysteinemia or with the T/T genotype were derived, if necessary the study was supplemented by personal communication with the original authors.

Result(s): Pooled risk estimates of 2.7 (1.4 to 5.2) and 4.2 (2.0 to 8.8) were calculated for fasting and afterload plasma homocysteine concentrations, respectively. For the MTHFR T/T genotype a pooled risk estimate of 1.4 (1.0 to 2.0) was found.

Conclusion(s): These data support hyperhomocysteinemia as a risk factor for recurrent early pregnancy loss. Further research should be focused on the pathophysiology of this relationship and on the clinical efficacy of B vitamin supplementation. (Fertil Steril® 2000;74:1196–9. ©2000 by American Society for Reproductive Medicine.)

Key Words: Abortion, early pregnancy loss, homocysteine, meta-analysis, miscarriage, MTHFR

Received February 2, 2000; revised and accepted June 8, 2000.

H.J.B. is an established Investigator of the Netherlands Heart Foundation (Grant no. D97.021). Study financially supported by the Dutch Preventiefonds, The Hague, The Netherlands (Grant no. 28.1006.2).

Reprint requests: Willianne L.D.M. Nelen, Ph.D., Department of Obstetrics and Gynecology, University Hospital Nijmegen St. Radboud, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands (FAX: +31 24 3541194; E-mail: w.nelen @obgyn.AZN.NL).

[a] Department of Obstetrics and Gynecology.

[b] Department of Pediatrics.

[c] Department of Internal Medicine.

0015-0282/00/$20.00
PII S0015-0282(00)01595-8

In 1985, Mudd et al. reported that women with classic homocystinuria, an autosomal recessive inherited deficiency of cystathionine β-synthase, experience fetal loss rates of almost 50% (1). Since 1992, mild elevations in the total plasma homocysteine (tHcy) concentrations, which has been described as a risk factor for arteriosclerosis (2), venous thrombosis (3), neural tube defects, placental abruption, and infarction and preeclampsia (4), have also been associated with an increased risk for recurrent early pregnancy loss (REPL) (5–9).

Recently, we reported that homozygosity for a common 677C→T mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, which is associated with higher tHcy concentrations, leads to a twofold to threefold higher risk of REPL in Dutch women (10). However, more recent studies could not confirm this association in other populations (7, 11–14).

From January 1992 to November 1999, a total of 10 case-control studies have been performed on homocysteine metabolism and recurrent early pregnancy loss. These studies are the subject of the present review (5–14) in which the risks of hyperhomocysteinemia and of the MTHFR 677 T/T genotype are quantitatively assessed.

MATERIALS AND METHODS

A MEDLINE-search of major subject headings from January 1992 through November 1999 was performed using these keywords: homocystein* or hyperhomocystein* or MTHFR; and abortion* or miscarriage* or pregnancy loss*

TABLE 1

Risk estimation for hyperhomocysteinemia and the MTHFR 677C→T mutation in recurrent early pregnancy loss.

Author and year (references)	Definition REPL		Homocysteine metabolism			OR (95% CI)
	Number of pregnancy losses	Menstrual age (wk)	Cut-off point (μmol/L)	Cases/ total cases	Control/ total controls	
Fasting tHcy						
Wouters et al. 1993 (6, 24)	≥2	≤16	>15[b]	22/180	3/46	2.0 (0.6–7.0)
Quere et al. 1998 (7)[a]	≥3	?	>10[c]	12/100	5/100	2.6 (0.9–7.7)
Nelen et al. 2000 (9)	≥2	≤16	>18.3[c]	19/123	5/103	3.6 (1.3–10.0)
Overall						2.7 (1.4–5.2)
Afterload tHcy						
Steegers-Theunissen et al. 1992 (5, 25)	≥2	≤16	>38[b]	4/14	1/15	5.6 (0.5–57.9)
Wouters et al. 1993 (6, 24)	≥2	≤16	>51[b]	29/180	1/46	8.6 (1.1–65.2)
Coumans et al. 1999 (8)	≥2	≤16	>51[b]	6/35	3/67[d]	4.4 (1.0–18.9)
Nelen et al. 2000 (9)	≥2	≤16	>61.5[c]	15/122	5/101	2.7 (0.9–7.7)
Overall						4.2 (2.0–8.8)
MTHFR 677C→T						
Nelen et al. 1997 (10)	≥2	≤16	T/T	29/185	6/113	3.3 (1.3–8.3)
Quere et al. 1998 (7)[a]	≥3	?	T/T	20/100	14/100	1.5 (0.7–3.2)
Grandone et al. 1998 (11)	≥2	<17	T/T	17/94	28/150	1.0 (0.5–1.9)
Holmes et al. 1999 (13)	≥3	≤12	T/T	11/129	6/67	0.9 (0.3–2.7)
Kutteh et al. 1998 (12)	≥3	first-trimester	T/T	4/50	2/50	2.1 (0.4–11.9)
Lissak et al. 1999 (14)	≥2	≤16	T/T	4/41	4/18	0.4 (0.1–1.7)
Overall						1.4 (1.0–2.0)

[a] Data were partly acquired by personal communication.
[b] 97.5th percentile.
[c] 95th percentile.
[d] Described in van Pampus et al. 1999 (26).

Nelen. Hyperhomocysteinemia and early pregnancy loss. Fertil Steril 2000.

or foetal loss* or fetal loss*. A total of 32 articles were identified of which 11 contained data from case-control studies on the risk of recurrent early pregnancy loss (defined as two or more spontaneous miscarriages within 16 weeks' menstrual age) in relation with disturbances of homocysteine metabolism. Two papers were excluded (15, 16): two papers reported the same study (10, 15), and another study (16) unrecognizably mixed the REPL patients with women who had suffered from second or third trimester fetal losses. The data from our most recent case-control study about this subject (9) were also included. Thus, the number of cases and controls with and without elevated tHcy concentrations fasting or afterload or the MTHFR T/T genotype were derived from each of the 10 studies (Table 1) (5–14). If necessary the data were completed by personal communication with the original authors. This study was approved by the institutional review board of the University Hospital Nijmegen.

The number of subjects with hyperhomocysteinemia was calculated on the basis of the definition used in each of the 10 component studies, that is, a plasma homocysteine concentration above either the 95th or 97.5th percentile of the control distribution.

We calculated the odds ratios for each of the studies with corresponding 95% confidence intervals, using Woolf's method (17) and tested for homogeneity as reported by Greenland (18). A pooled estimate was calculated by the Mantel-Haenszel method, and the 95% confidence intervals were calculated with use of Robins's method (19). In addition, the attributable risk was estimated by calculating the etiologic fractions and 95% confidence intervals according to Walter (20, 21). A P value of <0.05 was considered to be statistically significant.

RESULTS

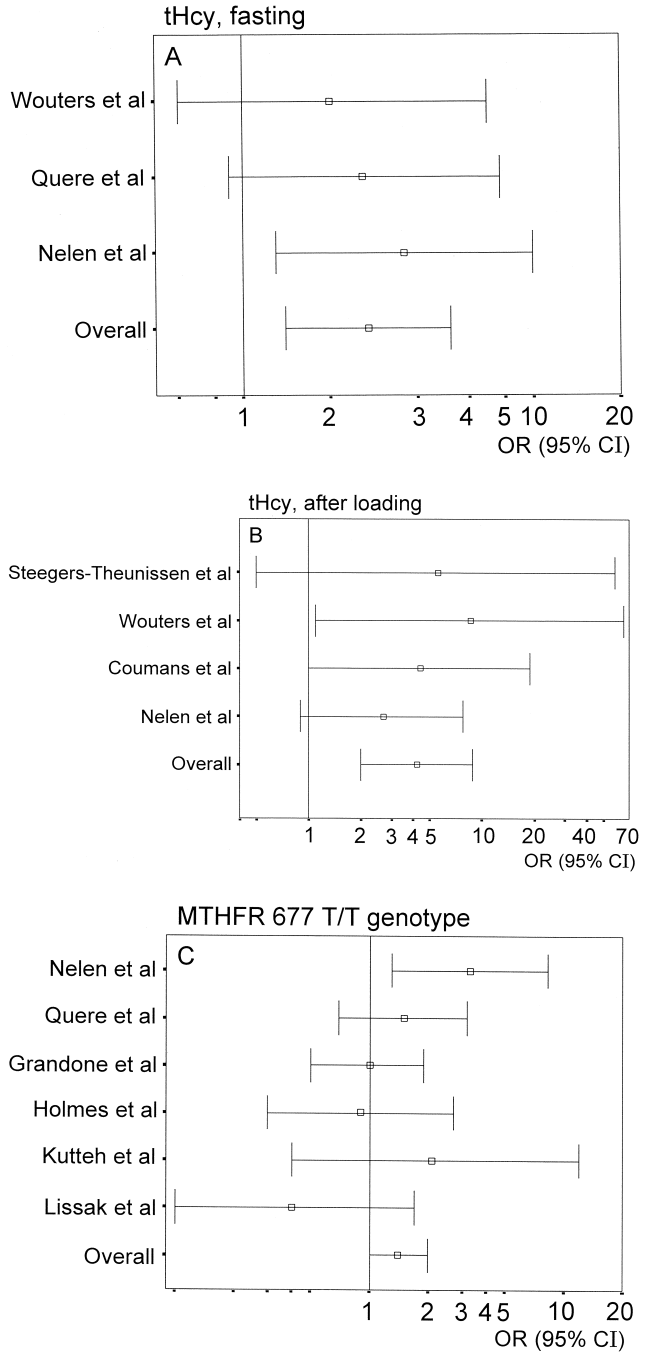
Ten case-control studies examined the relationship between homocysteine metabolism and recurrent early pregnancy loss (see Table 1). The odds ratios for hyperhomocysteinemia, fasting and after methionine loading, as well as homozygosity for the MTHFR C677T mutation are shown in Table 1 and Figure 1.

The test for heterogeneity did not reach significance for either the fasting or afterload tHcy concentration ($\chi^2 = 0.52$, $P>.05$, df = 2 and $\chi^2 = 1.19$, $P>.05$, df = 2, respectively) or for the MTHFR T/T genotype ($\chi^2 = 8.19$, $P>.05$, df = 5).

Three studies found hyperhomocysteinemia, fasting or afterload, to be a significant risk factor for REPL and two

FIGURE 1

(**A**), Individual and pooled odds ratios of elevated fasting homocysteine concentrations, (**B**), elevated homocysteine concentrations after methionine loading, or (**C**), the T/T genotype of the C677T mutation in the MTHFR gene in case-control studies on recurrent early pregnancy loss. Horizontal bars represent the 95% confidence intervals and □ the odds ratios.



Nelen. Hyperhomocysteinemia and early pregnancy loss. Fertil Steril 2000.

studies did not. Figure 1A and B, however, shows that all studies have odds ratio above 2.0. The pooled risk estimate for elevated fasting tHcy concentrations of three studies is 2.7 (95% CI 1.4–5.2) (see Fig. 1A). For the afterload tHcy concentrations we calculated a pooled risk estimate of 4.2 (95% CI 2.0–8.8) (see Fig. 1B).

One of the six studies found the MTHFR T/T genotype to be a significant risk factor for REPL. The remaining five studies showed nonsignificant odds ratios ranging from 0.4 to 2.1. The pooled estimate for the MTHFR T/T genotype was 1.4 (95% CI 1.0–2.0) (see Fig. 1C).

The calculated risks for REPL, attributable to elevated fasting and afterload tHcy concentrations, were 8.4% (95% CI 4.0%–12.7%) and 11.5% (95% CI 6.9%–16.2%), respectively. The etiologic fraction calculated for the MTHFR T/T genotype was 2.4% (95% CI −0.02%–6.9%).

DISCUSSION

The pooled estimates for elevated fasting and afterload tHcy concentrations support the postulation that hyperhomocysteinemia is a risk factor for REPL. It is hypothesized that an elevated fasting tHcy concentration is more associated with remethylation defects, whereas increased afterload tHcy concentrations reflect transsulfuration defects. Both significant pooled risk estimates point out that REPL may be related to defects in both remethylation and transsulfuration. The different definitions used for REPL should also be noted. Some studies used the traditional definition of three or more miscarriages, whereas others defined REPL as two or more consecutive miscarriages. Recently, our study found that Dutch hyperhomocysteinemic women with three or more miscarriages had a much higher risk of REPL than hyperhomocysteinemic women with only two miscarriages (9). In this meta-analysis, therefore, calculated pooled risks for REPL might be underestimated as a result of differences in the definitions used by the reviewed reports.

The question of whether women with REPL should undergo a methionine-loading test in addition to an assessment of their fasting tHcy concentration cannot be answered on the basis of this meta-analysis. However, in our previous study, fasting and afterload tHcy concentrations were observed as independent risk factors for REPL, and therefore it was concluded that a methionine-loading test had additional diagnostic value in identifying an extra subgroup of women at increased risk for REPL (9).

The role in REPL of the 677C→T mutation of the MTHFR gene is less clear. The largest study found the MTHFR T/T genotype to be a significant risk factor for REPL (10); however, the remaining five studies estimated no significant risk (7, 11–14). Although there was great variation in the odds ratios for the MTHFR T/T genotype in these studies—probably a result of the differences in sample size or in the geographic distribution of the T allele—the pooled

risk estimate for the MTHFR T/T genotype was 1.4 (95% CI 1.0–2.0). This means that homozygosity for the MTHFR 677C→T mutation represents a small increase in a woman's risk for REPL, but is a less convincing factor than elevated tHcy concentrations. Future research should be focused on the role of this mutation in the context of folate and tHcy concentrations.

Despite the epidemiologic evidence for a relationship between hyperhomocysteinemia and REPL, little is known about its pathophysiology. For cardiovascular disease, a lot of possible mechanisms related to hyperhomocysteinemia have been proposed. In cases of REPL, we described in a previous study a defective chorionic villous vascularization as a possible mechanism by which elevated homocysteine concentrations might be related to REPL (22).

The clinical relevance of the finding that hyperhomocysteinemia and the MTHFR T/T genotype are risk factors for REPL depends mainly on the question whether B vitamin supplementation will prevent new miscarriages. Folic acid on particular has a strong homocysteine lowering effect, which has also been achieved in women with REPL (23). However, data on clinical intervention studies are not available; because of the worldwide recommendations for periconceptional folic acid supplementation and extensive food fortification programs to prevent neural tube defects, the data will be impossible to obtain in the future. Therefore, it cannot be ruled out that homocysteine is a marker of disease rather than a cause, in which case B vitamin supplementation may be ineffective.

In conclusion, there is increasing evidence that mild hyperhomocysteinemia is a risk factor for REPL. Further research should be focused on the pathophysiology of hyperhomocysteinemia in REPL and on the clinical efficacy of homocysteine lowering by B vitamin supplementation.

References

1. Mudd HS, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al. The natural history of homocystinuria due to cystathionine β-synthase deficiency. Am J Hum Genet 1985;37:1–31.
2. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 1995; 274:1049–57.
3. Den Heijer M, Rosendaal FR, Blom HJ, Gerrits WBJ, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. Thromb Haemost 1998;80:874–7.
4. Obwegeser R, Hohlagschwandtner M, Sinzinger H. Homocysteine—a pathophysiological cornerstone in obstetrical and gynaecological disorders? Hum Reprod Update 1999;5:64–72.
5. Steegers-Theunissen RPM, Boers GHJ, Blom HJ, Trijbels FJM, Eskes TKAB. Hyperhomocysteinaemia and recurrent spontaneous abortion or abruptio placentae. Lancet 1992;339:1122–3.
6. Wouters MGAJ, Boers GHJ, Blom HJ, Trijbels FJM, Thomas CMG, Borm GF, et al. Hyperhomocysteinemia: a risk factor in women with unexplained recurrent early pregnancy loss. Fertil Steril 1993;60:820–5.
7. Quere I, Bellet H, Hoffet M, Janbon C, Mares P, Gris JC. A woman with five consecutive fetal deaths: case report and retrospective analysis of hyperhomocysteinemia prevalence in 100 consecutive women with recurrent miscarriages. Fertil Steril 1998;69:152–4.
8. Coumans ABC, Huijgens PC, Jakobs C, Schats R, De Vries JIP, van Pampus MG, et al. Haemostatic and metabolic abnormalities in women with unexplained recurrent abortion. Hum Reprod 1999;14:211–4.
9. Nelen WLDM, Blom HJ, Steegers EAP, Den Heijer M, Thomas CMG, Eskes TKAB. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol 2000;95:519–24.
10. Nelen WLDM, Steegers EAP, Eskes TKAB, Blom HJ. Genetic risk factor for unexplained recurrent early pregnancy loss. Lancet 1997;350: 861.
11. Grandone E, Margaglione M, Colaizzo D, d'Addedda M, D'Andrea G, Pavone G, et al. Methylene tetrahydrofolate reductase (MTHFR) 677T→C mutation and unexplained early pregnancy loss. Thromb Haemost 1998;79:1056–7.
12. Kutteh WH, Park VM, Deitcher SR. Hypercoagulable state mutation analysis in white patients with early first-trimester recurrent pregnancy loss. Fertil Steril 1998;71:1048–53.
13. Holmes ZR, Regan L, Chilcott I, Cohen H. The C677T MTHFR gene mutation is not predictive of risk for recurrent fetal loss. Br J Haematol 1999;105:98–101.
14. Lissak A, Sharon A, Fruchter O, Kassel A, Sanderovitz J, Abramovici H. Polymorphism for mutation of cytosine to thymine at location 677 in the methylenetetrahydrofolate reductase gene is associated with recurrent early fetal loss. Am J Obstet Gynecol 1999;181:126–30.
15. Nelen WLDM, Van der Molen EF, Blom HJ, Heil SG, Steegers EAP, Eskes TKAB. Recurrent early pregnancy loss and genetic-related disturbances in folate and homocysteine metabolism. Br J Hosp Med 1997;58:511–3.
16. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost 1999;82:6–9.
17. Schlesselman JJ. Basic methods of analysis. In: Schlesselman JJ, Stolley PD. Case-control studies: design, conduct, analysis. New York: Oxford University Press, 1982;176–7.
18. Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev 1987;9:1–30.
19. Rothman KJ. Modern epidemiology. Boston: Little, Brown, 1986.
20. Walter SD. The distribution of Levin's measure of attributable risk. Biometrika 1975;62:371–4.
21. Walter SD. Calculation of attributable risks from epidemiological data. Int J Epidemiol 1978;7:175–82.
22. Nelen WLDM, Bulten J, Steegers EAP, Blom HJ, Hanselaar AGJM, Eskes TKAB. Maternal homocysteine and chorionic vascularization in recurrent early pregnancy loss. Hum Reprod 2000;15:954–60.
23. Nelen WLDM, Blom HJ, Thomas CMG, Steegers EAP, Boers GHJ, Eskes TKAB. Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. J Nutr 1998;128:1336–41.
24. Wouters MGAJ. Recurrent miscarriage and hyperhomocysteinemia [dissertation]. Nijmegen: Catholic University 1996.
25. Steegers-Theunissen RPM. Homocysteine, vitamins and neural-tube defects [dissertation]. Nijmegen: Catholic University 1993.
26. Van Pampus MG, Dekker GA, Wolf H, Huijgens PC, Koopman MM, von Blomberg BM, et al. High prevalence of hemostatic abnormalities in women with a history of severe preeclampsia. Am J Obstet Gynecol 1999;180:1146–50.