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Association of MTHFR C677T Polymorphism With Recurrent Pregnancy Loss in a South Indian Population

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ABSTRACT

The C677T variant of the methylenetetrahydrofolate reductase gene (MTHFR) is one of the most studied candidate polymorphisms in reproductive genetics. It lowers the stability and activity of an enzyme at the centre of folate metabolism, and it has been proposed as a risk factor for recurrent pregnancy loss through raised homocysteine and disturbed methylation. This narrative review sets out what the published literature says about that proposal and how firmly it can be held. It draws on work on folate and homocysteine biochemistry, on the definition and causes of recurrent pregnancy loss, and on case-control studies, meta-analyses and the methodological literature on genetic association, and it reports no new data; the title's reference to a South Indian population is treated as a setting for the question, and no local data are presented. The biological case is plausible, and the effect of the variant on homocysteine is clearest where folate intake is low. The epidemiological case is less settled: summary estimates from pooled studies point to a modest association in some populations and none in others, and between-study heterogeneity, small samples, loosely defined cases and uncontrolled population structure weaken most individual reports. Routine testing of the variant is not supported by professional guidance. The review closes with the design features that a convincing study in this setting would need.

Keywords: MTHFR; C677T; Recurrent pregnancy loss; Folate; Homocysteine; Genetic association; Narrative review

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Article Class: Narrative Review

1. Introduction

Losing a pregnancy once is common and usually unexplained. Losing several in a row is rarer, much harder on the couple, and has long prompted a search for a cause

that can be tested and treated (1). Over the last two decades that search has included inherited variants in genes of folate and homocysteine metabolism, and none has attracted more attention than the C677T



variant of the methylenetetrahydrofolate reductase gene, MTHFR (2, 3).

The appeal is easy to see. The variant is common, it changes an enzyme that sits at a well-understood point in metabolism, and it has a plausible link to homocysteine, a compound that has been blamed for damage to blood vessels and to the placenta (4, 5). Yet a plausible mechanism is not a demonstrated cause, and a candidate-gene finding that cannot be reproduced has little clinical value (6, 7).

This article reviews the published literature on the question. The title refers to a South Indian population; no local data are reported here, and nothing in the review should be read as a finding from any one hospital or district. Instead we ask what the biology predicts, what pooled studies have found, how much weight individual reports can carry, and what a convincing study in a setting such as South India would need to contain. The aim is a fair account of what is known and what is simply repeated from one paper to the next.

2. Literature Review

2.1 Folate, Homocysteine and the MTHFR Enzyme

Folate carries single-carbon units for the synthesis of nucleotides and for the methylation of homocysteine to methionine, the precursor of the body's main methyl donor (8, 9). MTHFR makes 5-methyltetrahydrofolate, the form that donates the methyl group in that remethylation (Figure 1). When the enzyme works poorly, less methyl folate is available and homocysteine can accumulate, although diet, vitamin B12 and other genes also shape the result (8). Dietary folate comes mainly from leafy vegetables, pulses and, in some countries, fortified foods, and intake varies a great deal between households and regions (9).

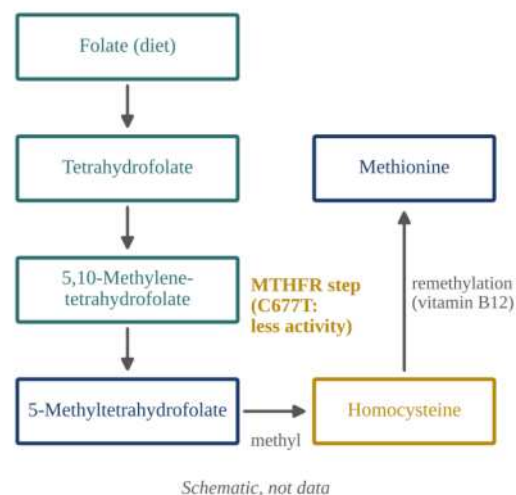


Figure 1. Folate and homocysteine pathway with the MTHFR step marked (schematic, not data).

Folic acid supplementation lowers plasma homocysteine in trials, which is the best evidence that folate status is a major determinant (10); lowering a marker is not the same as lowering a disease, however (5).

2.2 The C677T Variant

The gene was cloned in the mid-1990s, and a common substitution was soon found that changes an alanine to a valine and produces an enzyme that loses activity when warmed (2, 11). People with two copies of the T allele have clearly reduced activity and, in several studies, raised homocysteine, most evidently when folate intake is low (Table 1) (12, 13). A second common variant, A1298C, has a smaller effect (14, 15).

Two lines of clinical work gave the variant its reputation. A pooled analysis of cardiovascular studies found an association between the TT genotype and coronary disease that was clearer in populations with low folate intake (16), and work on neural tube defects tied the variant to a condition that folic acid supplementation prevents (17, 18, 19).

**Table 1.** The C677T genotypes and what is generally reported for each.

Genotype	Enzyme activity	Homocysteine tendency
CC	Typical	Reference group (2)
CT	Mildly reduced	Little change when folate is adequate (12)
TT	Clearly reduced; enzyme heat-labile (2, 11)	Higher, mainly when folate is low (12, 13)

Note: Qualitative summary of the cited literature. Effects depend on folate status and on the assay used for homocysteine.

2.3 Defining Recurrent Pregnancy Loss

Definitions differ. Some professional bodies count two or more clinical losses, others three or more consecutive losses, and the choice changes who is studied (20, 21). Known causes include parental chromosomal rearrangements, uterine anomalies and antiphospholipid syndrome, yet in many couples no cause is found (1, 22). Most early losses in the general population carry a chromosomal abnormality in the embryo, which matters because a gene variant that acts through the mother may be relevant only to the subset of losses with a normal karyotype (23). A group of women who have lost several pregnancies is not the same as the general population, and the comparison needs to respect that.

2.4 Thrombophilia, Methylation and Other Proposed Routes

Several mechanisms have been proposed (Table 2). Raised homocysteine could injure endothelium and trophoblast; reduced methyl supply could alter DNA methylation in the embryo and placenta; and impaired thymidylate synthesis could affect genome stability in rapidly dividing cells (8, 24). A separate idea treats the variant as a thrombophilia that predisposes to clotting in the placental circulation,

which fits the finding that inherited thrombophilias are over-represented among women with late complications (25, 26).

The thrombosis idea has been tested directly and has not held up well for this variant. Systematic reviews of inherited thrombophilias found associations for some factors, notably factor V Leiden, but little or none for MTHFR (27, 30). Trials of heparin and aspirin in unexplained recurrent miscarriage have not shown a clear gain in live birth (31, 32).

Table 2. Proposed routes from the variant to pregnancy loss and how well each is supported.

Route	Proposed mechanism	Support
Homocysteine	Toxicity to endothelium and trophoblast (4, 5)	Plausible; inconsistent
Methylation	Less methyl supply for DNA and histones (8, 9)	Mostly indirect
DNA synthesis	Thymidylate supply and genome stability (24)	Indirect
Thrombosis	Placental clotting (25, 26)	Weak for this variant (27)
Fetal genotype	Embryo carries the variant too (28, 29)	Little studied

Note: The last column is the authors' qualitative reading of the cited literature, not a measured quantity.

2.5 Population Variation and the Indian Setting

The frequency of the T allele varies considerably between populations, and reported genotype distributions depend on where the sample was drawn (33). India adds a particular complication. Its population is made up of many groups that have long married mostly within the group, so genetic structure is strong and allele frequencies can differ sharply between communities living in the same city (34). A control group assembled from a different community than the cases can



produce an apparent association with no biology behind it (35). This is one reason the same variant can look important in one report and unremarkable in the next.

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts: does the C677T variant plausibly raise the risk of recurrent pregnancy loss, and how much weight can the epidemiological evidence bear? It does not address treatment of individual patients, and it does not report data from any population. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

3.2 Literature Assembly

We drew on peer-reviewed work in four areas: folate and homocysteine biochemistry, the definition and causes of pregnancy loss, case-control studies and pooled analyses of the variant, and the methodological literature on genetic association. We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, the selection of sources reflects the authors' judgement, and the references predate the publication of this article. We kept a note of why papers were set aside. It is a habit worth keeping even when the answer seems obvious.

3.3 Appraisal Framework

To judge how far a study could be trusted we used five questions (Table 3). Were enough women studied? Was the case group defined and homogeneous? Were controls drawn from the same population? Was genotyping checked, for example against Hardy–Weinberg expectations (36)? Were all analyses reported, in line with published reporting guidance (37)? A reader of a case-control report should be able to tell who the controls were and how

they were chosen.

Table 3. Common weaknesses in association studies of the variant and what follows from them.

Weakness	Consequence	Remedy
Small samples	Unstable, often exaggerated effects (38)	Larger, pre-planned studies
Loose case definition	Mixed causes dilute any signal (20)	State the definition; exclude known causes
Population structure	Spurious differences (34, 35)	Matched controls; ancestry markers
Genotyping error	Misclassification (36)	Check Hardy–Weinberg; repeat samples
Selective reporting	Apparent consistency (7, 39)	Report all, follow STREGA (37)

Note: Weaknesses are those discussed in the cited methodological literature, and are not attributed to any single study.

4. Results

4.1 What Pooled Studies Show

Pooled analyses of the variant and recurrent pregnancy loss have generally reported a modest association between the T allele and risk, but the estimates differ between meta-analyses and between ethnic subgroups (29, 40). A pooled estimate can also drift upwards when small positive studies are published more readily than small null ones, which is why graphical tests for that pattern exist (39). Heterogeneity statistics in such analyses are often high, which signals that the studies are not estimating one common effect (41). Populations differ in allele frequency, folate intake and case selection, and the original reports seldom record the dietary and ancestry details that would explain the differences. None of this makes the variant biologically irrelevant; it makes it hard to interpret in any single clinic population.



	Biological plausibility	Consistency across studies	Study quality
Homocysteine route	moderate	weaker	weaker
Methylation route	moderate	weaker	weaker
Thrombosis route	weaker	weaker	moderate
Folate modifier	stronger	moderate	weaker

Authors' qualitative reading, not data

Figure 2. Qualitative reading of the evidence for each line of enquiry (schematic matrix, not data).

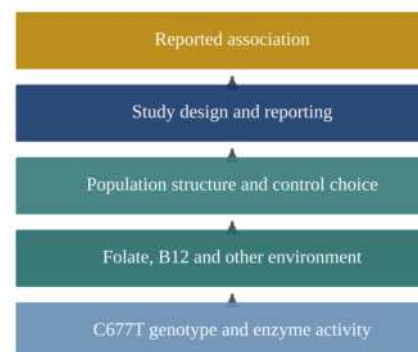
4.2 Folate Status as a Modifier

The biochemical literature points to nutrition as the key modifier. The rise in homocysteine associated with the TT genotype is most evident in people with low folate, and tends to shrink or disappear where folate is replete (10, 12). If the same holds for pregnancy outcomes, a study that ignores folate status may find an association in a poorly nourished sample and none in a well-nourished one, and both could be correct (Figure 2). A biochemical effect that vanishes when folate is replete is not one that a well-nourished patient would notice. The embryo carries its own copy of the variant too, and few studies have examined maternal and fetal genotypes together (28).

4.3 Quality of the Evidence

Applying the framework in Table 3 and Figure 3, most individual reports fall short on at least one count. Small samples produce unstable estimates, and a literature of small studies is easily distorted by selective publication (38). Case definitions vary, controls are often hospital attendees who have never been pregnant or had a single uncomplicated delivery, and the folate status of cases and controls is seldom recorded. Small differences in method can swing a result in either direction.

The wider genetic-association literature shows that initial positive findings are often not replicated, especially for common variants with small effects (6, 7). The C677T variant is a case in point. The point is not that a study is poor, only that its result travels less far than its abstract suggests. A null finding from a small study tells us little, because such a study had little chance of detecting a modest effect. Read this way, a null result from a small study is not evidence of absence.



Each layer can shift what is finally reported; schematic, not data

Figure 3. Layers that separate a genotype from a reported association (schematic, not data).

5. Discussion

5.1 Interpretation

The balance of evidence supports a plausible but unproven role. The biochemistry is sound, folate dependence is well documented, and pooled estimates tend to lean towards a modest increase in risk. Against that, the effect is inconsistent between populations, the mechanisms are largely inferred, and the thrombosis route has not been confirmed. Professional guidance on investigation of recurrent loss does not recommend routine testing for this variant (20, 21). That is a modest conclusion, and it is the one the literature currently supports. The genotype is better seen as a modifier of risk whose importance depends on nutrition and on what else is going on than as a cause that



acts alone. Nothing here argues against studying the variant; it argues for studying it with better designs.

5.2 Limitations

This is a narrative review without a formal search, so selection bias is possible and we make no claim of completeness. Almost all pooled data come from populations other than South Asian ones, and a rigorous Indian meta-analysis would be a useful contribution in its own right. These points apply to most candidate-gene studies, not only to this one.

5.3 Directions for Future Work

A convincing study would recruit adequate numbers of women with a stated, uniform definition of recurrent loss, exclude known causes including parental karyotype abnormalities, and draw controls from the same community. It would measure folate, vitamin B12 and homocysteine, check genotyping against Hardy–Weinberg expectations, and report according to STREGA (36, 37).

6. Conclusion

The C677T variant of MTHFR is a biologically credible candidate for a contributor to recurrent pregnancy loss, particularly where folate intake is low. The epidemiological evidence does not yet establish how large the contribution is, or whether it exists in every population. Readers should treat reports of strong associations with caution until they come from adequately sized studies with clear case definitions, matched controls and nutritional data.

For clinicians, the practical reading is that the variant does not on its own justify routine testing or special treatment. For researchers, the opportunity lies in carefully designed studies in populations such as those of South India, where genetic structure and diet vary greatly and good

evidence is still scarce.

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9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

V.D. conceived the review and wrote the sections on folate biochemistry and the variant. V.G. wrote the sections on pregnancy loss and proposed mechanisms. E.H. wrote the sections on population variation and study design. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or samples were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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