Association of folate-pathway gene polymorphisms with the risk of prostate cancer: A population-based nested case-control study, systematic review and meta-analysis

Simon M Collin¹, Luisa Zuccolo¹, Chris Metcalfe¹, Lina Chen¹, Sarah J Lewis¹, Michael Davis¹*, J Athene Lane¹*, Jenny Donovan¹*, George Davey Smith¹,²*, David E Neal³*, Freddie C Hamdy⁴*, Julius Gudmundsson⁵*, Patrick Sulem⁵*, Thorunn Rafnar⁵*, Kristrun R Benediktsdottir⁵*, Joanne M Murabito⁷,⁸*, Carol L Rosenberg⁸*, Shih-Jen Hwang⁷,⁹*, Rosalind A Eeles¹⁰,¹¹*, Michelle Guy¹⁰*, Kari Stefansson⁵*, Douglas F Easton¹²*, Richard M Martin¹,²

* these authors contributed equally to this study

1. Department of Social Medicine, University of Bristol, Canynge Hall, 39 Whatley Road, Bristol, BS8 2PS, UK
2. MRC Centre for Causal Analysis in Translational Epidemiology (CAiTE), University of Bristol, Oakfield House, Oakfield Grove, Bristol, BS8 2BN, UK
3. Department of Oncology, University of Cambridge, Box 279 (S4), Addenbrooke’s Hospital, Cambridge, CB2 0QQ, UK
4. Nuffield Department of Surgery, John Radcliffe Hospital, Headley Way, Headington, Oxford, OX3 9DU, UK
5. deCODE genetics, 101 Reykjavik, Iceland
6. Department of Pathology, Landspitali-University Hospital, 101 Reykjavik, Iceland
7. The National Heart, Lung, and Blood Institute’s Framingham Heart Study, Framingham, MA, USA
8. Section of General Internal Medicine and Sections of Hematology/Oncology, Department of Medicine, Boston University School of Medicine, Boston, MA, USA
9. National Heart, Lung, and Blood Institute, Bethesda, MD, USA
10. The Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK
11. The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey, SM2 5PT, UK
12. Cancer Research UK Genetic Epidemiology Unit, University of Cambridge, Strangeways Laboratory, Worts Causeway, Cambridge, CB1 8RN, UK
In 2008, more than 186,000 men will be diagnosed with prostate cancer, and more than 28,000 men will die from the disease. One new case occurs every 2.5 minutes and a man dies from prostate cancer every 19 minutes.
Lucy Wills (1888-1964)
Marmite

AWAKENS
THE
APPETITE.

MARMITE
The Pure Vegetable Food Extract.

AWARDED GOLD MEDALS
Universal Food and Cookery Exhibition,
Royal Albert Hall, 1903;
Grocery Exhibition, Crystal Palace, 1903 and 1906.

Registered
Trade Mark.

MANUFACTURED IN ENGLAND BY
The MARMITE FOOD EXTRACT Co., Ltd.

FACTORIES:
Camberwell, London, S.E.
and
Burton-on-Trent, Staffs.

OFFICES:
Mincing Lane House,
59, Eastcheap,
London, E.C.
Mr. Cooling, us imported to temp. Max 39.5 quite well. Writing with always aches & pains. Malaria for pregnancy. Malaria and I am returning that it has been inadequate.

17/12/37
I do not perform on a woman delivering. I am ready with a few infants. The infant is vigorous and clears the lip in a few minutes after birth. The baby is born and is breathing. The newborn is in good condition. The mother is well. The temperature is 37.0.

20/12/37
The baby is well. The baby is 1 day old. The temperature is 37.0. The baby is breathing. The baby is in good condition. The mother is well.

27/12/37
The baby is well. The baby is 2 days old. The temperature is 37.0. The baby is breathing. The baby is in good condition. The mother is well.
**Why Marmite is so good for all of us...**

MARMITE is a concentrated extract of yeast which supplies important B₂ vitamins essential for good health.

You should always have a jar in the larder and give it to the family regularly. It will increase the B₂ vitamin content of the diet, and grown-ups as well as children will approve of its appetising flavour.

MARMITE is a useful protective food which can help to keep people of all ages fit. It is particularly recommended for children and invalids.

**Breakfast**

**MARMITE ON TOAST**

Hot buttered toast, spread lightly with MARMITE is simple—and simply delicious. One teaspoonful is ample for 4 slices of toast, sandwich loaf size, with crusts cut off—serve hot, cut into fingers.

**Egg Cutlets**

1 oz. margarine  
1/2 oz. flour  
1 teaspoonful MARMITE  
1/pt. milk  
2 hard boiled eggs  
chopped parsley  
egg and breadcrumbs  
seasoning  
Melt margarine, stir in the flour, dissolve the MARMITE in the warm milk and add slowly to the flour, stir over a low heat until mixture thickens, season to taste. Add the parsley and eggs, chopped very finely. Allow to cool. Form into six balls and then shape into cutlets. Egg and crumb, fry in hot fat. If desired, serve with tomato sauce as for "Spaghetti in Tomato Sauce," see page 7.

**MARMITE ON FRIED BREAD**

A savoury slice of fried bread per person will help to make one rasher do the work of two. Spread the bread sparingly with MARMITE before frying, for extra flavour and healthfulness.

**MARMITE AND SCRAMBLED EGG**

Add a little MARMITE to the scrambled egg or put a little on the toast before putting the egg on it.

**Don't forget YOUR MARMITE**

Tastes good all ways does you good anyway
Sydney Farber (1903-1973)
tions obtained to date have been added to large quantities of oxalated bovine plasma, and subsequently, concentrates of serum Ac-globulin have been obtained in quantity and quality equal to those obtained from bovine serum itself.

The function of Ac-globulin in the clotting mechanism can then be outlined by use of the following equations:

1. Prothrombin + Thromboplastin $\xrightarrow{\text{Ca}^{++}}$ Thrombin
2. Plasma Ac-globulin $\xrightarrow{\text{Thrombin}}$ Serum Ac-globulin
3. Prothrombin + Thromboplastin $\xrightarrow{\text{Ca}^{++}}$ Serum Ac-globulin
4. Fibrinogen $\xrightarrow{\text{Thrombin}}$ Fibrin Clot

The clotting reaction is initiated by thromboplastin which comes from platelets and tissue juices. Some of the newly formed thrombin alters plasma Ac-globulin so that it becomes serum Ac-globulin. The latter intensifies the interaction of prothrombin and thromboplastin. Thrombin thus accelerates its own formation through an intermediate. This may be regarded as co-autocatalysis. These conclusions differ distinctly from those of Owren (3), but are in harmony with the old and well-known evidence presented in the literature to show that autocatalysis is involved in thrombin formation. This is, however, not autocatalysis but co-autocatalysis, because an intermediate is involved.

We have found that neither serum Ac-globulin nor plasma Ac-globulin can substitute for thromboplastin in the activation of prothrombin. On the contrary, a combination of all three substances is required for that purpose. The most active thrombin prepara-

The Action of Pteroylglutamic Conjugates on Man

SIDNEY FARBER, ELLIOTT C. CUTLER, JAMES W. HAWKINS, J. HARTWELL HARRISON, E. CONVERSE PEIRCE, 2ND, AND GILBERT G. LENZ

The Children’s Hospital, Peter Bent Brigham Hospital, New England Deaconess Hospital, Boston, and Departments of Pathology and Surgery, Harvard Medical School

In 1944, Leuchtenberger, Lewisohn, Lanzlo, and Leuchtenberger (4) reported that a “folic acid concentrate” and a fermentation L. casei factor inhibited the growth of sarcoma 180 transplanted in female Rockland mice. Further studies by Lewisohn and his co-workers (5) in 1945 showed complete regression in about one-third of the single spontaneous breast cancers observed in three different strains of mice treated with daily intravenous injections of 5 μg. of fermentation L. casei factor. This substance was thought at that time to be folic acid; it is now known that it was a conjugate of folic acid, pteroylglutamic acid (3). Subsequent work showed that pteroylglutamic acid (folic acid), when tested under similar conditions, was not effective in producing regression of these breast cancers (6).

In 1944, Hutchings, et al. (3) reported the isolation of the fermentation L. casei factor. This compound was shown to be 60–80 per cent as active when assayed with L. casei and 2–6 per cent as active when assayed with Strep. faecalis R as was the previously isolated liver L. casei factor, pteroylglutamic acid (8).

Degradative reactions have shown that the fermentation L. casei factor differs from pteroylglutamic acid in that the

We are grateful for the cooperation of Y. SubbaRow and his colleagues in the research division of the Lederle Laboratories Division, American Cyanamid Company, who are responsible for the chemical research which forms the foundation of these studies, and to Benjamin Carey, who made available these substances for experimental trial. The compounds were furnished in the form of dry material, yellow-orange in color, in sterile vials, under the names tereptherin and diopterin.

Thanks are due to the staffs of The Children’s Hospital, the Peter Bent Brigham Hospital, and the staff of the New England Deaconess Hospital.
Yellapragada Subbarow (1895-1948)
THE PRODUCTION of temporary remissions in the course of acute leukemia in children by the administration of the compound, 4-aminopteryoylglutamic acid (aminopterin)\(^1,2\)—a biologic antagonist to folic acid\(^*\)—has raised a number of theoretic and practical questions. Confirmation of this finding has been reported from several sources\(^3\); temporary remissions equally impressive have been obtained in adults with acute leukemia by Dameshek.\(^4\)

It is the purpose of this paper to summarize briefly the status of our observations\(^†\) on the action of folic acid antagonists on acute leukemia and other incurable forms of cancer for the interest of those now working with these agents, to state the nature of some of the problems which have arisen, and to indicate some directions of further research.

The demonstration by Lewisohn and his colleagues\(^4\) of the occurrence of complete regression in about one-third of single spontaneous breast cancers in three different strains of mice treated with fermentation L. casei factor, later shown to be pteroylglutamic acid (Hutchings et al.\(^6\)) and the subsequent synthesis of this compound by SubbaRow and his co-workers\(^7\) led to our study of the effect of pteroylglutamic acid on incurable cancer in man. Among the patients so treated were 11 children with acute leukemia. The occurrence of what we called an "acceleration phenomenon" in the viscera and bone marrow of these patients and an experience with folic acid deficiency experimentally produced in the rat suggested that it would be worth while to ascertain if this acceleration phenomenon might be employed to advantage in the treatment of acute leukemia in children, either by the use of radiation or nitrogen mustard therapy after pretreatment with folic acid or conjugates of folic acid, or by the immediate use of folic acid inhibitors or

From The Children's Medical Center, Boston, Mass., and The Department of Pathology, Harvard Medical School.


This study has been supported in part by Cancer Grant \#550-390 of the National Cancer Institute, United States Public Health Service, and in part by The Children's Cancer Research Foundation, Boston.

This paper is dedicated to Dr. George R. Minot. It was my privilege when a student to hear his lectures on diseases of the blood. In these he united in masterful fashion the fields of pathology, physiology, and clinical medicine to establish a logical approach to the nature of disease and so to therapy. His announcement, when I was a fourth year student, of the liver treatment of pernicious anemia fired the imagination of all who heard him to a consideration of the role of nutrition in other incurable diseases of unknown etiology. \(S.\ F.

\(^*\) By antagonist to folic acid is meant a substance which possesses the property of inhibiting the growth of \textit{Streptococcus Ficusalis R}, or \textit{L. casei} in the presence of marginal levels of folic acid. Reversal of inhibition occurs when the concentration of folic acid in the culture medium is elevated.

\(^†\) Our studies represent the accomplishment of a group of clinicians and laboratory workers who have joined forces to make possible rapid progress along the lines indicated in this paper. Detailed reports of clinical, experimental, toxicologic and pathologic studies are being prepared for publication.
I YAM WHAT I YAM!
## Associations of folate-pathway nutrients with risk of prostate cancer

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Measure</th>
<th>Increased risk</th>
<th>No association</th>
<th>Reduced risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Folate</strong></td>
<td>Intake</td>
<td></td>
<td>Vlajinac (1997)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stevens (2003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weinstein (2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hultdin (2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Johansson (2008)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pelucchi (2005)</td>
<td></td>
</tr>
<tr>
<td><strong>B6</strong></td>
<td>Intake</td>
<td></td>
<td>Vlajinac (1997)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pelucchi (2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weinstein (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>Hultdin (2005)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Johansson (2008)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Methionine</strong></td>
<td>Intake</td>
<td></td>
<td>Pelucchi (2005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weinstein (2006)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Homocysteine</strong></td>
<td>Blood</td>
<td></td>
<td>Weinstein (2003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hultdin (2005)</td>
<td></td>
</tr>
</tbody>
</table>
Association of folate-pathway gene polymorphisms with the risk of other cancers:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Cases</th>
<th>Odds ratio (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>MTHFR C677T</td>
<td>12,261</td>
<td>0.83 (0.75-0.93)</td>
</tr>
<tr>
<td></td>
<td>MTHFR A1298C</td>
<td>4,764</td>
<td>0.81 (0.69-0.96)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>MTHFR C677T</td>
<td>2,727</td>
<td>1.52 (1.31-1.77)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>MTHFR C677T</td>
<td>2,191</td>
<td>0.84 (0.71-0.99)</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>MTHFR Ex5 + 79T</td>
<td>4,121</td>
<td>1.17 (1.02-1.34)</td>
</tr>
</tbody>
</table>

Dong et al JAMA. 2008;299(20):2423-2436
Molecular effects of nutrients on gene expression and integrity through modulation of DNA methylation

- Decreased thymidylate synthesis -> increased uracil misincorporation -> altered DNA synthesis & repair

- Imbalance of dietary nutrient
  - Folate
  - Dietary methyl group
  - Zinc
  - Niacin
  - Vitamin C
  - Selenium

- Alteration of genomic DNA methylation

- Alteration of gene specific DNA methylation
  - promoter hypermethylation
  - promoter hypomethylation
  - exon site hypermethylation
  - exon site hypomethylation

- Cell growth, Tissue differentiation, Cancer, Aging
  - decrease in gene expression
  - increase in gene expression
  - decrease in gene transcription
  - increase in gene transcription
  - increase in DNA disruption


Copyright ©2002 American Society for Nutrition
## Included studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Design</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kimura</td>
<td>2000</td>
<td>Germany</td>
<td>case-control</td>
<td>132</td>
<td>150</td>
</tr>
<tr>
<td>Heijmans</td>
<td>2003</td>
<td>Netherlands</td>
<td>longitudinal</td>
<td>21</td>
<td>772</td>
</tr>
<tr>
<td>Singal</td>
<td>2004</td>
<td>USA</td>
<td>case-control</td>
<td>81</td>
<td>42</td>
</tr>
<tr>
<td>van Guelpen</td>
<td>2006</td>
<td>Sweden</td>
<td>longitudinal</td>
<td>299</td>
<td>617</td>
</tr>
<tr>
<td>Johansson</td>
<td>2007</td>
<td>Sweden</td>
<td>case-control</td>
<td>2,777</td>
<td>1639</td>
</tr>
<tr>
<td>Reljic</td>
<td>2007</td>
<td>Croatia</td>
<td>case-control</td>
<td>95</td>
<td>37</td>
</tr>
<tr>
<td>Marchal</td>
<td>2008</td>
<td>Spain</td>
<td>case-control</td>
<td>182</td>
<td>205</td>
</tr>
<tr>
<td>Stevens</td>
<td>2008</td>
<td>USA</td>
<td>case-control</td>
<td>1,144</td>
<td>1,144</td>
</tr>
<tr>
<td>CGEMS</td>
<td>2008</td>
<td>USA</td>
<td>GWAS</td>
<td>1,188</td>
<td>1,110</td>
</tr>
<tr>
<td>Framingham</td>
<td>2008</td>
<td>USA</td>
<td>GWAS</td>
<td>172</td>
<td>231</td>
</tr>
<tr>
<td>deCODE</td>
<td>2008</td>
<td>Iceland</td>
<td>GWAS</td>
<td>1,619</td>
<td>30,779</td>
</tr>
<tr>
<td>UKGPCS</td>
<td>2008</td>
<td>UK</td>
<td>GWAS</td>
<td>1,854</td>
<td>1,894</td>
</tr>
<tr>
<td>ProtecT</td>
<td>2008</td>
<td>UK</td>
<td>case-control</td>
<td>1,600</td>
<td>1,855</td>
</tr>
</tbody>
</table>

## Cases and controls in the meta-analysis for each SNP

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>SNP ID</th>
<th>Studies</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td>677C&gt;T</td>
<td>rs1801133</td>
<td>12</td>
<td>10,745</td>
<td>40,158</td>
</tr>
<tr>
<td>MTHFR</td>
<td>1298A&gt;C</td>
<td>rs1801131</td>
<td>5</td>
<td>3,176</td>
<td>4,829</td>
</tr>
<tr>
<td>MTR</td>
<td>2756A&gt;G</td>
<td>rs1805087</td>
<td>8</td>
<td>7,810</td>
<td>37,543</td>
</tr>
<tr>
<td>MTRR</td>
<td>66A&gt;G</td>
<td>rs1801394</td>
<td>4</td>
<td>3,032</td>
<td>4,515</td>
</tr>
<tr>
<td>MTHFD1</td>
<td>1958A&gt;G</td>
<td>rs2236225</td>
<td>6</td>
<td>7,493</td>
<td>36,941</td>
</tr>
<tr>
<td>SLC19A1</td>
<td>80G&gt;A</td>
<td>rs1051266</td>
<td>4</td>
<td>6,222</td>
<td>35,821</td>
</tr>
<tr>
<td>SHMT1</td>
<td>C1420T</td>
<td>rs1979277</td>
<td>2</td>
<td>2,689</td>
<td>4,110</td>
</tr>
<tr>
<td>FOLH1</td>
<td>1561C&gt;T</td>
<td>rs202676</td>
<td>5</td>
<td>6,314</td>
<td>35,190</td>
</tr>
</tbody>
</table>
MTHFR C677T – all cases (n=10,745) vs controls (n=40,158)

MTHFR C677T, per T allele, pooled OR = 1.03 (0.98 - 1.09)
MTR A2756G – all cases (n=7,810) vs controls (n=37,543)

MTR A2756G, per G allele, pooled OR = 1.05 (0.99 - 1.71)
MTR A2756G – localized cases (n=2,187) vs controls (n=4,414)

MTR A2756G, per G allele, pooled OR = 1.10 (1.01 - 1.21)
MTR A2756G – advanced cases (n=1,780) vs controls (n=4,414)

MTR A2756G, per G allele, pooled OR = 1.00 (0.91 - 1.11)
SHMT1 C1420T – all cases (n=2,689) vs controls (n=4,110)

SHMT1 C1420T, per T allele, pooled OR = 1.08 (1.00 - 1.16)
So what?

If MTR A2756G were an activating polymorphism, having same effect as elevated B12, then prostate cancer risk could be increased via hypermethylation of tumour suppressor genes. Opposite of rat model (low B12 -> low MTR activity -> hypomethylation)*

But folate-pathway gene polymorphisms are unlikely to be major determinants of susceptibility to prostate cancer on a population basis.