Thermolabile MTHFR genotype and retinal vascular occlusive disease

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Abstract

**Background**—Raised levels of total plasma homocysteine (tHcy) are associated with an increased risk of retinal vascular occlusive disease. A thermolabile form of a pivotal enzyme in homocysteine metabolism, methylenetetrahydrofolate reductase (MTHFR), has been associated with vascular occlusive disease and raised tHcy levels. The relation between thermolabile MTHFR genotype, tHcy, and retinal vascular occlusive disease has not been determined.

**Methods**—A retrospective case-control study involving hospital based controls and cases with retinal vascular occlusions in whom tHcy levels had been determined was undertaken. Genotyping for the MTHFR 677 C-T mutation that specifies the thermolabile form of the enzyme was performed by established methods in all subjects. The relation between homozygosity for thermolabile MTHFR genotype (TT), raised tHcy levels, and risk of retinal vascular occlusive disease was examined.

**Results**—87 cases of retinal vascular occlusive disease (mean age 68.7 years) comprising 26 cases of retinal artery occlusion and 61 of retinal vein occlusion were compared with 87 controls (mean age 70.2 years). The TT genotype did not confer a significantly increased risk of retinal vascular occlusive disease. The mean tHcy level was significantly higher in the cases than in the controls (p<0.0001). Overall, and in both the cases and controls, the frequency of the TT genotype was higher in those with normal tHcy levels than in those with increased levels of tHcy. However, the TT genotype did not significantly alter the risk of increased tHcy levels in these patients.

**Conclusions**—The TT genotype is not associated with an increased risk of retinal vascular occlusive disease or increased tHcy levels in this group of elderly patients. In older patients, nutritional rather than genetic factors may be more important in increasing tHcy levels, a known risk factor for retinal vascular occlusive disease.

(Abram M) Homocysteine metabolism may be caused by inherited defects in the controlling enzymes of homocysteine metabolism, one of which is 5,10-methylenetetrahydrofolate reductase (MTHFR). MTHFR catalyses the conversion of methylenetetrahydrofolate to methylenetetrahydrofolate, the methyl donor in the remethylation of homocysteine to methionine, and to date nine loss of function mutations have been documented in the MTHFR gene. Of these, point mutation 677C-T which results in the substitution of alanine for valine results in a less active, thermolabile form of the MTHFR enzyme.

The prevalence of homozygotes for the thermolabile MTHFR genotype (TT) in an Irish population is approximately 11%.

A higher prevalence of TT genotype has been found in some reports of patients with coronary artery disease, ischaemic stroke, and venous thrombosis when compared with controls. Furthermore, raised plasma levels of homocysteine (tHcy) may be a result of TT genotype and raised tHcy is an independent risk factor for atherosclerosis and venous thrombosis. Possible mechanisms of action of homocysteine on vascular endothelium include promotion of platelet activation, enhanced coagulation, and smooth muscle proliferation. However, not all previous reports have found that the TT genotype is itself significantly associated with vascular occlusive disease.

A recent report has shown a higher prevalence of TT genotype in patients with retinal vein occlusion than that expected in the general population, but tHcy levels in the affected patients were not measured. Raised tHcy was found to be an independent risk factor for retinal vascular occlusive disease in a recent case-control report of typical older patients, as well as in an uncontrolled case series and in two case reports of unusual cases. The present study was undertaken to assess the relation between TT genotype, retinal vascular occlusive disease, and tHcy levels.

**Methods**

**CASES AND CONTROLS**

As previously described, a retrospective case-control study was performed and patients with retinal vascular occlusion were identified from the records of the photographic department of the Royal Victoria Eye and Ear Hospital. To minimise the influence of the vascular event on plasma tHcy levels and to reduce bias caused by risk factor treatment, only cases diagnosed between 3 and 12 months before the study were included. Age matched controls included patients attending for routine surgery and a small number of hospital staff; the exclusion criteria have been described previously.
Table 1  Frequencies of MTHFR genotypes in cases and controls

<table>
<thead>
<tr>
<th>MTHFR genotype</th>
<th>Controls (n=87)</th>
<th>Cases (n=87)</th>
</tr>
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<tbody>
<tr>
<td>CC</td>
<td>36 (41.4%)</td>
<td>36 (41.4%)</td>
</tr>
<tr>
<td>CT</td>
<td>36 (41.4%)</td>
<td>44 (50.6%)</td>
</tr>
<tr>
<td>TT</td>
<td>15 (17.2%)</td>
<td>13 (14.9%)</td>
</tr>
</tbody>
</table>

VARIABLES EXAMINED
Demographic, cardiovascular risk factor, and diagnostic data were recorded for each subject based on a previously described standardised format.21 Diagnostic data from fasting blood samples, analysed using standard automated laboratory techniques, consisted of biochemical, haematological, and endocrine parameters that are known to alter tHcy levels while tHcy was determined by high performance liquid chromatography and fluorescence detection.26 Molecular genetic analysis for the MTHFR 677C-T mutation was performed using heteroduplex technology.27

STATISTICAL ANALYSIS
Logarithmic transformations and geometric means were used for tHcy values, which showed a marked positive skew. Univariate analysis using the Mann-Whitney U test was carried out to determine the significance of association between the cases and controls with regard to tHcy levels. Odds ratios for retinal vascular disease and raised tHcy levels (>12 µmol/l) in cases and controls conferred by TT genotype were calculated by logistic regression.

Results
Eighty seven patients with retinal vascular occlusive disease (mean age 68.7 years) comprising 26 cases of retinal artery occlusion and 61 of retinal vein occlusion were compared with 87 controls (mean age 70.2 years); 48% of the study participants were men (n=84) and, while there were similar proportions of men to women in the control and venous occlusion groups, there was a significantly higher proportion of men with retinal artery occlusions (p=0.009).

The frequency of the three genotypes in the cases and controls is shown in Table 1. On univariate analysis the TT genotype did not confer a significantly increased risk of retinal vascular occlusive disease (Table 2). On univariate analysis the TT genotype did not confer a significantly increased risk of retinal vascular occlusive disease (Table 2). Overall, 65 participants (37%) in the study had raised tHcy levels as indicated by a fasting tHcy level of >12 µmol/l; 43 subjects (49%) with retinal vascular occlusive disease had raised tHcy levels while the mean tHcy level in all cases was 12.9 µmol/l. In the control group 22 subjects (25%) had tHcy levels of >12 µmol/l and the mean level in all the controls was 10.7 µmol/l. The mean tHcy level was significantly higher in the cases than in the controls (p<0.0001).

Overall, the frequency of the TT genotype was higher in those with normal tHcy levels (19.2%) than in those in whom the tHcy levels were raised (9.2%). This trend was seen in both the cases (11.6% TT genotype with raised tHcy and 18.1% TT genotype with normal tHcy) and the controls (9.0% TT genotype with raised tHcy compared with 20% TT genotype with normal tHcy; Table 2). However, on univariate analysis the TT genotype did not confer a significantly increased risk of increased tHcy levels in either the cases or controls (Table 2).

When broken down according to whether the patients presented with venous or arterial occlusion, there was no significant difference in the impact of TT genotype on disease (Table 2). Cardiovascular risk factors of smoking (p=0.85), hypertension (p=0.66), diabetes (p=0.06), or treatment for glaucoma did not significantly modify the risk of disease conferred by TT genotype in this population, as judged by a test for interaction of genotype and risk factor. None of the eight patients who underwent carotid surgery carried the TT genotype.

Discussion
This study has found that the TT genotype is not associated with retinal vascular occlusive disease. This is in contrast with a recent report which showed that retinal vein occlusion may be associated with the TT mutation in a middle aged Israeli population.28 It may be that their finding reflects chance, or possibly poor matching of ethnic origin between cases and controls since the Israeli population is unlikely to be uniform in its genetic history. Larger studies of the genetics of retinal vascular occlusion disease may help to explain this disparity between the findings of these two studies.

The effect of MTHFR genotype on disease risk, found in some studies, is thought to be mediated through its effect on tHcy levels.6–11 In this study the TT genotype was not associated with increased levels of tHcy. It is not possible to compare this finding with the Israeli population with retinal vein occlusions as it is not known whether or not they had raised levels of tHcy.20 However, this does contrast with the results of two other studies in the Irish population, one of which examined relatively young patients with cardiovascular disease and the other assessed tHcy levels in relation to MTHFR genotype in a middle aged population.1,2 Both of these studies found that the TT genotype conferred a significantly increased risk of raised tHcy levels.1,2 Furthermore, the

Table 2  Odds ratios for retinal vascular occlusive disease and increased total plasma homocysteine (tHcy) levels conferred by TT genotype

<table>
<thead>
<tr>
<th></th>
<th>TT* Odds ratio</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Cases (n=87)</td>
<td>13 (14.9%)</td>
<td>0.84 to 1.90</td>
</tr>
<tr>
<td>Venous (n=61)</td>
<td>5 (5.7%)</td>
<td>0.37 to 3.51</td>
</tr>
<tr>
<td>Arterial (n=26)</td>
<td>8 (9.2%)</td>
<td>0.29 to 1.83</td>
</tr>
<tr>
<td>versus Controls (n=87)</td>
<td>15 (7.2%)</td>
<td></td>
</tr>
<tr>
<td>Cases with increased tHcy (n=43)</td>
<td>5 (11.6%)</td>
<td>0.18 to 1.98</td>
</tr>
<tr>
<td>versus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases with normal tHcy (n=44)</td>
<td>8 (18.1%)</td>
<td>0.40 to 2.04</td>
</tr>
<tr>
<td>Controls with increased tHcy (n=22)</td>
<td>2 (9.0%)</td>
<td></td>
</tr>
<tr>
<td>versus Controls with normal tHcy (n=65)</td>
<td>13 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>All subjects with increased tHcy (n=65)</td>
<td>7 (9.2%)</td>
<td>0.20 to 1.26</td>
</tr>
<tr>
<td>All subjects with normal tHcy (n=109)</td>
<td>21 (19.2%)</td>
<td></td>
</tr>
</tbody>
</table>

*TT = homozygous for thermolabile MTHFR genotype.
odds ratios for the association between raised homocysteine and genotype for these studies fall outside the 95% confidence interval reported here, indicating that the difference between studies is not merely a consequence of small sample sizes but may reflect a real difference.5 8

These contrasting reports of the relation between TT genotype and tHcy levels may be related to the age of the patients studied. The population studied in this report had a median age of approximately 70 years whereas the population mean age was lower in the two previous Irish studies which showed an association between TT genotype and tHcy levels.5 8 Interestingly, a number of studies of elderly patients with late onset vascular disorders also failed to find an association between MTHFR and tHcy levels.16 18 19

Both nutritional and genetic factors are important determinants of increased tHcy levels and dietary deficiency of the vitamin co-factors B6, B12, and folate can cause an increase in levels of tHcy.5 8 10 28–32 A recent report found that supplementation with folic acid reduced tHcy levels and individuals with the TT genotype may require higher levels of folate for adequate tHcy regulation.31 32 Genetic determinants of tHcy may be more important in younger patients with premature vascular disease, whereas nutritional factors may be more important in older patients as evidenced by a recent study in which two thirds of elderly patients had raised tHcy levels because of dietary deficiency.32 It is possible that the mechanism whereby the TT genotype contributes to raised levels of tHcy is down-regulated in older patients so that the genotype no longer exerts an influence on tHcy levels.

In conclusion, the TT genotype is not associated with an increased risk of retinal vascular occlusive disease or with raised levels of tHcy in this group of elderly patients. In older patients, nutritional rather than genetic factors may be more important in increasing tHcy levels, a known risk factor for retinal vascular occlusive disease.

The Research Foundation of the Royal Victoria Eye and Ear Hospital, Dublin provided a grant for this study. Maria Karabatzaki was supported by a student grant provided by the Health Research Board of Ireland. Raymond Meleady was supported by an Irish Heart Foundation/Health Research Board of Ireland Unit Grant and by the European Union.

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doi: 10.1136/bjo.85.1.88

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