Original Investigation

Associations Between Methylenetetrahydrofolate Reductase Polymorphisms, Serum Homocysteine Levels, and Incident Cortical Cataract

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IMPORTANCE Methylenetetrahydrofolate reductase (MTHFR) polymorphisms have been shown to influence homocysteine levels; homocysteine has been implicated as a cataractogenic stressor.

OBJECTIVE To investigate the associations of MTHFR polymorphisms and serum homocysteine levels with incident cortical cataract in an older population.

DESIGN, SETTING, AND PARTICIPANTS From 1992 to 1994, a population-based cohort study, the Blue Mountains Eye Study, was conducted with 3654 residents (82.4% of eligible participants) of the Blue Mountains region aged 49 years and older. At the second (1997-1999, 5-year follow-up) and third (2002-2004, 10-year follow-up) surveys, 2334 (75.8% of survivors) and 1952 (76.7% of survivors) were examined, respectively. For this report, the second survey serves as baseline when homocysteine levels were assessed, and 5-year incidence of cataract refers to incidence estimated from the second to the third survey. After excluding participants with no follow-up data or DNA or who had previous cortical cataract or cataract surgery, 757 participants were included in gene and environment analyses. This current project on associations with cataract was designed initially March 19, 2013, and completed April 14, 2014. Cataract was assessed using the Wisconsin Cataract Grading system. Two MTHFR polymorphisms, C677T (rs1801133) and A1298C (rs1801131), were included. Serum homocysteine levels were assessed following standard methods.

MAIN OUTCOMES AND MEASURES Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals for incident cortical cataract, after adjusting for age, sex, smoking status, hypertension, diabetes, education, and myopia. Path analysis was performed to explore a possible pathway of MTHFR polymorphisms via homocysteine levels to cortical cataract.

RESULTS The mean (SD) age of the 1726 participants in the Blue Mountains Eye Study 2 cohort with normal homocysteine levels was 68.3 (8.1) years and 73.2 (8.5) years for those with elevated homocysteine levels. Both the C677T polymorphism (CT/TT vs CC: OR = 1.50; 95% CI = 1.01-2.23) and elevated homocysteine levels (>15 μmol/L: OR = 2.24; 95% CI = 1.38-3.63) were independently associated with increased risk of cortical cataract. Path analysis showed that the genetic effect on cortical cataract was partially mediated via homocysteine levels. Combined CT/TT genotypes and elevated homocysteine levels were associated with a 3-fold risk of cortical cataract (OR = 3.74; 95% CI = 1.79-7.80). The synergy index of both exposures was 1.34 (95% CI = 0.44-4.01).

CONCLUSIONS AND RELEVANCE MTHFR polymorphism and elevated homocysteine levels contributed separately and jointly to increased risk of cortical cataract. If these findings are confirmed, homocysteine levels may be a therapeutic target to reduce risk of cortical cataract in persons carrying genetic risk.

Published online March 17, 2016.
Cortical cataract is a common type of age-related cataract. Previous studies have shown a strong familial aggregation for cortical cataract\(^1,2\) and have estimated the heritability to be between 24% and 74%,\(^3,4\) suggesting a strong genetic component for this type of cataract.

Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms have been linked to other eye diseases, such as diabetic retinopathy,\(^5\) primary open-angle glaucoma,\(^6\) and closed-angle glaucoma.\(^6\) However, there are limited data on the role of MTHFR polymorphisms in cataract formation.\(^7\) MTHFR polymorphisms also influence homocysteine levels.\(^8,9\) Two common polymorphisms in MTHFR (chromosome 1p36.22), C677T (rs1801133) and A1298C (rs1801131), are associated with reduced enzyme activity and result in subsequent increases in homocysteine levels.\(^10,11\)

Recently, homocysteine has been implicated as a cataractogenic stressor, inducing a cascade of processes in lens epithelial cells that may result in cortical cataract formation.\(^12\) Elevated homocysteine levels are an indicator of a disruption in homocysteine metabolism. Thus, we aimed to assess the associations among MTHFR polymorphisms, serum homocysteine levels, and the 5-year incidence of cortical cataract in a population-based cohort.

Methods

Study Population
The Blue Mountains Eye Study (BMES) is a population-based study of common eye diseases in an Australian population aged 49 years and older living in the Blue Mountains region, west of Sydney. Baseline examinations were conducted from 1992 to 1994, with 3654 participants (82.4% of those eligible) recruited. Follow-up examinations were conducted 5 (1997-1999, BMES 2) and 10 (2002-2004, BMES 3) years later, and 2334 (75.8% of survivors) and 1952 (76.7% of survivors) participants were seen during the 5- and 10-year follow-up examinations, respectively.\(^13\) For this report, the BMES 2 survey serves as the baseline when homocysteine levels were assessed, and the 5-year incidence of cataract refers to the incidence estimated from the BMES 2 to the BMES 3 survey.

Detailed examination procedures have been described previously.\(^14,15\) The same procedures were used for all 3 examinations. Briefly, after pupil dilatation, participants underwent a detailed eye examination, including lens photography. Interviewer-administered questionnaires were used to collect medical and demographic information. The study adhered to the tenets of the Declaration of Helsinki and was approved by the human ethics committees of the University of Sydney and the Western Sydney Area Health Service. Written informed consent was obtained from each participant.

Cataract Grading
Detailed grading procedures have been described previously.\(^15\) Briefly, lens photographs taken at the examinations were assessed in a masked manner for cataract using the Wisconsin Cataract Grading System.\(^16\) Retroillumination (Neitz CT-R; Neitz Instruments) photographs were used to determine cortical cataract. Total area of involvement was estimated using a grid overlay. Cortical cataract was defined as a cortical opacity of 5% or more of the total lens area in at least one eye. Inter- and intragrade reliabilities for cataract grading were high.\(^17\)

DNA Genotyping
DNA was extracted from whole blood. Genotyping was performed using the Illumina Human 670-Quadv1 custom genotyping array at the Wellcome Trust Centre for Human Genetics (Sanger Institute) as part of the Wellcome Trust Case Consortium 2. Both the MTHFR single-nucleotide polymorphisms (SNPs), C677T (rs1801133) and A1298C (rs1801131), were directly genotyped and not imputed. Quality control procedures were performed at the Sanger Institute and at the Centre for Clinical Epidemiology and Biostatistics at the University of Newcastle and have been described previously.\(^18\) Briefly, the quality control procedures applied before data analysis involved excluding SNPs with low call rate, pronounced deviation from Hardy-Weinberg equilibrium, or low minor allele frequency and excluding individuals with low call rate, discrepancy in clinical and genotypic sex, evidence of cryptic relatedness based on identical by state sharing (I member of pair excluded), or outlying continental ancestry based on principal component analysis.\(^19\)

Serum Assays
Fasting blood samples were collected at the study site during BMES 2 and processed the same day at the Institute of Clinical Pathology and Medical Research. Serum homocysteine assays were conducted on an IMx Analyzer (Abbott Laboratories). Serum folate assays were conducted on a Beckman Access Analyzer (Beckman Coulter, Inc).

Statistical Analysis
All analyses were performed using SAS version 9.3 (SAS Institute Inc). Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals for the associations between MTHFR polymorphism, elevated homocysteine levels (>15 μmol/L [to convert to milligrams per liter, divide by 7.397]), and cortical cataract. Risk factors adjusted for in the models included age, sex, smoking status, hyperten-
Table 1. Characteristics of the BMES 2 Population With Blood Information According to Serum HCY and Folate Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal HCY Level (≤15 μmol/L; n = 1390)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>68.3 (8.1)</td>
</tr>
<tr>
<td>Female</td>
<td>828 (59.6)</td>
</tr>
<tr>
<td>Current smoking habit</td>
<td>117 (8.4)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1080/1382 (78.1)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>152 (10.9)</td>
</tr>
<tr>
<td>Higher education*</td>
<td>813/1337 (60.8)</td>
</tr>
<tr>
<td>Myopia</td>
<td>213 (15.3)</td>
</tr>
<tr>
<td>MTHFR 677 rs1801133</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>638 (45.9)</td>
</tr>
<tr>
<td>CT</td>
<td>602 (43.3)</td>
</tr>
<tr>
<td>TT</td>
<td>150 (10.8)</td>
</tr>
<tr>
<td>MTHFR 1298 rs1801131</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>673/1389 (48.5)</td>
</tr>
<tr>
<td>AC</td>
<td>579/1389 (41.7)</td>
</tr>
<tr>
<td>CC</td>
<td>137/1389 (9.9)</td>
</tr>
</tbody>
</table>

Abbreviations: BMES, Blue Mountains Eye Study; HCY, homocysteine, MTHFR, methylenetetrahydrofolate reductase.

SI conversion factors: To convert homocysteine to micrograms per liter, multiply by 2.266. To convert folate to nanomoles per liter, multiply by 2.66.

* Defined as trade certificate or higher qualification.

The SIab was calculated as:

$$S_{Iab} = \frac{RR_{ab} - 1}{(RR_{a} + RR_{b}) - 2}$$

where RRab is the relative risk of combined exposures, RRa is the relative risk of exposure to elevated homocysteine levels, and RRb is the relative risk of exposure to MTHFR genotypes. An SI of 1 indicates an additive joint effect, whereas an SI greater than 1 indicates more than an additive effect. Confidence interval estimation of additive interaction measures was performed as described by Hosmer and Lemeshow.

Results

Of the 2334 participants in the BMES 2 cohort, 1726 (73.9%) had blood samples for homocysteine analysis and genetic information, and 1691 had samples for folate analysis. Comparisons between those with and without blood information, ie, homocysteine levels and genetic information, in the BMES 2 cohort indicated no significant differences between the 2 groups except for age; participants without blood information were slightly older than those with blood information (eTable in the Supplement).

Table 1 shows the characteristics of the BMES 2 population according to homocysteine and folate status. Participants with elevated homocysteine levels (n = 336 [19.5%]) were more likely to be older, male, current smokers, and have his-
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Diabetes and elevated homocysteine levels (rhombus were more likely to be older and to have a history of diabetes, hypertension, and myopia, and were 49.5% for AA, 41.0% for AC, and 9.5% for CC for the 677CT/TT genotype but less likely to have the 677CC genotype (Table 1). The genotype frequencies for MTHFR SNP C677T rs1801133 were 45.0% for CC, 42.8% for CT, and 12.2% for TT and were 49.5% for AA, 41.0% for AC, and 9.5% for CC for A1298C rs1801131.

Of the 1726 participants who had blood information at BMES 2, 1337 (77.5%) attended the BMES 3 examination. After excluding participants (n = 580) with prior cortical cataract or cataract surgery or with missing or ungradable photos in either eye, there were 757 participants included in the analyses incorporating homocysteine level and genetic information. A comparison of the baseline characteristics of these 757 participants showed that those with incident cortical cataract were more likely to be older and to have a history of diabetes and elevated homocysteine levels (Table 2). Hardy-Weinberg equilibrium P values showed no deviation between the observed and expected values in either cases or controls for both MTHFR SNP C677T rs1801133 (P = .85 for cases, P = .20 for controls) and A1298C rs1801131 (P = .22 for cases, P = .79 for controls).

Cortical cataract developed in 17.1% (n = 130) of participants over the 5-year period. After adjusting for age, sex, smoking status, hypertension, diabetes, education, and myopia (not including folate or homocysteine level), only the MTHFR C677T polymorphism (rs1801133) was associated with incident cortical cataract in both the additive (OR for each additional T allele = 1.34; 95% CI = 1.01-1.78) and dominant (CT/TT genotypes vs CC: OR = 1.50; 95% CI = 1.01-2.23) models (Table 3). Additional adjustment for homocysteine levels in the dominant model resulted in a slightly stronger association between the MTHFR C677T polymorphism and incident cortical cataract (CT/TT genotypes vs CC: OR = 1.71; 95% CI = 1.13-2.58). The MTHFR A1298C polymorphism was not associated with incident cortical cataract in either the additive or dominant models (Table 3). After adjusting for age, sex, smoking status, hypertension, diabetes, education, myopia, and folate level, elevated homocysteine level at baseline was associated with a 2-fold increased risk of 5-year incident cortical cataract (adjusted OR = 2.24; 95% CI = 1.38-3.63) (Table 3). Additional adjustment for the MTHFR C677T SNP resulted in a slight attenuation of the association between homocysteine levels and incident cortical cataract (OR = 1.96; 95% CI = 1.20-3.20).

The Figure shows that the OR for the MTHFR C677T direct pathway to incident cortical cataract was 1.42 (95% CI = 1.07-1.90), whereas the OR for its indirect pathway via elevated homocysteine levels was 1.19 (95% CI = 0.86-1.63). The proportion of risk contribution from the MTHFR C677T polymorphism on cortical cataract that is attributed to the indirect pathway is 33% (Figure: 0.17/0.52 = 0.33). In other words, 33% of the risk from the MTHFR C677T polymorphism is mediated through the indirect pathway of homocysteine, and 67% of the risk is via a direct pathway between the MTHFR polymorphism and cortical cataract.

Because we found that the genetic effect was not wholly mediated by homocysteine level, we then looked at the joint effects of the MTHFR C677T polymorphism and homocysteine level. After adjusting for age, sex, smoking status, hypertension, diabetes, education, myopia, and folate level, those with risk genotypes of the C677T polymorphism (CT/TT alone had an 88% increased risk of incident cortical cataract (OR = 1.88; 95% CI = 1.16-3.06), those with elevated homocysteine levels were 49.5% for AA, 41.0% for AC, and 9.5% for CC for the 677CT/TT genotype but less likely to have the 677CC genotype (Table 1). The genotype frequencies for MTHFR SNP C677T rs1801133 were 45.0% for CC, 42.8% for CT, and 12.2% for TT and were 49.5% for AA, 41.0% for AC, and 9.5% for CC for A1298C rs1801131.

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Table 3. Associations Between MTHFR SNPs C677T rs1801133 and A1298C rs1801131, Elevated HCY Levels, and Incidence of Cortical Cataract

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cortical Cataract, OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated HCY level, &gt;15 μmol/L</td>
<td>2.24 (1.38-3.63)</td>
</tr>
<tr>
<td>SNP: dominant modelb</td>
<td></td>
</tr>
<tr>
<td>C677T rs1801133</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>CT/TT</td>
<td>1.50 (1.01-2.23)</td>
</tr>
<tr>
<td>A1298C rs1801131</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>AC/CC</td>
<td>0.73 (0.50-1.08)</td>
</tr>
<tr>
<td>SNP: additive modelb</td>
<td></td>
</tr>
<tr>
<td>677 T</td>
<td>1.34 (1.01-1.78)</td>
</tr>
<tr>
<td>1298 C</td>
<td>0.85 (0.63-1.14)</td>
</tr>
</tbody>
</table>

Abbreviations: HCY, homocysteine; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; SNP, single-nucleotide polymorphism.

a Adjusted for age, sex, smoking status, hypertension, diabetes, education, myopia, and folate level.

b Adjusted for age, sex, smoking status, hypertension, diabetes, education, and myopia.

Figure. Direct and Indirect Pathways to Incident Cortical Cataract

HCY indicates homocysteine; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; SNP, single-nucleotide polymorphism.

* Adjusted for age, sex, smoking status, hypertension, diabetes, education, myopia, and folate level.

** Adjusted for SNP-additive model.

teine levels alone had a 2-fold increased risk of incident cortical cataract (OR = 2.17; 95% CI = 1.01-4.68), and those with both the risk genotypes and elevated homocysteine levels had nearly a 4-fold increased risk of incident cortical cataract (OR = 3.74; 95% CI = 1.79-7.80) compared with persons without these factors (Table 4). The SI was 1.34 (95% CI = 0.44-4.01) for combined exposures of C677T risk genotypes (CT/TT) and elevated homocysteine levels on the risk of cortical cataract.

Discussion

We observed in this older Australian population that both the C677T polymorphism and elevated homocysteine levels were associated with an increased risk of incident cortical cataract. Path analysis showed that only a third of the risk of cortical cataract due to the MTHFR polymorphism could be attributed to the indirect pathway via elevated homocysteine levels. The joint exposure to MTHFR risk genotypes and elevated homocysteine levels was associated with a nearly 4-fold increased risk of cortical cataract.

We could not find previous reports of significant association between the MTHFR genetic polymorphism and cortical cataract in the literature. The MTHFR gene encodes for the MTHFR enzyme, an essential enzyme in homocysteine metabolism.9 Of the 2 polymorphisms investigated, C677T had reduced enzyme activity compared with A1298C;10,11 677TT conferred a 60% to 70% reduction in MTHFR enzyme activity, while 1298CC conferred a 30% to 40% reduction.90 We observed an increased risk of incident cortical cataract among those with the 677CT/TT genotypes only and no association for the A1298C polymorphism with cortical cataract incidence. Two previous case-control studies have reported on frequencies of the polymorphisms, and 1 study5 found that the 677CC (wild) genotype was more prevalent among cataract cases (63%), while the other study7 found that the 3 genotypes had similar frequencies in cataract cases and controls and found no associations between MTHFR genotypes and cataract. In comparison, we found 37% of those with incident cortical cataract had the CC genotype in our population.

To our knowledge, there has been only 1 other observational study25 that has investigated the association between homocysteine levels and cataract. This case-control study found significantly higher serum homocysteine levels among cases than controls. Although the type of cataract was not established in the case-control study, this finding keeps with our observation. A number of experimental studies have shown that elevated homocysteine levels induce stress and generate reactive oxygen species in animal and human lens epithelial cells.12,24 Prolonged exposure to high levels of homocysteine has been shown to induce apoptosis and suppress antioxidant protection in the lens epithelial cells, which can lead to the formation of cortical cataract.12,24 It has also been shown previously that aqueous levels of homocysteine correlate with plasma levels.25 These results from experimental studies lend support to the biological plausibility of the observed effect of homocysteine levels on incident cortical cataract in our population.
Biologically, the MTHFR polymorphism is associated with reduced MTHFR enzyme activity, which results in a subsequent increase in homocysteine levels.\(^9^{10}\) We observed a significant association of elevated homocysteine levels with the presence of each additional 677T allele (indirect pathway in the Figure). This is consistent with other studies; a meta-analysis of MTHFR gene mutation, homocysteine levels, and cardiovascular disease showed that those with the TT genotype had a 25% higher mean total homocysteine level than those with the CC genotype.\(^2^{6}\)

The path analysis revealed a strong genetic component in this pathway to cortical cataract formation. Although 33% of the risk for incident cortical cataract from the MTHFR polymorphism could be attributed to the direct pathway via elevated homocysteine levels, two-thirds of the risk was attributed to the direct pathway of this polymorphism. There are likely other pathways involved in the association between MTHFR polymorphism and cortical cataract in addition to the pathway of homocysteine level. MTHFR gene expression has been detected in lens cells of mouse embryos.\(^2^{7}\) However, other possible mechanisms of the MTHFR gene in the lens are unclear.

There are other cofactors involved in homocysteine metabolism that may influence the interrelationships reported in this study. Riboflavin has been recently shown to affect the relationship between MTHFR polymorphisms and the serum level of homocysteine.\(^2^{8}\) However, in this population, there were limited numbers of participants with low riboflavin levels, and thus, we were unable to investigate this association further. Folate levels play an integral part in the metabolism of homocysteine.\(^9^{19}\) Previously, we reported a protective relationship between the use of supplements containing folate and cortical cataract prevalence in the BMES population.\(^2^{9}\) In the current study, we did not find a significant association between folate levels and incident cortical cataract (data not shown). However, because there was a previous association of folate levels with cortical cataract prevalence and the known effect of folate on homocysteine levels, we adjusted for folate to ensure the associations observed were independent of folate levels.

A combined exposure to both the MTHFR C677T polymorphism and elevated homocysteine levels had a strong association with incident cortical cataract, as demonstrated by the higher OR for incidence of cortical cataract when both factors are present (Table 4). While a Rothman SI of 1.34 suggests a possible synergistic effect when both factors are present, we could not confirm this possibility in our study sample because the confidence interval of the SI includes 1, which indicates an additive effect.

Strengths of our study include a population-based cohort with a reasonable follow-up of participants (76.5% of surviving participants), standardized examination methods, and masked grading procedures of lens photographs used at each visit. There are limitations in our study. Although we have adjusted for a number of known confounding factors associated with cortical cataract, there may be other unidentified confounding factors that we have not accounted for in the models. We had 1 measurement of homocysteine level available from this cohort. As such, a single measurement may not represent the average level over the long term or lifetime and therefore may be insufficient for determining the degree to which the genetic effect is mediated by homocysteine levels. As with other complex diseases, multiple pathways may contribute to the increased risk of cortical cataract. The fact that we focus on 1 pathway in this report should be viewed as simplified in regard to the etiology of cortical cataract. We excluded participants who had cataract surgery in either eye by the time BMES 3 was conducted, which may have underestimated cortical cataract incidence in our population. However, because we do not know the type of cataract prior to surgery, this exclusion should have minimized misclassification in cortical cataract phenotype. Most importantly, we cannot exclude the possibility of chance findings in our study, and replications in other different study samples are essential before a conclusion can be drawn.

Conclusions

We have investigated the interrelationships between the MTHFR C677T polymorphism, elevated homocysteine levels, and incident cortical cataract in a population-based cohort. Findings suggest that both the MTHFR gene and elevated homocysteine levels are separately and jointly associated with cortical cataract incidence, and when in combination, their joint effect is nonsignificantly stronger than the sum of individual effects of the gene and elevated homocysteine levels. Future studies are warranted to confirm these findings. If replicated, homocysteine levels can be a therapeutic target to reduce the risk of cataract in those with the genetic risk.
Singapore, Singapore (Cheng); Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio (Iyengar); Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, Ohio (Iyengar); University Hospitals Eye Institute, Cleveland, Ohio (Iyengar); Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, Ohio (Iyengar); Center for Clinical Investigation, Case Western Reserve University, Cleveland, Ohio (Iyengar).

Author Contributions: Dr Wang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Tan, Mitchell, Cumming, Jun, Cheng, Wang.

Acquisition, analysis, or interpretation of data: Tan, Kiffey, Mitchell, Rochtchina, Flood, Cumming, Jun, Holliday, Scott, Teo, Klein, Iyengar, Wang.

Drafting of the manuscript: Tan.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Kiffey, Rochtchina, Cumming, Jun, Holliday, Teo, Iyengar.


Administrative, technical, or material support: Tan, Mitchell, Scott.


Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Jun is employed by Eisai Inc. No other disclosures were reported.

Funding/Support: This work was supported by grants 974159, 211069, and 1031058 from the Australian National Health & Medical Research Council (NHMRC). Ms Tan is supported by postgraduate research scholarship GNT1049049 from NHMRC. Dr Wang is supported by grants 358702 and 632909 (2005-2015) from an NHMRC Senior Research Fellowship.

Role of the Funder/Sponsor: The NHMRC had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES


