DNA Methylation in Peripheral Blood and Risk of Gastric Cancer: A Prospective Nested Case–control Study

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ABSTRACT

DNA methylation in peripheral blood is a potential biomarker of gastric cancer risk which could be used for early detection. We conducted a prospective case–control study nested within the Melbourne Collaborative Cohort Study. Genomic DNA was prepared from blood samples collected a median of 12 years before diagnosis for cases ($N = 168$). Controls ($N = 163$) were matched to cases on sex, year of birth, country of birth, and blood sample type using incidence density sampling. Genome-wide DNA methylation was measured using the Infinium HumanMethylation450K Beadchip. Global measures of DNA methylation were defined as the median methylation M value, calculated for each of 13 CpG subsets representing genomic function, mean methylation and location, and reliability of measurement. Conditional logistic regression was conducted to assess associations between these global measures of methylation and gastric cancer risk, adjusting for *Helicobacter pylori* and other potential confounders. We tested nonlinear associations using quintiles of the global measure distribution. A genome-wide association study of DNA methylation and gastric cancer risk was also conducted ($N = 484{,}989$ CpGs) using conditional logistic regression, adjusting for potential confounders. Differentially methylated regions (DMR) were investigated using the R package *DMRcate*. We found no evidence of associations with gastric cancer risk for individual CpGs or DMRs ($P > 7.6 \times 10^{-6}$). No evidence of association was observed with global measures of methylation (OR 1.07 per SD of overall median methylation; 95% confidence interval, 0.80–1.44; $P = 0.65$). We found no evidence that blood DNA methylation is prospectively associated with gastric cancer risk.

Prevention Relevance: We studied DNA methylation in blood to try and predict who was at risk of gastric cancer before symptoms developed, by which stage survival is poor. We did not find any such markers, but the importance of early diagnosis in gastric cancer remains, and the search for markers continues.

Introduction

When incidence of gastric cancer was first estimated in 1975, it was the most common cancer globally. Since then, the incidence and mortality have decreased substantially, as a result of a decline in the prevalence of risk factors such as *Helicobacter pylori (H. pylori)*, use of salt for food preservation, tobacco smoking, and improvements in diet, with more fresh fruit and vegetables consumed (1). In the latest international figures from 2018 (2), gastric cancer was the fifth most common malignancy worldwide, and the third leading cause of cancer mortality.

Although Australia is among the countries with a relatively low incidence of gastric cancer, mortality is relatively high, with 5-year survival reported to be 30% in 2011–2015 (3). This is consistent with European data for gastric cancers diagnosed between 2000 and 2007 (4) and reflects the lack of early signs and symptoms, making early detection difficult, leading to the search for biomarkers that could be used to assess risk (5).

DNA methylation is one such biomarker that has been widely studied for several cancers. Global hypomethylation is associated with genomic instability and cancer risk, while

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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Cancer Prev Res 2021;14:233–40

doi: 10.1158/1940-6207.CAPR-20-0003

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hypermethylation of tumor suppressor gene promoters may also increase risk by reducing expression of the tumor suppressor genes (6, 7). Previous analyses using data from the Melbourne Collaborative Cohort Study (MCCS) have suggested that variations in genome-wide DNA methylation measured in DNA extracted from peripheral blood collected before diagnosis are associated with risk of prostate (8), breast (9), and urothelial cell carcinoma (10) and B-cell neoplasms (11). Thus, there is potential for using peripheral blood DNA methylation as a biomarker of risk for gastric cancer.

A recent review of molecular studies to identify biomarkers for early diagnosis noted aberrant DNA methylation in a number of genes in the serum or gastric mucosa of people diagnosed with gastric cancer (5). To our knowledge, very few prospective studies of DNA methylation and gastric cancer have been conducted, and many of the existing studies have used DNA extracted from gastric tissue rather than peripheral blood.

The aim of this study was to identify methylation biomarkers of gastric cancer risk. This was done by assessing the prospective association of peripheral blood DNA methylation at individual CpGs, in CpG clusters, or globally, with the incidence of gastric cancer

Materials and Methods

Study sample

Participants were selected from the MCCS, a prospective study of 41,513 men and women ages 27 to 76 years (99% were ages 40–69) recruited between 1990 and 1994 (12). Italian and Greek migrants were oversampled to extend the range of lifestyle exposures. Peripheral blood was drawn from study participants at recruitment, and stored as dried blood spots (DBS) on Guthrie cards, mononuclear cells or as buffy coats. The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol (IEC 9001). Participants gave written consent to participate and for the investigators to obtain access to their medical records.

Questionnaire and anthropometric measures

At baseline, information was obtained on country of birth, smoking status, and dietary intake using interviewer-administered questionnaires. For all participants, anthropometric measures including weight, height, and waist circumference were taken by trained staff using standard methods (13). The alternative healthy eating index (AHEI; ref. 14) was calculated for each participant using responses to a 121-item Food Frequency Questionnaire specifically designed for the MCCS (15), and included as a covariate to reflect overall diet in the analysis.

Case ascertainment

Gastric cancer cases were identified by record linkage to the Victorian Cancer Registry as participants with incident invasive (or metastatic) adenocarcinoma between the gastroesophageal junction and the pylorus (ICD-O 160–169; morphology codes: 8140, 8142, 8143, 8144, 8145, 8211, 8255, 8261, 8263, 8323, 8480, 8481, or 8490) during follow-up to June 30, 2014. Participants with other gastric cancer diagnoses were considered ineligible.

Study design

A case–control study nested in the MCCS was conducted (**Fig. 1**). Participants were excluded whether they had a confirmed cancer diagnosis other than keratinocyte cancer prior to blood collection, had no baseline blood sample available or had missing data for any of the matching variables. One control was selected for each case using incidence density sampling with age as the time axis, matching on sex, year of birth, country of birth (Australia/New Zealand/UK/other, Greece, Italy) and blood sample type [DBS, peripheral blood mononuclear cell (PBMC), buffy coat].

DNA extraction and bisulphite conversion

DNA was extracted from frozen lymphocytes and buffy coat samples using QIAamp mini spin columns (Qiagen) and stored at 4°C. DNA from DBSs collected on Diagnostic Cellulose filter paper (Whatman) and stored in airtight containers at room temperature was extracted using the method described by Joo and colleagues (16). Briefly, 21 blood spots of 3.2 mm diameter each were punched and lysed in PBS and protease using TissueLyser (Qiagen). The resulting lysate was processed using Qiagen mini spin columns according to the manufacturer's protocol. The DNA was quantified using the Quant-iT Picogreen dsDNA assay and measured on the Qubit Fluorometer (Life Technologies), with a minimum of 500 ng of DNA considered acceptable for methylation analysis. Bisulphite conversion was performed using the EZ DNA Methylation-Gold Kit (Zymo Research) according to the manufacturer's instructions. Postconversion, quality control was performed using an in-house SYBR Green-based bisulphite-specific PCR, designed to determine the success of bisulphite conversion by comparing the amplification efficiency of the test sample with an unconverted high molecular weight control DNA (17). Bisulphite-converted samples that amplified five or more quantitative cycles earlier than the unconverted control were progressed to the Infinium HumanMethylation450 (HM450K) BeadChip assay (Illumina Inc). For all case–control pairs, the DNA was progressed through the experimental workflow at the same time.

DNA methylation assay

Samples were processed in batches of 96 (8 Infinium HM450K BeadChips per batch). To minimize potential plate and chip effects, samples from each matched case–control pair were plated to adjacent wells on the same BeadChip, with plate, chip, and position assigned randomly (18). The HM450K BeadChip assay was performed according to the manufacturer's instructions. A total of 200 ng of bisulphite converted DNA was Whole Genome Amplified and hybridized onto the BeadChips. The TECAN automated liquid handler (Tecan Group Ltd) was used for single-base extension and staining.

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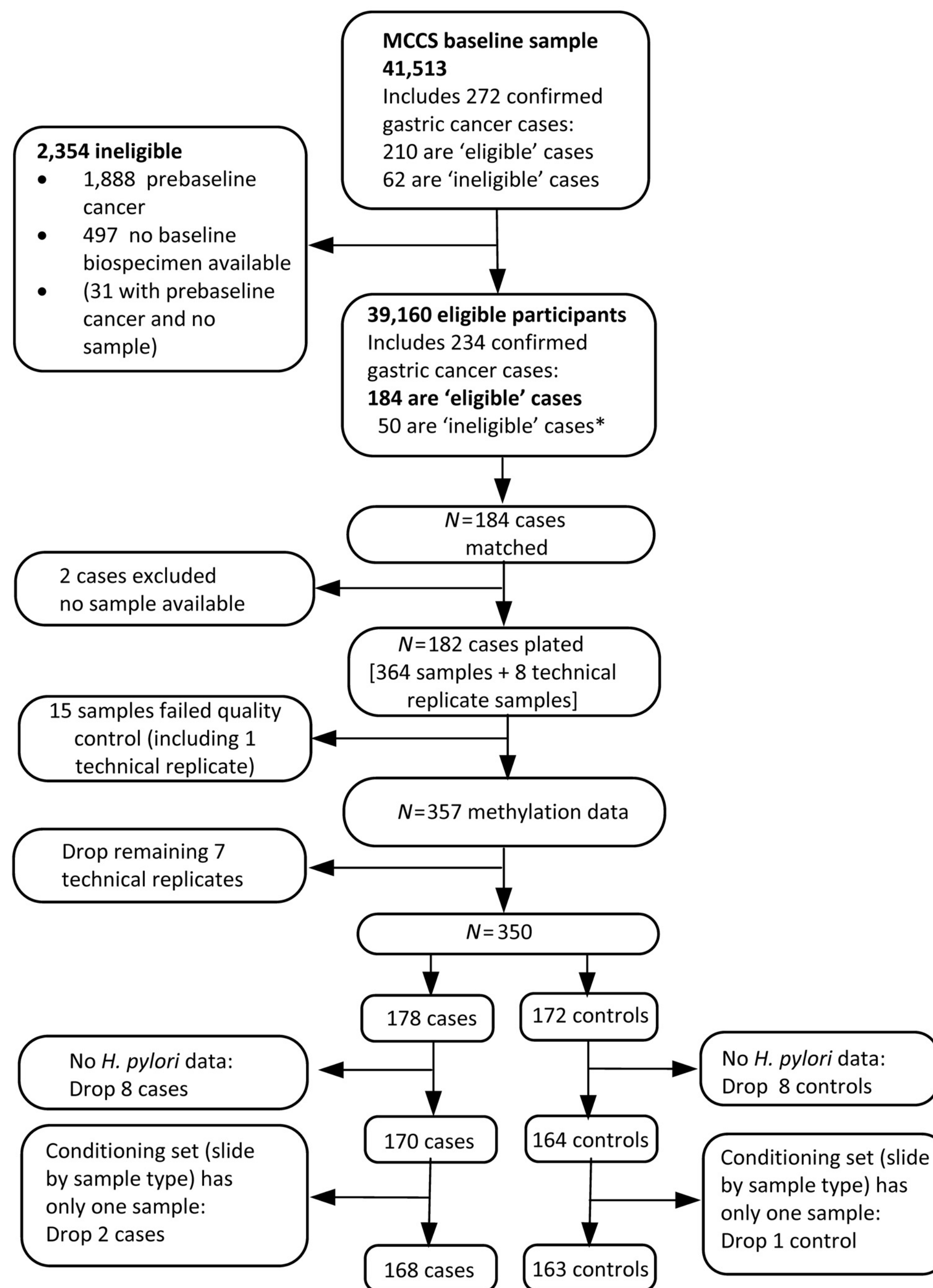

Figure 1.

Flow chart of sample selection. *Gastric cancer cases were defined as incident invasive (or metastatic) adenocarcinoma between the gastro-esophageal junction and the pylorus (ICD-O 160–169; morphology codes: 8140, 8142, 8143, 8144, 8145, 8211, 8255, 8261, 8263, 8323, 8480, 8481, or 8490);other gastric cancer diagnoses were considered ineligible.

Data processing

A β-value (interpreted as fraction of methylation) was calculated for each CpG site for each sample using the R package *minfi*. β-values were transformed into M values using $M = \log_2(\beta/(1-\beta))$ (19). Methylation measures with a detection *P* value higher than 0.01 were considered missing. Samples with >5% of CpG methylation measures missing were excluded, and CpGs with values missing for more than 20% of samples were excluded, resulting in 484,989 CpG sites remaining for subsequent analysis.

H. pylori Assay

A commercially available Immunoblotting Kit (Helicoblot 2.1; Genelabs Diagnostics) which has 96% sensitivity and 93%

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specificity for the detection of *H. pylori* infection (20) was used to assess the *H. pylori* status of cases and controls in plasma collected at baseline. Sixteen participants were excluded from the analysis due to missing data for *H. pylori* infection status.

Statistical analysis

Genome-wide measures of DNA methylation

We first excluded from the analysis all CpG sites found to be measured inaccurately by the HM450K assay (21). The median methylation M value was used as a global measure of methylation and calculated for the following 13 CpG subsets: all CpGs; all CpGs that also had higher reliability [intraclass correlation >0.1, based on sample replicate analysis (22)]; four subsets with higher reliability and categorized according to genomic region (CpG islands, shores, shelves, and open sea), four subsets with higher reliability and categorized according to gene function (gene promoters, 3′UTR, gene bodies, and intergenic), and three subsets according to mean β-value in the controls (lowly methylated, mean $\beta \leq 0.2$; moderately methylated with $0.2 < \text{mean } \beta \leq 0.8$; and highly methylated with $0.8 < \text{mean } \beta \leq 1.0$). Quintiles of each global measure were also calculated to investigate nonlinear relationships, with cut-offs based on controls, and the lowest quintile as the reference category. For all global methylation measures, the association with gastric cancer risk was investigated using conditional logistic regression with the median methylation as the exposure and case–control status as the outcome.

Genome-wide study of DNA methylation

ORs for gastric cancer were estimated for individual CpGs on the HM450K array using conditional logistic regression with statistical significance indicated by $P < 10^{-7}$.

For both the global measures and the individual CpGs, conditional logistic regression models were adjusted for sex, year of birth, country of birth, white blood cell proportions estimated using the Houseman algorithm (23), *H. pylori*, AHEI diet score, smoking, and waist circumference, conditioning on combinations of slide by sample type. Because *H. pylori* data were missing from individual cases or controls within matched pairs, rather than conditioning on case–control pairs, we conditioned on chip and sample-type combination to maximize the sample size, excluding two cases and one control due to only one participant being present in the relevant conditioning set. For the full adjustment set, we examined the correlations among the predictors, and the fit of the models as measured by the Akaike information criterion. This analysis showed that the models with the full adjustment set were affected by multicollinearity and overfitting. Consequently, we undertook additional analyses: (i) unadjusted analysis; (ii) adjusted for *H. pylori* status and white blood cell proportions (Model 1), and (iii) using an offset method (24) to adjust for *H. pylori* and cell proportions only (Model 2). Model 1 uses a set of adjustment variables that still gives good prediction of gastric cancer but omits the variables which contribute little to its prediction. In Model 2, we define an offset variable (effectively fixing the coefficients of the adjustment variables) calculated from a model of gastric cancer predicted by *H. pylori* and white blood cell proportions only, and eliminate multicollinearity by using this offset variable as an adjustment, thereby obtaining a less biased estimate of the unique effect of methylation on the prospective risk of gastric cancer. Given the multicollinearity and overfitting in the fully adjusted model and the potential for confounding in the raw model, we regard Model 1 and Model 2 as more informative than the raw or fully adjusted models. Model 1 achieved similar prediction of gastric cancer risk to other models while omitting variables that contributed to multicollinearity and overfitting but little to prediction.

As sensitivity analyses, (i) we conducted subgroup analyses by sample type (PBMC and DBS), and (ii) we assessed the effect of time since blood draw on the association between methylation and gastric cancer by adding a main effect of continuous time since blood draw, along with an interaction of methylation with time since blood draw, to Model 1 and Model 2. The time since blood draw was calculated using the date of diagnosis for cases and, for controls, the date at which they reached the age at which their matched case was diagnosed.

Differentially methylated regions

We tested for genomic regions of contiguous CpGs associated with gastric cancer risk using the *DMRcate* R package (25) with default settings (bandwidth of $\lambda = 1{,}000$ base pairs (bp) and a scaling factor $C = 2$ for the Gaussian kernel smoother). We performed the analysis using two adjustment sets (i) fully adjusted, adjusting for year of birth, country of birth, white blood cell proportions, *H. pylori*, AHEI diet score, smoking, and waist circumference; and (ii) adjusting for *H. pylori* and cell proportions only, corresponding to Model 1 above. All CpGs were considered to be part of a potentially significant cluster, and clusters with regionwise Stouffer *P* value less than 10^{-7} were considered statistically significant (25).

Analyses were carried out using R version 3.4.4 and Stata 14.2 (StataCorp).

Results

The analysis included 168 cases and 163 controls with complete data for all covariates and methylation. The baseline characteristics of the sample are shown in **Table 1**. A total of 84% of cases and 64% of controls were *H. pylori* positive. The BMI distribution was different for cases when compared with controls, with obesity being more common in cases (30%) than controls (23%). The median time between blood draw and diagnosis for cases was 12 years.

There were no statistically significant associations for the 13 global measures of DNA methylation, for either the linear analysis of association with risk (**Table 2**; Supplementary Table S1), or the analysis including time since blood draw ($P > 0.17$ for the analyses using adjustment set as in

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Table 1. Baseline characteristics of study participants.

Variable	Controls $N = 163$	Cases $N = 168$
DNA source		
Buffy coat	6 (4%)	6 (4%)
Dried blood spots	99 (61%)	102 (61%)
Peripheral blood mononuclear cells	58 (36%)	60 (36%)
Sex (male)	105 (64%)	110 (65%)
Country of birth		
Australia/NZ/UK	87 (53%)	87 (52%)
Italy	41 (25%)	41 (24%)
Greece	35 (21%)	40 (24%)
Age at blood draw, median (IQR)	62 (56–65)	61 (56–65)
H. pylori status positive	105 (64%)	141 (84%)
Smoking		
Never	75 (46%)	81 (48%)
Current	24 (15%)	25 (15%)
Former	64 (39%)	62 (37%)
Alcohol		
None	61 (39%)	62 (37%)
Low[a]	70 (44%)	83 (50%)
Moderate[b]	16 (10%)	16 (10%)
High[c]	11 (7%)	5 (3%)
BMI		
<25 kg/m^2	36 (22%)	41 (24%)
25–30 kg/m^2	89 (55%)	77 (46%)
≥30 kg/m^2	38 (23%)	50 (30%)
Waist circumference (cm), median (IQR)	91 (83–98)	91 (84–100)
AHEI diet score, median (IQR)	65 (57–71)	64 (57–71)
Time between blood draw and diagnosis (years), median (IQR)		12 (6–17)
Gastric Cancer subtype		
Cardia		51 (30%)
Noncardia		117 (70%)
Age at diagnosis, median (IQR)		73 (67–77)

[a]1–39 g/day (men), 1–19 g/day (women).
[b]40–59 g/day (men), 20–39 g/day (women).
[c]60+ g/day (men), 40+ g/day (women).

Table 2. OR for gastric cancer per 1 SD increment in median M-value methylation.

CpG region[a]	*N* CpGs	Analysis	OR[b] (95% CI)	*P*
All	294,519	Model 1	1.18 (0.74–1.88)	0.49
		Model 2	1.07 (0.80–1.44)	0.65
All reliable[c]	195,358	Model 1	1.12 (0.75–1.67)	0.57
		Model 2	1.08 (0.78–1.51)	0.64
Location[c]				
Island	58,503	Model 1	0.98 (0.59–1.62)	0.93
		Model 2	0.99 (0.73–1.36)	0.96
Shore	52,907	Model 1	1.14 (0.71–1.82)	0.59
		Model 2	1.05 (0.79–1.40)	0.74
Shelf	16,701	Model 1	1.00 (0.66–1.54)	0.98
		Model 2	1.00 (0.73–1.38)	0.99
Open Sea	67,247	Model 1	0.96 (0.62–1.50)	0.87
		Model 2	0.98 (0.72–1.34)	0.90
Function[c]				
Promoter[d]	82,285	Model 1	1.02 (0.66–1.57)	0.93
		Model 2	1.01 (0.75–1.36)	0.95
3′UTR	7,616	Model 1	0.99 (0.65–1.51)	0.96
		Model 2	0.99 (0.70–1.40)	0.96
Body	64,054	Model 1	1.03 (0.68–1.57)	0.89
		Model 2	1.02 (0.73–1.43)	0.91
Intergenic	50,187	Model 1	1.12 (0.75–1.68)	0.59
		Model 2	1.08 (0.77–1.52)	0.65
Mean β level				
Low (0–0.2)	57,518	Model 1	0.88 (0.48–1.62)	0.69
		Model 2	0.96 (0.66–1.38)	0.81
Moderate (0.2–0.8)	68,344	Model 1	1.17 (0.76–1.79)	0.48
		Model 2	1.08 (0.80–1.44)	0.63
High (0.8–1.0)	69,496	Model 1	1.04 (0.70–1.55)	0.84
		Model 2	1.03 (0.74–1.44)	0.86

Note: Conditional logistic regression analyses: Model 1 adjusted for *H. pylori* status and white blood cell proportions and Model 2 adjusted for *H. pylori* status and white blood cell proportions using an offset method to estimate the unique effect of methylation. Raw and fully adjusted estimates are in Supplementary Table S1.
[a]All subsets only have Naeem high quality probes.
[b]Per 1 SD (based on controls) median M-value increase.
[c]Only includes reliable probes with intraclass correlation (ICC) > 0.1.
[d]Defined as CpGs overlapping potential promoter regions, including those located within 1,500 bp upstream from transcription start sites and 5′UTR. Previously defined promoter associated probes were also categorized into this group according to Illumina annotation v1.2.

Model 1 and Model 2). Subgroup analyses of PBMC and DBS (Supplementary Table S2), and the nonlinear analysis based on quintiles also showed no evidence of associations (**Fig. 2**).

The only adjustment variable with consistent statistically significant prediction of gastric cancer was *H. pylori* status, with OR typically around 4. The variables dropped from the initial adjustment set to form the adjustment set for Model 1 and Model 2 were individually not strong predictors of gastric cancer with $P > 0.1$ and Pseudo-$R^2 < 0.01$ for each of the variables dropped.

There were no statistically significant results for the genome-wide association study of DNA methylation, with all *P* values greater than 10^{-7} ($P > 7.6 \times 10^{-6}$). This is illustrated by the quantile–quantile plot in **Fig. 3**, which compares the distribution of *P* values obtained in the epigenome-wide association study with the uniform distribution of *P* values expected for an overall null association, showing no evidence of genomic inflation or of associations stronger than expected by chance. The *DMRcate* analysis did not identify differentially methylated regions significantly associated with risk of gastric cancer.

Discussion

In this prospective nested case–control study we found no evidence for any association between DNA methylation and gastric cancer risk. No associations were observed for measures of global methylation nor methylation at individual CpGs, or at CpG clusters.

Although we included 168 gastric cancer cases in the analysis, which is more than many studies of gastric cancer in the literature, it was a relatively small sample and our estimates were accordingly imprecise. Our previous case–control studies nested within the MCCS that have suggested associations between blood DNA methylation and cancer, included more than 400 incident cases of urothelial and prostate cancer, and mature B-cell lymphoma (8–11). Because of the limited sample size, we did not attempt to study cardia and noncardia tumors

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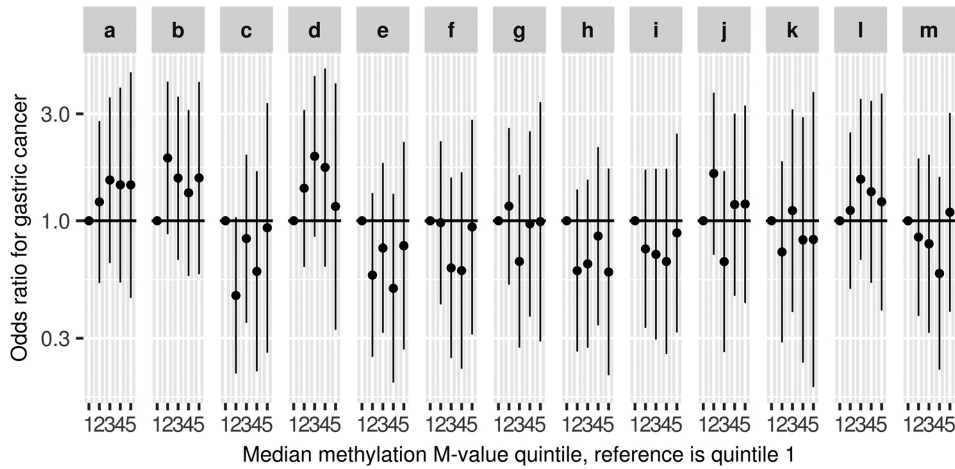

Figure 2.

ORs for gastric cancer risk according to quintiles of DNA methylation by CpG subgroup: **a** all **b** all reliable **c** island **d** shore **e** shelf **f** open sea **g** promoter **h** 3′UTR **i** body **j** intergenic **k** lowly methylated (mean β in controls ≤ 0.2) **l** moderately methylated (0.2 < mean β ≤ 0.8) **m** highly methylated (0.8 < mean β ≤ 1.0). Results are from conditional logistic regression adjusted for *H. pylori* status and white blood cell proportions (Model 1). Promoter is defined as CpGs overlapping potential promoter regions, including those located within 1,500 bp upstream from transcription start sites and 5′UTR. Previously defined promoter associated probes were also categorized into this group according to Illumina annotation v1.2.

separately, although they may have different biology and aetiology (26). Heterogeneity of sample type may also be a limitation if methylation markers associated with gastric cancer risk were detectable only in one type of blood sample, but we have assessed this in the two main sample types DBS and PBMC, finding no associations.

The strengths of the study include the large number (>450,000) of CpGs examined, the variety of global methylation measures investigated, the prospective design, and restricting the analysis of global measures to reliable and high-quality probes (21, 22). As in our previous studies, cases were plated on the same chip as controls and at random consecutive positions

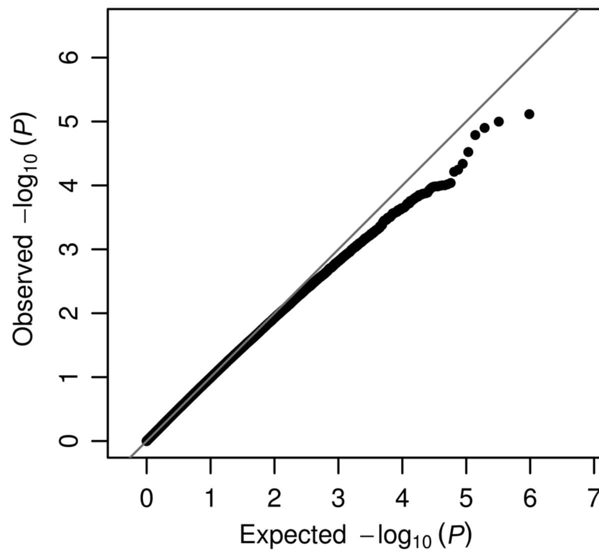

Figure 3.

Quantile–quantile plot for the association between methylation M values at individual CpGs and risk of gastric cancer. The plot uses a reverse log scale such that larger values of $-\log_{10}(P)$ correspond to smaller *P* values. The points on the right-hand side of the graph, representing the smaller *P* values over the multiple tests, are below the diagonal line and have observed *P* values not less than what we would expect with an overall null association.

to reduce technical variability. We used a rigorous adjustment set to deal with confounding, including *H. pylori* status which has been associated with methylation in peripheral blood leukocytes (27, 28) and is a key risk factor for gastric cancer (29). Blood cell composition varies with age and illness and this may affect the measurement of DNA methylation (23). We have minimized the possible effect of this in our analysis by adjusting for white blood cell proportions. An additional strength was that we considered four configurations of the adjustment variables to address overfitting and multicollinearity in the fully adjusted model.

We originally hypothesized that DNA methylation might mediate the effect of diet, particularly intake of nutrients associated with one carbon metabolism and hence methylation of DNA, as a risk factor for gastric cancer. However, we found no association with gastric cancer risk for dietary intake of B vitamins and related nutrients of the one carbon pathway using the same cases and controls as in this analysis and adjusting for *H. pylori* (30). We also found little evidence of dietary intake of these nutrients being associated with blood DNA methylation using cross-sectional data from several other nested case–control studies within the MCCS (31).

Australia does not have a high incidence of gastric cancer (3, 26) but the MCCS includes around 25% of participants from southern Europe who tend to be at increased risk of gastric cancer due to higher rates of *H. pylori* infection (29). This is consistent with our observation that almost 50% of the gastric cancer cases were southern European migrants. Seventy percent of the gastric cancers in the MCCS were noncardia cancers, which is also more characteristic of high-risk populations (26). Thus, our sample may not be representative of the general Australian population or populations of other westernized nations due to the relatively high burden of *H. pylori*. Neither is our population similar to the high-risk mainly Asian populations typically studied in this context. For example, Tahara and Arisawa (32) identified methylation markers for gastric

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cancer in different sample types, mainly plasma, serum, or gastric wash fluid. However of the 8 studies referred to, 6 were in Japan, Hong Kong, or China, one in Russia and one in cell lines from America and Japan.

In this study, we tested (i) CpG sites individually, (ii) CpG sites by region (genome-wide measures), and (iii) CpG sites by cluster. Other measures assessing DNA methylation could be tested, for example we previously assessed five measures of epigenetic aging (33) and found no association for gastric cancer [e.g., Horvath age acceleration: OR = 0.95, 95% confidence interval (CI): 0.80–1.12 and Hannum age acceleration: OR = 1.08, 95% CI: 0.88–1.31]. Three additional methylation-based measure of age—PhenoAge, GrimAge, and predicted telomere length—were subsequently investigated for associations with cancer of seven types in this cohort (34) showing strong associations with colorectal, kidney, lung and, mature B-cell cancers, but no evidence of association with gastric cancer.

A 2013 review by Qu and colleagues (35) identified a number of genes that contribute to distinct biological pathways and are commonly methylated in gastric tumors, and others for which methylation was found to be correlated with gastric cancer prognosis. In a study including plasma and tissue samples from South Korean (high-risk population) and the U.S. (low-risk population) participants, methylated DNA markers were identified that could discriminate between cases and controls and which tended to increase from normal mucosa, through metaplasia, adenoma, and gastric adenocarcinoma (36). These studies provide some evidence for the importance of DNA methylation and its effect on activation of oncogenes and the silencing of tumor suppressor genes in gastric carcinogenesis (36). Identifying a consistent genetic signature for gastric cancer would contribute to diagnosing gastric cancer earlier (37), which is likely to be important in improving outcomes in gastric cancer, although this was not supported by our study.

The follow-up time for cases in our study was up to 21 years with a median of 12 years. We did not have sufficient gastric cancer cases to stratify the present analysis by time since blood draw but using a continuous interaction term we found no evidence that any associations between methylation and gastric cancer were stronger as blood was collected closer to the time of diagnosis.

In conclusion, we found no evidence of an association between DNA methylation in peripheral blood and risk of gastric cancer. The need to identify a marker of gastric cancer allowing earlier diagnosis, and potentially better outcomes remains. Future studies assessing potential blood DNA methylation biomarkers for gastric cancer risk prediction need a larger sample size to investigate associations over different time periods following blood draw as well as different gastric cancer subtypes.

Authors' Disclosures

The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414, 1074383, and 1087683. No disclosures were reported by the other authors.

Authors' Contributions

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Acknowledgments

Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database.

The MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414, 1074383, and 1087683 and by infrastructure provided by Cancer Council Victoria. The funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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Received January 2, 2020; revised June 4, 2020; accepted September 15, 2020; published first September 21, 2020.

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