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MTHFR Gene Polymorphism-Mutations and Air Pollution as Risk Factors for Breast Cancer

A Metaprediction Study

Mildred C. Gonzales ▼ Pojui Yu ▼ S. Pamela K. Shiao

Background: The methylenetetrahydrofolate reductase gene (*MTHFR*) is one of the most investigated genes associated with breast cancer for its role in epigenetic pathways.

Objectives: The objectives of this metaprediction study were to examine the polymorphism-mutation risk subtypes of *MTHFR* and air pollution as contributing factors for breast cancer.

Methods: For triangulation purposes in metapredictive analyses, we used a recursive partition tree, nonlinear association curve fit, and heat maps for data visualization, in addition to the conventional comparison procedure and pooled analyses.

Results: We included 36,683 breast cancer cases and 40,689 controls across 82 studies for *MTHFR* 677 and 23,252 cases and 27,094 controls across 50 studies for *MTHFR* 1298. *MTHFR* 677 TT was a risk genotype for breast cancer ($p = .0004$) and in the East Asian subgroup ($p = .005$). On global maps, the most polymorphism-mutations on *MTHFR* 677 TT were found in the Middle East, Europe, Asia, and the Americas, whereas the most mutations on *MTHFR* 1298 CC were located in Europe and the Middle East for the control group. The geographic information system maps further revealed that *MTHFR* 677 TT mutations yielded a higher risk of breast cancer for Australia, East Asia, the Middle East, South Europe, Morocco, and the Americas and that *MTHFR* 1298 CC mutations yielded a higher risk in Asia, the Middle East, South Europe, and South America. Metapredictive analysis revealed that air pollution level was significantly associated with *MTHFR* 677 TT polymorphism-mutation genotype.

Discussion: We present the most comprehensive analyses to date of *MTHFR* polymorphism-mutations and breast cancer risk. Future nursing studies are needed to investigate the health impact on breast cancer of epigenetics and air pollution across populations.

Key Words: air pollution • breast cancer • geographic information systems • meta-analysis • *MTHFR* gene

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Breast cancer is the most common malignancy and the second leading cause of cancer death among women (American Cancer Society, 2016). Genome-wide association studies (Zhang, Beeghly-Fadiel, Long, & Zheng, 2011) have shown that the methylenetetrahydrofolate reductase gene (*MTHFR*) is one of the most investigated genes in breast cancer for its role in epigenetic modification. Using a metapredictive approach, we investigated whether the *MTHFR* gene polymorphism-mutations and environmental factors such as air pollution could increase the risk of breast cancer susceptibility.

DNA methylation, as one of the epigenetic mechanisms affecting the control of gene transcription and expression, has been a specific target of research for cancer treatment and prevention (Eccles et al., 2013). The two most common loci of polymorphism-mutations in the *MTHFR* gene are C677T (rs1801133) and A1298C (rs1801131), which are both associated with reduced enzymatic activity. Homozygote 677 TT has been associated with approximately 70% loss of enzymatic function, and heterozygote 677 CT with 35% loss of function, compared to homozygote wild-type 677 CC (100% full enzymatic

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activity; Frosst et al., 1995). Individuals with the 677 TT genotype had significantly elevated homocysteine levels, with a decline in methylation of homocysteine to methionine in the plasma, adversely channeling the homocysteine metabolism into a transsulfuration pathway, leading to toxicities. Thereby, these polymorphism-mutations predispose individuals to multiple disease conditions such as thrombosis, coronary artery disease, myocardial infarction (Mehlig et al., 2013; Yadav et al., 2013), and cancers (Teng et al., 2013; You et al., 2013).

Compared to *MTHFR* 677 mutations, the functional relevance of *MTHFR* 1298 AC variant is less well defined, and its enzymatic function is less abnormal. *MTHFR* 1298 CC (homozygote) has been associated with 30% loss of function, and 1298 AC (heterozygote) has 15% loss of function in enzymatic activity compared to 1298 AA wild type (100% full enzymatic activity; Weisberg, Tran, Christensen, Sibani, & Rozen, 1998). *MTHFR* 1298 mutations are, however, associated with neurotransmitter pathways implicated in multiple neurological disease conditions such as autism, Alzheimer's, Parkinsonism, and in cardiovascular diseases, recurrent miscarriages, and cancers (Pérez-Sepúlveda et al., 2013; Wu, Ding, Sun, Yang, & Sun, 2013; Zidan, Rezk, & Mohammed, 2013). Growing research has focused on interventions aimed at optimizing MTHFR enzymatic function, which can be preventive or therapeutic for multiple disease conditions.

The MTHFR enzyme is inactivated by heat (i.e., it is thermolabile). As environmental temperature rises, the *MTHFR* heterozygous or homozygous mutation state correlates with further reduced enzymatic activity (Frosst et al., 1995). Global warming brought about by air pollution can lead to epigenetic modification critically affecting gene expression (Hoffmann & Willi, 2008); thereby, it may further harm individuals with health problems.

Air pollution, causing damage like that from cigarette smoking, has been classified as carcinogenic to humans. Exposure to air pollution in urban settings has been specifically associated with changes in DNA methylation (epigenetic modification), inflammation, immune and oxidative stress response, and gene expression for DNA damage and repair leading to cancer (DeMarini, 2013). Exposure to fine particulate matter ($PM_{2.5}$) and nitrogen dioxide (NO_2), markers of traffic-related air pollution, was associated with the development of breast cancer (Chen & Bina, 2012). Therefore, metapredictive analyses of air pollution on the *MTHFR* polymorphism-mutations and risk of breast cancer are needed to fill the knowledge gap in understanding the complex interactions of genetics and environment with the development of breast cancer (Figure 1 shows a conceptual framework for the associations of air pollution affecting epigenetic modification and *MTHFR* gene polymorphisms-mutations, and susceptibility to breast cancer).

Significance and Objectives

Previous meta-analyses (Pooja et al., 2015; Xie et al., 2015; Zhong et al., 2014) on the association between *MTHFR* polymorphisms and breast cancer susceptibility have reported inconclusive results across various racial and ethnic groups. Inconsistent findings could be due to heterogeneity of ethnic heritage, migration, geographic locations, environmental factors, and complex epigenetic pathways leading to carcinogenesis. This study focuses on a significant public health question that many nurses are interested in: Could the mutations associated with breast cancer be associated with levels of air pollution? Answers to this question fill a gap in the literature. Specifically, the authors pose the question as to whether epigenetic modifications in the *MTHFR* gene have been associated with air pollution in published case–control studies done across the world. The significance of this study is using metapredictive analysis as an applicable method in approaching heterogeneity of previous meta-analysis findings, thus bridging the knowledge gap in the literature (Pereira, Denise, & Lespinet, 2014; Shiao & Yu, 2016). Therefore, the primary objective of this study is to examine the polymorphism-mutation patterns and risk subtypes of *MTHFR* gene for breast cancer across the globe. The secondary objective is to investigate air pollution as a contributing factor for *MTHFR* gene polymorphisms and risk for breast cancer through metapredictive analysis.

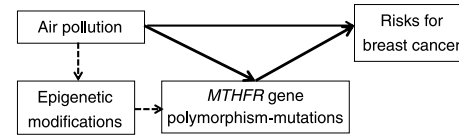

FIGURE 1. A conceptual framework of gene–environment interaction for increased susceptibility to breast cancer. The model shows hypotheses that air pollution affects epigenetic modification and *MTHFR* gene polymorphism-mutations as risk factors for breast cancer. Solid lines depict the variables that are measured in this metaprediction, and dotted lines represent what are known in the literature.

METHODS

Design

This study is a meta-analysis to determine *MTHFR* gene polymorphism-mutations as risk factors for breast cancer. In addition, to explore the source of heterogeneity from divergent mutation rates of *MTHFR* polymorphisms and breast cancer risk, metapredictive analytics were used to explore multiple predictors including air pollution for breast cancer susceptibility. For triangulation purpose, geographic information system (GIS) maps, recursive partition analysis, nonlinear association curve fit, and heat maps were used to enhance visualization and representation of data.

Included Studies

Following the guidelines for preferred reporting items for systematic review and meta-analysis (PRISMA; Moher, Liberati, Tetzlaff, Altman, & PRISMA Group, 2009), searches were made using all available databases of PubMed and Airiti Library (leading Chinese e-content provider of academic e-journals) to

identify and access all available studies. Search terms used combined *breast cancer, MTHFR, environment,* and/or *air pollution*. Without publication date filter, the searches resulted in 168 articles from 1999 (first related study was published) to July 2015 (see Figure 2). For about a year, multiple online searches, three months apart, were conducted to ensure all published studies were found. Previous meta-analyses were reviewed and references were cross-checked to trace all original studies. Abstracts were read and analyzed for their relevance. The inclusion criteria were (a) relevant study of breast cancer and *MTHFR*, (b) case–control design, (c) clear presentation of genotype allele count data, and (d) detailed quality results of data analysis. Fifty-eight articles were identified as not case–control studies and were excluded. A further 110 articles were retrieved for evaluation. More studies were excluded because they combined various carcinomas and lacked specific data for breast cancer ($n = 8$), had incomplete and absence of MTHFR genotype allele data and/or data not clearly presented ($n = 16$), and used duplicate or subsidiary data from other studies ($n = 4$). Eighty-two articles were finally included in this meta-analysis (see Table S1, Supplemental Digital Content 1, http://links.lww.com/NRES/A215, Characteristics of Studies—Studies Included in the Meta-analysis). Throughout the course of the study, data extraction and entry were conducted and repeatedly checked for accuracy between raters for 100% consensus on the data coding.

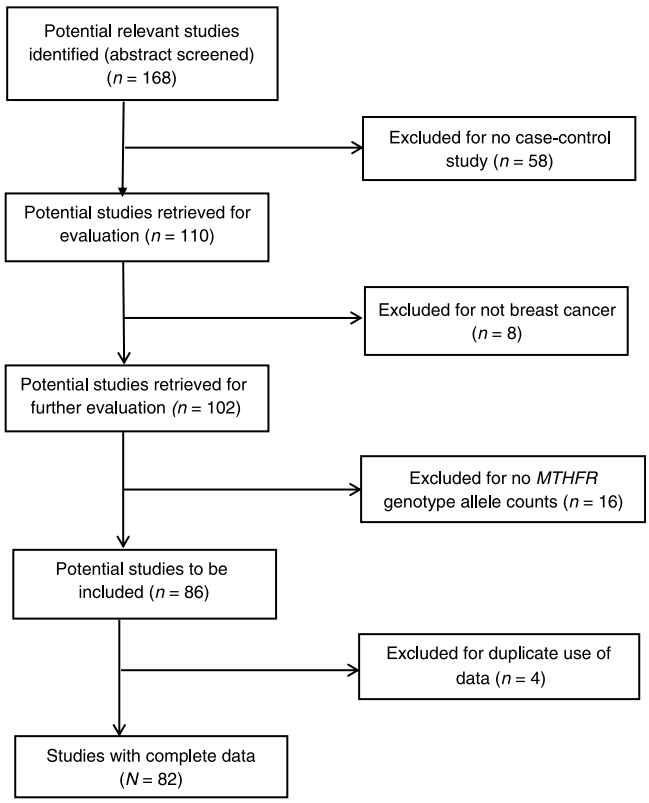

FIGURE 2. Selection of studies for inclusion in the meta-analysis. Databases of PubMed and Airiti Library (leading Chinese e-content provider of academic e-journals) were used to identify potential studies.

The studies were evaluated for quality using the scoring tool from Shiao and Yu (2016). The tool was developed by integrating sets of criteria adapted from multiple sources on assessment of studies, such as U.S. QUOROM consensus process on the quality of meta-analysis (Moher et al., 1999), quality reporting for observational studies (Stroup et al., 2000), and criteria in related studies (Kennedy et al., 2012; Shiao & Yu, 2016). Utilizing the quality scoring tool, we determined three areas for scoring: (a) external validity, with 10 items on the selection of cases and controls (score range of 0–11); (b) internal validity, with 12 items on genomic research methods and procedure (score range of 0–12); and (c) quality of reporting, with six items on the data and study results (score range of 0–6). The total possible score ranged from 0 to 29.

Characteristics of Original Studies

These 82 studies were conducted across the globe, including Australia, Europe, North and South America, Asia, the Middle East, and Africa. From the 82 studies with *MTHFR* 677 genotype counts, 50 studies also had 1298 genotype data. Each study was reviewed for race and ethnicity to clearly identify subgroup compositions that could explain possible heterogeneous results. The most investigated racial-ethnic groups for *MTHFR* and breast cancer were White (28 studies), followed by Asian (25 studies for East Asian and 8 studies for South Asian), Middle Eastern (6 studies), U.S. mixed (8 studies), Brazil mixed (4 studies), Mexico (1 study), Ecuador (1 study), and Morocco (1 study; see Table S1, Supplemental Digital Content 1, http://links.lww.com/NRES/A215, Characteristics of Studies). We also reviewed air quality data globally from the reports of the World Health Organization (2009, 2015). The rates of death from air pollution (APD) were categorized per level in deaths per million population: (a) Level 2 = *100 and under*, (b) Level 3 = *101–250*, and (c) Level 4 = *251 and above*.

In each study, we reviewed the frequency distributions of genotype allele counts for *MTHFR* loci (677 and 1298). They were within the expected distribution ranges per genotype. The control and case groups in the studies were aggregated per country to visualize the bigger picture of genotype percent distribution (see Figure S1, Supplemental Digital Content 2, http://links.lww.com/NRES/A216, *MTHFR* 677, % Mutations; see Figure S2, Supplemental Digital Content 3, http://links.lww.com/NRES/A217, MTHFR 1298, % Mutations). In each study, DNA samples had been collected from blood, tissue, and buccal swabs or salivary samples and analyzed via established guidelines. The reported accuracy with quality control was 100%, reported in all studies.

Using the total score, quality of the studies ranged from 9 to 24 out of a possible 29 points. Of the 82 studies, 15 (18%) were below the 50% mark for the total possible score because of deficient details and missing information required by the

criterion for quality scoring. We conducted comparative pooled analyses on all studies with quality scores below 15 and again on those with scores above 15. Outcome results were similar; thus, all studies were included in the meta-analysis.

A goodness-of-fit χ^2 test was used on all studies to evaluate the Hardy–Weinberg equilibrium (HWE). HWE was suggested to evaluate the distribution of data for the control group populations. Deviations from HWE have been handled in previous reports by meta-analytic approach and by presenting confidence intervals (CI; Ziegler, Steen, & Wellek, 2011). Therefore, we reviewed HWE among controls in all studies and recomputed the results for verification. We considered $p < .05$ representative of a departure or deviation from HWE. After verifying the reported HWE of the studies, we noted that, in 15 studies, the reports of within-HWE consistency showed discrepancy (deviation from HWE; see Table S1 for HWE status of studies). However, exclusion of these 15 studies did not significantly alter the outcome results; thus, all studies regardless of HWE status were included in the meta-analysis.

Data Synthesis and Analysis

Multiple data sources were merged using applicable statistical programs for analyses. Data were entered using Excel, and StatsDirect version 3.0.158 (StatsDirect, 2015) was used for pooled analyses of risk ratio (*RR*) and odds ratio (*OR*), adjusting the weight of sample sizes from each original study. Many previous meta-analyses on this subject used *OR*. A recognized problem with *OR* is that, when the outcome is common, the *OR* may not properly approximate the relative risk (overstatement of the effect size). Thus, there is a danger that *OR* could exaggerate the relative risk (Viera, 2008). In this study, we preferred *RR* as it could provide a conservative, standardized ratio and clear understanding on the measure of association. An *RR* of 1 means "no effect," an *RR* of <1 indicates a protective effect for breast cancer, and an *RR* of >1 indicates increased risk of breast cancer; 95% CI was calculated for the comparisons. Significant findings were defined as those with *p*-values of <.05. Assessment of heterogeneity was performed using Cochran's *Q* test and I^2 to determine whether the differences in results were due to chance. Heterogeneity exists when the Cochran's *Q* is significant with a *p*-value of <.10. The I^2 statistic is the percentage of variability in the effect estimates due to heterogeneity rather than chance. An I^2 statistic value over 50% indicates that substantial heterogeneity may be present (Deeks, Higgins, & Altman, 2011, Section on Identifying and Measuring Heterogeneity section, para. 5). When there was significant heterogeneity, we used a random effects model instead. Conversely, a fixed effects model was chosen when Cochran's *Q* was not significant, with a *p*-value of >.10 and I^2 value was less than 50% (Deeks et al., 2011, Section on Identifying and Measuring Heterogeneity section, para. 5).

A metapredictive method integrates multiple statistical models for triangulation purposes, so it can be more robust and accurate for multiple predictors and polymorphism-mutation genotype analyses (Pereira et al., 2014; Shiao & Yu, 2016). Through the process of triangulation, a source of heterogeneity could be explored and identified for divergent mutation rates of *MTHFR* polymorphisms and breast cancer risk. GIS maps were generated using JMP 12.1 software (SAS, 2015) to manage metadata specification of the geospatial data set. These maps helped associate regional patterns of polymorphism-mutations and breast cancer risk with the level of air pollution per country (Albrecht, 2007). In addition, a partition tree model was used to examine the associations between multiple predictors and outcome variables. Recursive partition analysis using JMP 12.1 software created a decision tree that classified groups of population (*MTHFR* polymorphism-mutation rates in cases and control groups and risks) by splitting data into subgroups based on levels of APD, their independent variable (Strobl, Malley, & Tutz, 2009). In each analysis, Akaike's Information Criterion (AIC) was used to select the optimal number of subgroups; the model that yields the smallest value of AIC is selected (Akaike, 1985). We conducted Tukey's test for pairwise comparisons to identify any difference between two means that exceeded the expected standard error (Abdi & Williams, 2010, p. 1565). We used heat maps (SAS JMP Program) as a graphical representation of data to visualize the matrix values represented on a color scale. The heat map cluster results revealed rows (levels of APD) and columns (*MTHFR* polymorphism-mutation genotype rates) of hierarchical cluster structure in a data matrix that supplemented detailed analysis of the underlying associations between variables.

RESULTS

Pooled Analyses

For pooled analysis of *MTHFR* genotypes, we included 82 studies in our *MTHFR* 677 group, with 36,683 breast cancer cases and 40,689 controls (Table 1). Using the control group as the reference for the general healthy population, the rank order of subgroups with the *MTHFR* 677 TT (homozygous) mutation was Middle East (13.57%) followed by U.S. mixed (12.63%), East Asian (12.22%), Caucasian (11.28%), Brazil mixed (10.69%), and South Asian (2.97%; for specific percent mutations per control and case groups, see Figure S1, Supplemental Digital Content 2, http://links.lww.com/NRES/A216, *MTHFR* 677). On the test of association, *MTHFR* 677 TT was a risk genotype for breast cancer in the total sample (*RR* = 1.13, 95% CI [1.06, 1.21], $p = .0004$) and for the East Asian subgroup (*RR* = 1.22, 95% CI [1.06, 1.40], $p = .005$). Mexico, Ecuador, and Morocco each had one study; these three also showed 677 TT as a risk genotype (Figure 3). *MTHFR* 677 CC (wild type) was a protective genotype for the total sample (*RR* = 0.97, 95% CI [0.95, 0.99], $p = .007$), as well as for the East Asian subgroup (*RR* = 0.95, 95% CI [0.90, 0.10], $p = .04$). With the combined model of *MTHFR* 677 TT and CT genotypes, both

TABLE 1. Pooled Meta-analysis: *MTHFR* 677 Genotypes and Risks of Breast Cancer

		Cases[a] (n = 36,683)		Controls (n = 40,689)					
Genotype/group	j[b]	n	(%)	n	(%)	Model[c]	*RR*	95% CI	p
TT	82	4,568	(12.5)	4,652	(11.4)	Random	1.13	[1.06, 1.21]	.0004
Caucasian	28	1,707	(11.9)	1,573	(11.3)	Random	1.10	[0.98, 1.23]	.11
East Asian	25	1,298	(14.1)	1,310	(12.2)	Random	1.22	[1.06, 1.40]	.005
South Asian	8	58	(2.6)	71	(3.0)	Fixed	1.02	[0.72, 1.43]	.92
Mideastern	6	286	(14.2)	318	(13.6)	Fixed	0.99	[0.86, 0.15]	.92
U.S. mixed	8	1,016	(14.0)	1,236	(12.6)	Random	1.09	[0.98, 1.22]	.12
Brazil mixed	4	80	(9.5)	92	(10.7)	Fixed	0.89	[0.67, 1.18]	.41
CT	82	15,443	(42.1)	16,984	(41.7)	Random	1.00	[0.98, 1.03]	.66
Caucasian	28	6,389	(44.4)	6,076	(43.6)	Fixed	1.01	[0.98, 1.04]	.54
East Asian	25	3,835	(41.6)	4,431	(41.3)	Fixed	1.01	[0.97, 1.04]	.74
South Asian	8	555	(24.7)	574	(24.0)	Random	1.10	[0.94, 1.30]	.24
Mideastern	6	850	(42.3)	1,045	(44.6)	Random	0.91	[0.76, 1.07]	.25
U.S. mixed	8	3,139	(43.3)	4,220	(43.1)	Random	1.01	[0.95, 1.07]	.79
Brazil mixed	4	371	(44.0)	344	(40.0)	Fixed	1.10	[0.99, 1.23]	.09
CC	82	16,672	(45.5)	19,053	(46.8)	Random	0.97	[0.95, 0.99]	.007
Caucasian	28	6,310	(43.8)	6,290	(45.1)	Fixed	0.99	[0.96, 1.01]	.27
East Asian	25	4,078	(44.3)	4,979	(46.5)	Random	0.95	[0.90, 0.10]	.04
South Asian	8	1,637	(72.8)	1,744	(73.0)	Random	0.97	[0.92, 1.03]	.37
Mideastern	6	876	(43.5)	980	(41.8)	Random	1.07	[0.96, 1.20]	.22
U.S. mixed	8	3,098	(42.7)	4,330	(44.3)	Fixed	0.98	[0.94, 1.01]	.21
Brazil mixed	4	393	(46.6)	425	(49.4)	Fixed	1.02	[0.93, 1.12]	.65
CC+CT	82	32,114	(87.6)	36,037	(88.6)	Random	0.98	[0.97, 1.00]	.08
TT+CT	82	20,011	(54.6)	21,636	(53.2)	Random	1.03	[1.01, 1.05]	.003
Genotype/subgroup									
TT risk > 1		12,821	(35.0)	13,499	(33.2)				
TT	39	1,885	(14.7)	1,555	(11.5)	Random	1.35	[1.22, 1.49]	<.0001
CT	39	5,496	(42.9)	5,772	(42.8)	Random	0.99	[0.96, 1.03]	.74
CC	39	5,440	(42.4)	6,172	(45.7)	Random	0.93	[0.90, 0.97]	.0006
CC+CT	39	10,936	(85.3)	11,944	(88.5)	Random	0.96	[0.95, 0.97]	<.0001
TT+CT	39	7,381	(57.6)	7,327	(54.3)	Random	1.06	[1.03, 1.10]	.0001
TT risk < 1		9,042	(24.7)	7,900	(19.4)				
TT	15	941	(10.4)	869	(11.0)	Fixed	0.90	[0.82, 0.98]	.02
CT	15	3,837	(42.4)	3,191	(40.4)	Random	1.04	[0.98, 1.11]	.20
CC	15	4,264	(47.2)	3,840	(48.6)	Random	1.00	[0.95, 1.05]	.92
CC+CT	15	8,101	(89.6	7,031	(89.0)	Fixed	1.01	[1.00, 1.02]	.03
TT+CT	15	4,778	(52.8)	4,060	(51.4)	Random	1.00	[0.94, 1.05]	1.00
TT risk ~ 1		14,820	(40.4)	19,290	(47.4)				
TT	28	1,742	(11.8)	2,228	(11.6)	Random	1.05	[0.95, 1.16]	.38
CT	28	6,110	(41.2)	8,021	(41.6)	Random	1.00	[0.97, 1.04]	.88
CC	28	6,968	(47.0)	9,041	(46.9)	Random	0.99	[0.96, 1.02]	.40
CC+CT	28	13,078	(88.3)	17,062	(88.5)	Random	1.00	[0.99, 1.00]	.37
TT+CT	28	7,852	(53.0)	10,249	(53.1)	Random	1.01	[0.98, 1.04]	.48

Note. J = 82 studies. CI = confidence interval; *RR* = risk ratio. [a]Breast cancer diagnosis. [b]Number of studies. [c]Random effects models were used when Cochran's Q, $p < .10$ and $I^2 > 50\%$ (substantial heterogeneity is present); otherwise, fixed effects models were used (minimal heterogeneity).

polymorphism-mutations were noted to be risk genotypes for breast cancer (*RR* = 1.03, 95% CI [1.01, 1.05], $p = .003$).

GIS maps enhanced the visualization of geographic regional patterns of *MTHFR* 677 polymorphisms-mutations and breast cancer risks in countries worldwide (see Figure S3, Supplemental Digital Content 4, http://links.lww.com/NRES/A218, Combined *MTHFR* 677 TT and CT Polymorphism-Mutation Genotypes). GIS maps identify populations geographically, whereas racial-ethnic data may be mixed because ethnic groups are scattered in various countries (see Figure S4, Supplemental Digital Content 5, http://links.lww.com/NRES/A219, for *MTHFR* 677 TT homozygous mutation genotype; see Figure S5, Supplemental Digital Content 6, http://links.lww.com/NRES/A220, for CT heterozygous

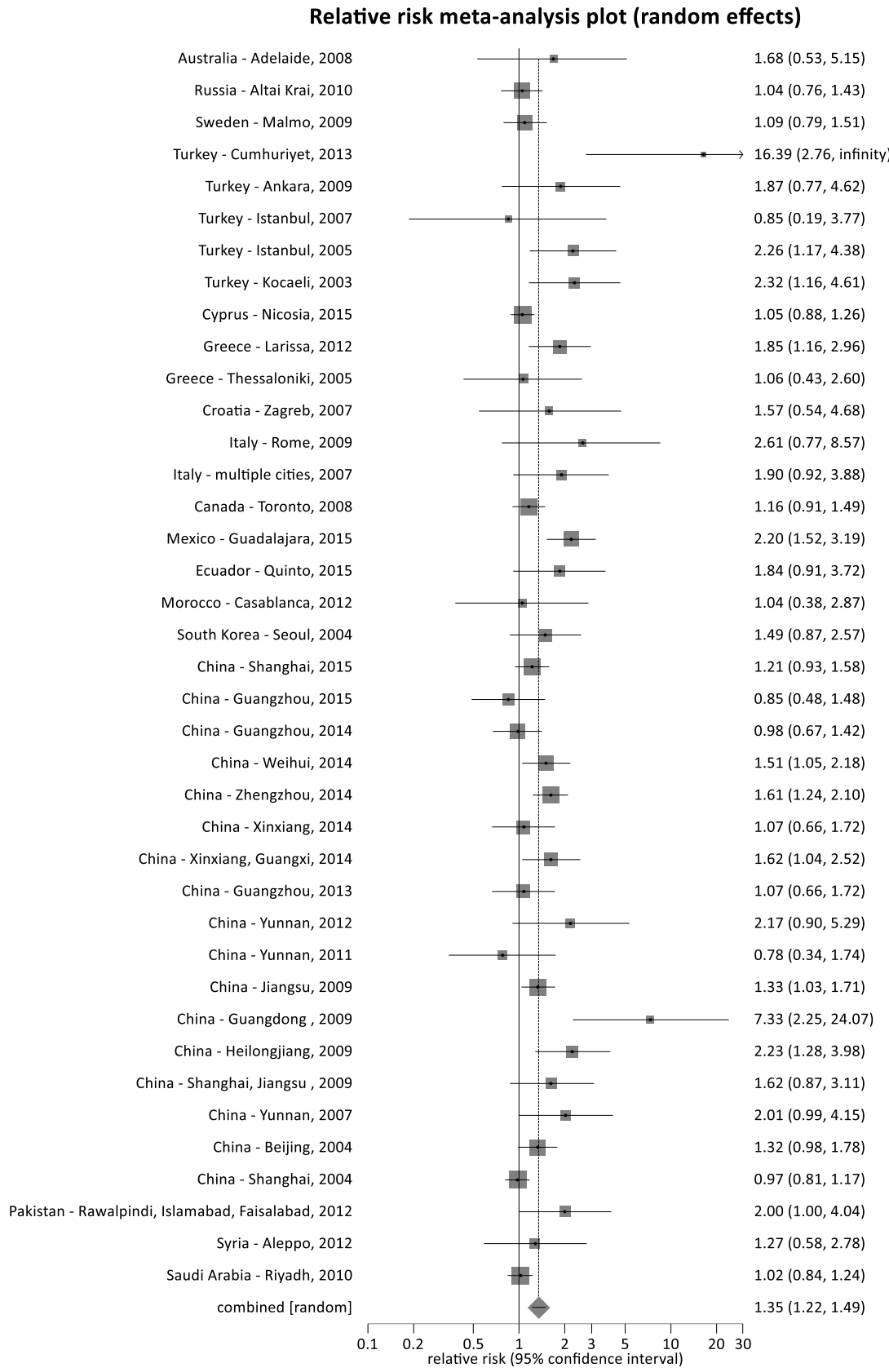

FIGURE 3. Forest plot for meta-analysis of *MTHFR* 677 TT showing countries and cities with risk of >1.

mutation genotype). Further use of global visualization showed that the highest polymorphism-mutation rates on *MTHFR* 677 TT were found in the Middle East (Iran and Saudi Arabia), Europe (Cyprus, Spain, Germany, Slovenia, and United Kingdom), Asia (Japan and China), and North America (Canada and United States) for the control group (see Figure S4, Supplemental Digital Content 5, http://links.lww.com/NRES/A219). As noted in the map for risk of breast cancer from *MTHFR* 677 TT mutations (see Figure S4, Supplemental Digital Content 5, http://links.lww.com/NRES/A219, the third map), the darkest color (red) depicted the highest breast cancer risks in Australia, South Korea, China, Pakistan, Turkey, Syria, Italy, Cyprus, Greece, Croatia, Poland, Canada, Mexico, and Ecuador.

To identify the sources of heterogeneity in racial-ethnic subgroups, studies per country were grouped together with TT as risk genotype (*RR* > 1) or protective genotype (*RR* < 1). This strategy clearly depicted groups of countries with the same trends (Figures 2 and 3) or those with heterogeneous variations within each country (see Figure S6, Supplemental Digital Content 7, http://links.lww.com/NRES/A221; *RR* = 1.35, 95% CI [1.22, 1.49], $p \leq .0001$) and protective (*RR* = 0.90, 95% CI [0.82, 0.98], $p = .02$) genotypes (Table 1). Countries or regions with *MTHFR* 677 TT as risk genotype were Australia, Russia, South and East Europe (Sweden, Turkey, Cyprus, Greece, Croatia, and Italy), America (Canada, Mexico, Ecuador), East Asia (South Korea and China), Middle East (Pakistan, Syria, and Saudi Arabia), and Morocco (Figure 3). Conversely, countries or regions with *MTHFR* 677 TT as a protective genotype were North Europe (Finland, Slovenia, Germany, and United Kingdom), Brazil, Southeast Asia (Singapore and Thailand), and other parts of the Middle East (Kazakhstan, Iran, and Jordan; Figure 4). Comparative results of *OR* and *RR* were presented. As projected, *RR*s presented more conservative results than *OR*s (see Table S2a, Supplemental Digital Content 8, http://links.lww.com/NRES/A222).

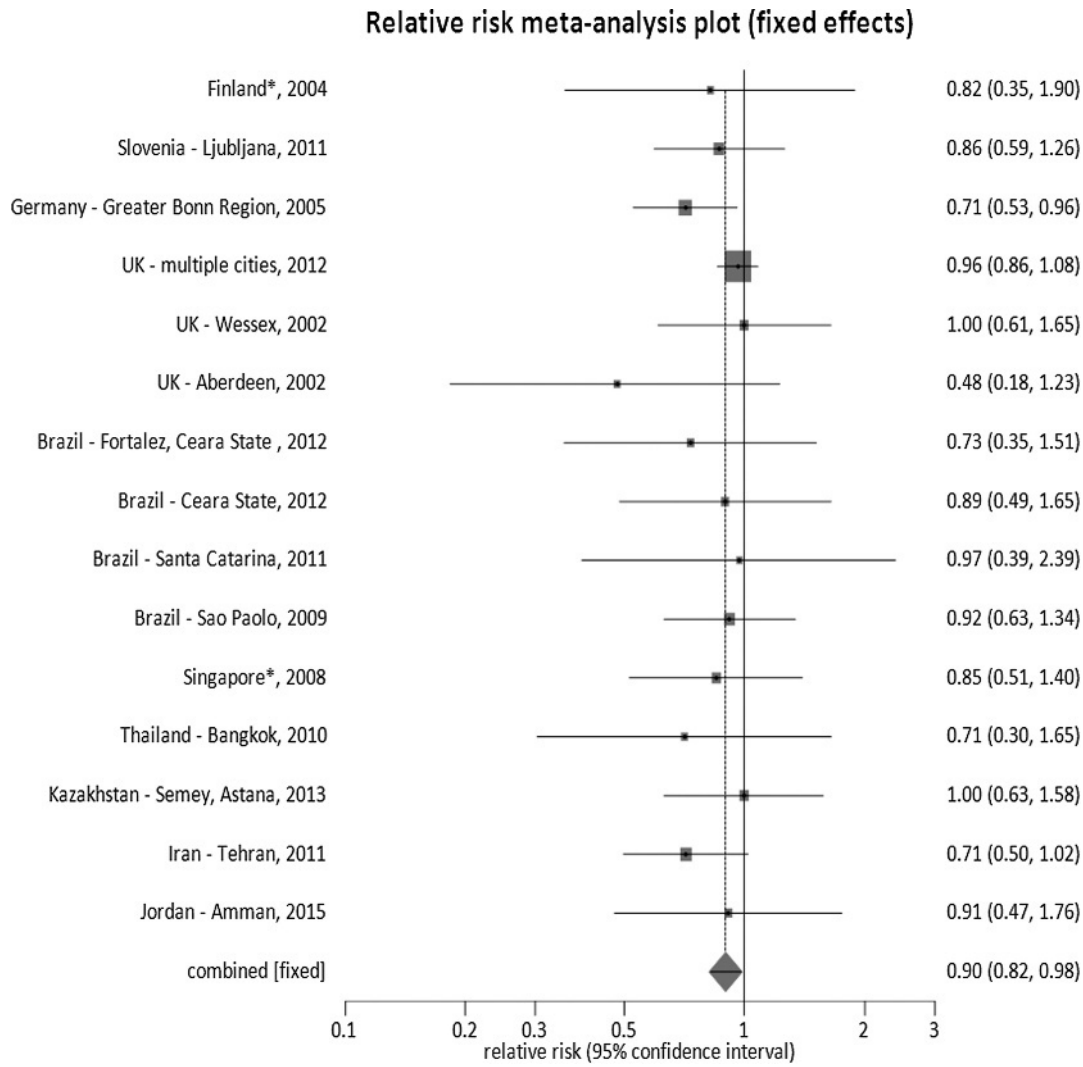

FIGURE 4. Forest plot for meta-analysis of *MTHFR* 677 TT showing countries and cities with risk of <1.

On the pooled analysis of *MTHFR* 1298 genotypes, 50 studies were included with a total of 23,252 cases and 27,094 controls (Table 2). Using the control group as a reference for the general healthy population, the rank order of *MTHFR* 1298 CC (homozygous) mutation was Caucasian (14.58%) followed by Middle East (10.59%), U.S. mixed (8.38%), East Asian (5.57%), South Asian (5.32%), and Brazil mixed (5.10%; for specific percent mutations per case and control groups, see Figure S2, Supplemental Digital Content 3, http://links.lww.com/NRES/A217, *MTHFR* 1298). For visual examination, GIS maps were also generated (see Figure S7, Supplemental Digital Content 9, http://links.lww.com/NRES/A223, Combined *MTHFR* 1298 CC and AC Polymorphism-Mutation Genotypes; see Figure S8, Supplemental Digital Content 10, http://links.lww.com/NRES/A224,

TABLE 2. Pooled Meta-analysis: *MTHFR* 1298 Genotypes and Risks of Breast Cancer

		Cases[a] (*n* = 23,252)		Controls (*n* = 27,094)					
Genotype/group	***j*[b]**	***n***	**(%)**	***n***	**(%)**	**Model[c]**	***RR***	**95% CI**	***p***
CC	50	2,290	(9.8)	2,529	(9.3)	Fixed	1.03	[0.98, 1.08]	.27
Caucasian	16	1,152	(14.9)	1,227	(14.6)	Fixed	1.02	[0.95, 1.09]	.62
East Asian	16	404	(5.8)	420	(5.6)	Fixed	1.05	[0.92, 1.20]	.45
South Asian	4	61	(5.7)	68	(5.3)	Fixed	1.07	[0.76, 1.53]	.69
Mideastern	5	131	(13.3)	135	(10.6)	Random	1.43	[0.80, 2.55]	.23
U.S. mixed	5	487	(8.6)	639	(8.4)	Fixed	0.97	[0.87, 1.09]	.66
Brazil mixed	3	54	(7.1)	39	(5.1)	Fixed	1.38	[0.93, 2.06]	.11
AC	50	9,116	(39.2)	10,534	(38.9)	Fixed	1.00	[0.98, 1.02]	.85
Caucasian	16	3,357	(43.5)	3577	(42.5)	Fixed	1.03	[0.99, 1.07]	.13
East Asian	16	2,338	(33.7)	2,593	(34.4)	Fixed	0.98	[0.94, 1.03]	.51
South Asian	4	414	(38.8)	488	(38.2)	Fixed	0.99	[0.90, 1.10]	.89
Mideastern	5	406	(41.3)	564	(44.2)	Random	0.90	[0.73, 1.11]	.33
U.S. mixed	5	2,279	(40.3)	2,996	(39.3)	Fixed	1.00	[0.96, 1.04]	.94
Brazil mixed	3	319	(41.7)	313	(40.9)	Fixed	1.02	[0.91, 1.15]	.75
AA	50	11,846	(51.0)	14,031	(51.8)	Random	0.99	[0.97, 1.02]	.55
Caucasian	16	3,214	(41.6)	3,612	(42.9)	Fixed	0.97	[0.93, 1.00]	.06
East Asian	16	4,199	(60.5)	4,531	(60.1)	Fixed	1.00	[0.97, 1.03]	.78
South Asian	4	591	(55.4)	721	(56.5)	Fixed	1.00	[0.93, 1.07]	.96
Mideastern	5	447	(45.4)	576	(45.2)	Random	1.03	[0.76, 1.41]	.83
U.S. mixed	5	2,893	(51.1)	3,987	(52.3)	Fixed	1.01	[0.97, 1.04]	.75
Brazil mixed	3	392	(51.2)	413	(54.0)	Fixed	0.95	[0.86, 1.04]	.27
AA+AC	50	20,962	(90.2)	24,565	(90.7)	Random	0.99	[0.98, 1.01]	.67
CC+AC	50	11,406	(49.1)	13,063	(48.2)	Random	1.01	[0.98, 1.04]	.42
Genotype/subgroup									
CC risk > 1		6,560	(28.2)	7,603	(28.1)				
CC	18	1,045	(15.9)	1,059	(14.0)	Random	1.20	[1.05, 1.37]	.006
AC	18	2,802	(42.7)	3,180	(41.8)	Random	1.00	[0.94, 1.07]	.98
AA	18	2,713	(41.4)	3,364	(44.2)	Random	0.95	[0.90, 1.00]	.06
AA+AC	18	5,515	(84.1)	6,544	(86.1)	Fixed	0.98	[0.97, 0.99]	.007
CC+AC	18	3,847	(58.6)	4,239	(55.8)	Random	1.05	[0.99, 1.11]	.08
CC risk < 1		3,715	(16.0)	3,947	(14.6)				
CC	9	315	(8.5)	387	(9.8)	Fixed	0.84	[0.73, 0.97]	.02
AC	9	1,497	(40.3)	1,568	(39.7)	Fixed	1.01	[0.96, 1.07]	.66
AA	9	1,903	(51.2)	1,992	(50.5)	Random	1.04	[0.95, 1.14]	.38
AA+AC	9	3,400	(91.5)	3,560	(90.2)	Fixed	1.02	[1.00, 1.03]	.02
CC+AC	9	1,812	(48.8)	1,955	(49.5)	Random	0.97	[0.89, 1.06]	.53
CC risk ~ 1		12,977	(55.8)	15,544	(57.4)				
CC	23	930	(7.2)	1,083	(7.0)	Fixed	1.02	[0.94, 1.11]	.60
AC	23	4,817	(37.1)	5,786	(37.2)	Fixed	0.99	[0.96, 1.02]	.64
AA	23	7,230	(55.7)	8,675	(55.8)	Fixed	1.00	[0.98, 1.02]	.85
AA+AC	23	12,047	(92.8)	14,461	(93.0)	Fixed	1.00	[0.99, 1.00]	.61
CC+AC	23	5,747	(44.3)	6,869	(44.2)	Fixed	1.00	[0.97, 1.02]	.85

Note. *J* = 50 studies. CI = confidence interval; *RR* = risk ratio. [a]Breast cancer diagnosis. [b]Number of studies. [c]Random effects models were used when Cochran's *Q*, $p < .10$ and $I^2 > 50\%$ (substantial heterogeneity is present); otherwise, fixed effects models were used (minimal heterogeneity).

MTHFR 1298 CC Homozygous Mutation Genotype; see Figure S9, Supplemental Digital Content 11, http://links.lww.com/NRES/A225, AC Heterozygous Mutation Genotype). For the control group, the highest mutation rates on MTHFR 1298 CC were located in Europe (Cyprus, United Kingdom, Finland, Germany, Poland, Greece, Sweden, and Russia) and the Middle East (Iran, Syria, and Jordan; see Figure S8, Supplemental Digital Content 10, http://links.lww.com/NRES/A224). On the maps for breast cancer risk from MTHFR 1298 CC mutations (see Figure S8, Supplemental Digital Content 10, http://links.lww.com/NRES/A224, third map), the darkest red depicted the highest breast cancer risks, in China, Thailand, Pakistan, Kazakhstan, Syria, Jordan, Slovenia, Italy, Spain, Brazil, and Ecuador.

Additional subgroup analyses based on risk groups were more revealing for *MTHFR* 1298 (see Figure S10, Supplemental Digital Content 12, http://links.lww.com/NRES/A226, Figure S11, Supplemental Digital Content 13, http://links.lww.com/NRES/A227, and Figure S12, Supplemental Digital Content 14, http://links.lww.com/NRES/A228, Forest Plots). Pooled analyses showed statistically significant results on opposing subgroups of *MTHFR* 1298 CC as risk (*RR* = 1.20, 95% CI [1.05, 1.37], p = .006) and protective (*RR* = 0.84, 95% CI [0.73, 0.97], p = .02) genotypes (Table 2). Countries or regions with *MTHFR* 1298 CC mutation as a risk genotype were North and South Europe (Sweden, Poland, Turkey, Cyprus, Greece, Slovenia, Italy, and Spain), Ecuador, Southeast Asia (Thailand and India), and the Middle East (Kazakhstan, Pakistan, Syria, and Jordan; see Figure S10, Supplemental Digital Content 12, http://links.lww.com/NRES/A226). Contrarily, countries or regions with *MTHFR* 1298 CC as a protective genotype were Australia, Russia, North Europe (Finland, Germany, and United Kingdom), Canada, Japan, Singapore, and Iran (see Figure S11, Supplemental Digital Content 13, http://links.lww.com/NRES/A227). Comparison of *RR*s and *OR*s for *MTHFR* 1298 also presented results similar to those for *MTHFR* 677, with *RR*s being more conservative than *OR*s

TABLE 3. Metaprediction: Effect of Death From Air Pollution on *MTHFR* 677 Genotypes for Controls and Breast Cancer Cases, and Breast Cancer Risks

	Partition tree				Tukey's test			
Variable	APD[a,b]	Count[c]	*M*	(*SD*)	Comparison	*D*	(*SED*)	*p*
TT%ct	2	21	7.0	(4.52)	3 vs. 2	5.1	(1.38)	.001
	3 and 4	61	11.3	(4.61)	4 vs. 2	3.8	(1.25)	.009
					3 vs. 4	1.3	(1.21)	.54
TT%ca	2	21	8.2	(6.20)	3 vs. 2	6.1	(1.85)	.004
	3 and 4	61	14.0	(6.05)	4 vs. 2	5.66	(1.67)	.003
					3 vs. 4	0.41	(1.62)	.97
CT%ct	2	21	34.2	(13.46)	3 vs. 2	10.5	(2.60)	<.001
	3 and 4	61	41.9	(6.52)	4 vs. 2	5.9	(2.34)	.04
					3 vs. 4	4.6	(2.28)	.11
CT%ca	2	21	37.8	(14.36)	3 vs. 2	4.7	(2.91)	.25
	3 and 4	61	40.7	(7.45)	3 vs. 4	2.8	(2.54)	.52
					4 vs. 2	1.9	(2.62)	.75
CC%ct	2	21	58.8	(16.47)	2 vs. 3	15.6	(3.38)	<.001
	3 and 4	61	46.8	(9.09)	2 vs. 4	9.7	(3.04)	.006
					4 vs. 3	5.9	(2.96)	.12
CC%ca	2	21	54.1	(18.46)	2 vs. 3	10.8	(3.90)	.02
	3 and 4	61	45.3	(10.40)	2 vs. 4	7.6	(3.51)	.09
					4 vs. 3	3.2	(3.41)	.62
RR 677TT	2 and 3	44	1.1	(0.55)	4 vs. 2	0.34	(0.23)	.28
	4	38	1.4	(1.11)	4 vs. 3	0.1	(0.23)	.70
					3 vs. 2	0.2	(0.26)	.78
RR 677CT	2	21	1.1	(0.35)	2 vs. 3	0.2	(0.06)	.009
	3 and 4	61	1.0	(0.13)	2 vs. 4	0.2	(0.06)	.02
					4 vs. 3	0.0	(0.05)	.81
RR 677CC	2	21	0.9	(0.18)	3 vs./2	0.082	(0.04)	.14
	3 and 4	61	1.0	(0.13)	3 vs. 4	0.054	(0.04)	.32
					4 vs. 2	0.028	(0.04)	.75

Note. %ct = control rate; %ca = case rate; APD = air pollution death rate; D = mean difference; *SD* = standard deviation; *SED* = standard error of the mean difference. [a]APD was scored in three groups, based on the levels in deaths per million population: 2 = *≤100,* 3 = *101–250,* 4 = *≥251*. [b]Partitioning for each outcome was based on Akaike Information Criterion values (corrected). [c]Number of studies.

(see Table S2b, Supplemental Digital Content 8, http://links.lww.com/NRES/A222).

Air Pollution, *MTHFR* Mutations, and Breast Cancer Risks

On the metapredictive analysis, although all potential risk factors including quality score, source of controls, and types of breast cancer were explored, the level of APD was the only significant contributing factor for the polymorphism-mutations and breast cancer risks (e.g., see Figure S13, Supplemental Digital Content 15, http://links.lww.com/NRES/A229). To show metaprediction, we used partition trees (split groups) and the Tukey's test by levels of APD to predict *MTHFR* 677 genotype mutation rates and breast cancer risk (Table 3). The partition tree and Tukey's test results converged and showed significant differences between APD Levels 2 and 3 (p = .001) and between Levels 2 and 4 (p = .009) for *MTHFR* 677 TT rate by APD for control group. We noted the same trend of statistical significance by APD on the 677 TT in breast cancer cases and by APD on *MTHFR* 677 CT and CC genotype rates in both control and breast cancer cases. Furthermore, on the *RR* for *MTHFR* 677 CT, we identified significant differences between Levels 2 and 3 (p = .009) and Levels 2 and 4 (p = .02) APD, with the smallest AIC of 22.66 (smallest value is the best model). For *MTHFR* 1298, we conducted the same sequence of analyses. The partition tree and Tukey's test did not render any statistically significant differences among the tested associations (see Table S3, Supplemental Digital Content 16, http://links.lww.com/NRES/A230, 677 TT and CT M).

We further explored the nonlinear fit between our potential contributing factor—levels of APD—and percent *MTHFR* 677 TT homozygous mutation per control and breast cancer case groups (see Figure S14a, Supplemental Digital Content 17, http://links.lww.com/NRES/A231). As the level of APD increased from Level 2 to Level 3, TT genotype percent rate increased; however, we noted a slight decline on the mutation genotype rate when the level further increased to Level 4. We noted a similar trend in *MTHFR* 1298 CC (homozygous) mutation genotype rate although the curve was noticeably flatter (see Figure S14b, Supplemental Digital Content 17, http://links.lww.com/NRES/A231). Another illustration of associations between variables was presented through heat maps. On percent *MTHFR* 677 TT by levels of APD, data density with red blocks depicted higher concentration of 677 TT with air pollution Level 4 (see Figure S15, Supplemental Digital Content 18, http://links.lww.com/NRES/A232, *MTHFR* 677 TT Genotype). For percent *MTHFR* 1298 CC, red blocks with high data concentration dropped as air pollution progressed from Level 3 to Level 4 for both case and control groups (see Figure S15, Supplemental Digital Content 18, http://links.lww.com/NRES/A232, *MTHFR* 1298 CC Genotype).

DISCUSSION

Compared to previous meta-analyses, this metaprediction study presents the most comprehensive report on *MTHFR* and breast cancer in that this study employed triangulation techniques beyond the conventional pooled and subgroup analyses. This study clearly addresses heterogeneity as a factor causing inconsistent, conflicting results in previous studies, and this study presents the potential source of that heterogeneity. Consistent with recent meta-analyses (Xie et al., 2015; Zhong et al., 2014), overall pooled analysis in this study showed that the *MTHFR* 677 TT was a risk genotype for breast cancer susceptibility (p = .0004) across all populations and specifically for East Asians (p = .005). However, there was heterogeneity with opposing findings for regions, findings that we summarized and integrated here across studies using different analytics. The countries and regions that presented opposing findings included Northern Europe, Southeast Asia, the Middle East, and Brazil. Global maps showed that the highest polymorphism-mutation rates on *MTHFR* 677 TT were found in the Middle East, Europe, Asia, and America for the control group. On *MTHFR* 1298, the highest mutation rates were located in Europe and the Middle East for the control group. The GIS maps further revealed higher risks of breast cancer for the countries or regions including Australia, East Asia, the Middle East, South Europe, Morocco, and the Americas based on *MTHFR* 677 TT mutations and Asia, Middle East, South Europe, and South America based on *MTHFR* 1298 CC mutations. Essentially, the GIS maps presented the potential source of heterogeneity in regional patterns across the world. The results from GIS maps and conventional pooled analysis to identify subgroups of risk regions converged with slight differences. In addition to results that were vividly presented in color, GIS maps pooled mutation rates and risks without weighting the sample sizes of each study. The conventional pooled analyses, however, weighted the sample sizes of each study when calculating the risks.

To further understand the source of heterogeneity in pooled analyses, we added metapredictive analyses, and we included graphical data for visualization. We used recursive partition trees, nonlinear curve fit, and heat maps to examine complex associations in nonlinear exposure-response patterns. These techniques were most helpful not only to visually detect the regional geographical patterns of the increased mutation rates and risks but also to triangulate the findings with multiple prediction methods to validate the results across the methods.

For associated environmental factors, this metapredictive analysis revealed that air pollution level was significantly associated with *MTHFR* 677 TT polymorphism-mutation and an increased trend toward breast cancer risk. Studies have shown that exposure to air pollution is associated with the development of breast cancer and increased mortality rates for breast

cancer (Chen & Bina, 2012; Gorham, Garland, & Garland, 1989; Reding et al., 2015). Air pollution due to industrialization could be adding to the effect of global warming, which could further exacerbate the decreased enzymatic function of MTHFR 677 TT in warm environmental temperature, leading to increased breast cancer susceptibility. Thus, air pollution as an exogenous factor could not only detrimentally affect the *MTHFR* gene expression that results from genotoxicity (DeMarini, 2013), but air pollution could also be a factor in *MTHFR* gene mutations and associated risks for breast cancer. Therefore, the findings from this metaprediction support regulatory initiatives for clean air to attain global health.

Other sources of heterogeneity for gene mutations have been identified and associated with human migration and gene–environment interactions (Chen & Bina, 2012; Gaudet et al., 2013; Xu et al., 2011). Findings from this analysis uncovered the potential source of that heterogeneity from geographical regions as it affects *MTHFR* polymorphism-mutations and breast cancer risk. This new scientific discovery accrued from our analysis via SAS JMP. To illustrate comprehensive standardized risk ratios, we presented a comparative analysis of *RR* and *OR* using the total counts of the three genotypes (homozygous mutation, heterozygous mutation, and wild type). The results from the tests of heterogeneity and associations were comparable, but *RR* presented more conservative results than *OR* in the pooled analyses.

Conclusion

We have presented the most comprehensive meta-analyses of *MTHFR* 677 and 1298 genotype polymorphism-mutations and breast cancer risk by presenting the sources of potential heterogeneity using metapredictive techniques. In this study, air pollution was notably the most significant contributing factor associated with *MTHFR* polymorphism-mutations and potential breast cancer susceptibility. These findings provided new understanding that will guide future epigenetics research into the effects of air pollution on the development of breast cancer. Nurses in the community could play a significant role in primary prevention as they advocate for clean air and engagement of our profession in environmental regulations. We recommend future studies to examine the potential ways to detoxify and mitigate the systemic effects of pollution to improve health outcomes, thereby promoting health for the world's population.

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