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ORIGINAL ARTICLE

Distribution of *MTHFR* C677T Gene Polymorphism in Healthy North Indian Population and an Updated Meta-analysis

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Abstract Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme of folate pathway. Several polymorphisms were reported in *MTHFR* gene but C677T polymorphism is most studied and it has been reported to be risk factor for several diseases/disorders. The present study was designed to explore the frequency of *MTHFR* C677T polymorphism in North Indian healthy population. In addition to this a meta-analysis of published articles was also performed to estimate the global prevalence of *MTHFR* C677T polymorphism. A total of 1000 unrelated healthy subjects were selected for *MTHFR* C677T polymorphism analysis. Different databases were searched for eligible articles. Prevalence proportion with 95 % CI was used to determine global prevalence of T allele and TT genotype. Meta-analysis was performed by Open meta-analyst. In 1000 blood samples analyzed, the frequency of T allele and TT genotype was 11 and 1 % respectively. Results of the meta-analysis showed that the global prevalence of T allele and TT genotype were 24.0 % (95 % CI 21.7–26.5) and 7.7 % (95 % CI 6.5–8.9) respectively. In sub-group meta-analysis, the lowest frequency of T allele was found in Africans (10.3 %; 95 % CI 3.8–16.8), and highest in Europeans (34.1 %; 95 % CI 31.9–36.3). The frequency of T allele in the North India is 11 %. The results of the meta-analysis showed that the frequency of the T allele and the TT genotype of C677T is highest in the Caucasian population.

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Introduction

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in folate/homocysteine pathway. It catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which donates methyl group for the conversion of homocysteine to methionine. *MTHFR* gene is located at chromosome 1p36.3 [1]. A number of single nucleotide polymorphisms (SNPs) are reported in *MTHFR* gene but C677T mutation (rs1801133) is the most studied and clinically important. C677T polymorphism lies in exon 4, in which cytosine is replaced by thymine at 677th position, which resulted in an alanine to valine substitution at position 222 in protein (A222V) [2, 3]. C677T mutation was shown to render the enzyme thermolabile [2]. MTHFR enzyme functions as dimer or tetramer and Flavin adenine dinucleotide (FAD) is the co-factor. The C677T mutation changes the secondary structure of the peptide and interactions between monomers. The A → V mutation increases the rate of dissociation of FAD and loss of FAD is linked to changes in quaternary structure and enzymatic activity reduces [4–6]. By docking study it is established that the mutant enzyme (222V) has less affinity towards its cofactor FAD than the normal enzyme (222A) [7]. The variant protein loses its cofactor FAD more quickly and has lower stability. C677T polymorphism effect can be suppressed by addition of folate, which causes a higher FAD affinity [8].

MTHFR C677T polymorphism has been reported as a risk factor for several diseases/disorders such as—Down syndrome [9, 10], neural tube defects [11], orofacial clefts [12],

type I diabetes [13], cardiovascular diseases [2, 14], male infertility [15], schizophrenia [16], bipolar disorder [16] and cancer [17] etc. The frequency of C677T polymorphism has been described from several world populations. But from India very limited case–control studies were published. So, it is very important to know the frequency of such a clinically important gene polymorphism in the healthy Indian population. Together with this the second objective of present study was to estimate the global prevalence of C677T polymorphism by meta-analysis.

Materials and Methods

Random Sample Analysis

Sample Collection

3 ml blood sample was collected in EDTA coated vials from 1000 unrelated healthy subjects which were domicile of Eastern Uttar Pradesh (UP). The study was approved by the Institutional Ethics Committee of the VBS Purvanchal University, Jaunpur. Blood sample was collected after getting informed written consent. Genomic DNA was extracted by the method of Bartlett and White [18].

Genotyping

MTHFR C677T genotyping was carried out by PCR–RFLP method of Frosst et al. [2]. Briefly 100 ng of genomic DNA was amplified in a final volume of 15 µl with 4 pM of each of forward and reverse primers, 250 µl of dNTPs mix, 1X *Taq* DNA polymerase buffer and 1u of *Taq* DNA polymerase. PCR program was initial denaturation at 94 °C for 4 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. The amplicons (198 bp) were digested with *Hinf*I as the C677T mutation creates a restriction site for it, and resolved in a 4 % agarose gel. For quality control, 10 % of samples (randomly selected) were re-genotyped and no discrepancy in genotypes were found.

Statistical Analysis

Allele frequencies were calculated by the gene counting method. χ^2 test was performed to test the Hardy–Weinberg Equilibrium(HWE). All statistical analysis was performed by DeFinetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Meta-analysis

Searched Strategy and Identification of Studies

For meta-analysis Pubmed, Science Direct, Springer link and Google scholar databases were searched for the suitable articles using keywords "*MTHFR*", "*methylenetetrahydrofolate reductase*", "*C677T*". Large number of articles were retrieved so that only those articles were selected for the inclusion in which C677T polymorphism in

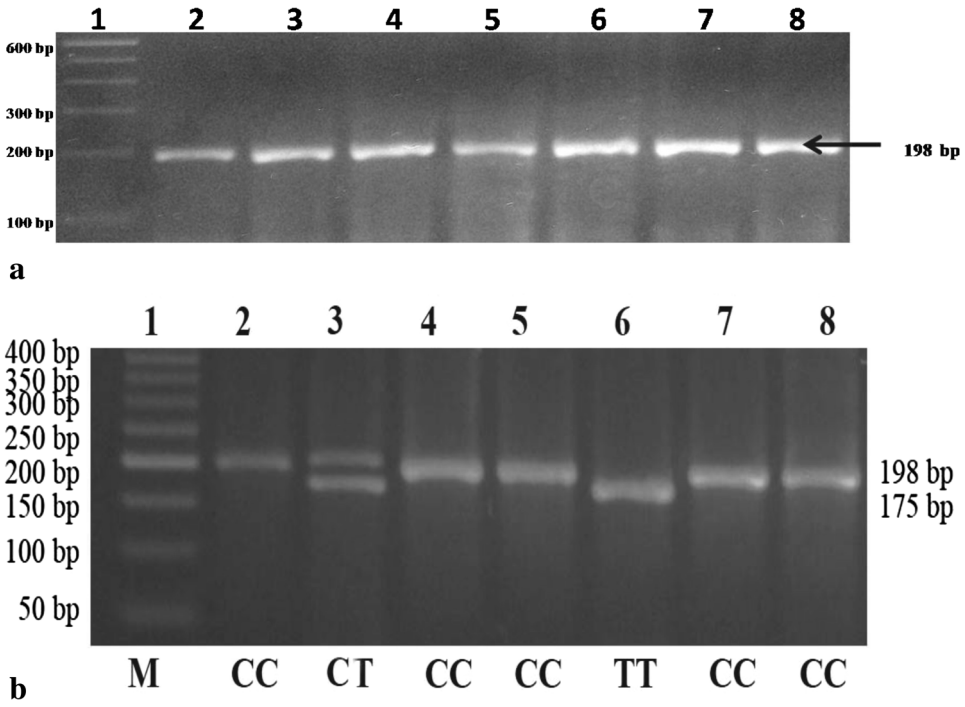

Fig. 1 **a** *MTHFR* C677T amplicon with 100 bp DNA ladder in *lane 1*; **b** Gel showing *Hinf*I digested different *MTHFR* C677T genotypes

Table 1 *MTHFR* C677T genotype distribution in randomly collected samples and gender wise distribution of samples, number of genotypes, number of alleles, HWE *p* value

Sample	Number	Age (mean ± SD)	Genotype						Number of Alleles				HWE *p* value
			CC		CT		TT		C		T		
			No.	Freq	No.	Freq	No.	Freq	No.	Freq	No.	Freq	
All													
Male	528	36.68 ± 16.49	416	0.79	103	0.19	9	0.02	935	0.89	121	0.11	0.37
Female	472	36.89 ± 17.70	381	0.81	89	0.18	2	0.004	851	0.90	93	0.10	0.18
Total	1000	36.44 ± 15.03	797	0.80	192	0.19	11	0.01	1786	0.89	214	0.11	0.88

Freq frequency

healthy subjects were analyzed. The included articles were also hand searched for additional studies which may be included in present meta-analysis.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: studies should: (1) be original, (2) selected only healthy subjects for C677T analysis and (3) reported *MTHFR* genotypes. Studies were excluded if they were: (1) case–control, (2) case reports, and (3) review, editorial etc.

Data Extraction

For all the eligible studies following information were extracted: first authors' family name, year of publication, journal name, country name, population/ethnic group, number of alleles and/or genotypes. If in a study samples were taken from multiple countries or different race/caste then information was abstracted separately for each country/race/caste.

Statistical Analysis

Prevalence proportions (PP) was computed from the number of alleles/genotypes and total number of alleles/sample sizes (N) with the corresponding 95 % confidence interval (CI). A pooled PP was then estimated on the basis of the individual PPs. The PP was estimated either by fixed effect [19] or random effect [20] model depending upon heterogeneity. The heterogeneity between studies was tested using the Q-statistics and was quantified using the I^2 statistic [21]. If $I^2 > 50$ % then random effect model was used [22], otherwise fixed effect model was adopted. We have also done sub-group analysis based on geographical area i.e. the region from where the study belonged (Africa, North America, South America, Asia, Australia and Europe). All *p* values are two tailed with a significance level at <0.05. All statistical analyses were undertaken by Open Meta-analyst [23].

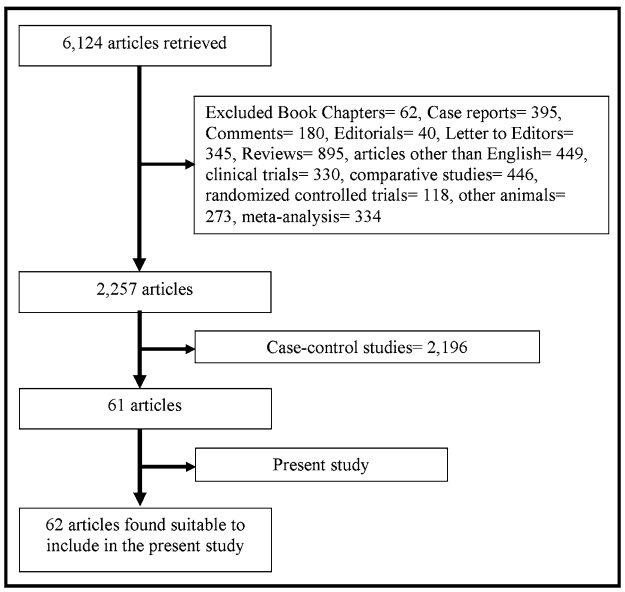

Fig. 2 Flow diagram of study search and selection process

A global C677T frequency map was created by StatPlanet software [24] by pooling the prevalence of *MTHFR* C677T gene polymorphism in different countries.

Results

MTHFR C677T Polymorphism Analysis

Total 1000 blood samples were collected from healthy individuals selected from Eastern UP and analyzed for *MTHFR* C677T polymorphism. The 198 bp long *MTHFR* amplicon was digested with *Hinf*I and resolved in 2 % agarose gel. The C677T substitution at nucleotide 677 creates a *Hinf*I digestion site. T allele was digested into 2 fragments (175 and 23 bp), whereas C allele remains uncut (Fig. 1). In total 1000 samples the number of CC, CT and

Table 2 Eligible included studies

Author	Country	No. of population studies	Sample size
Harmon et al. [44]	Ireland	1	625
Stevenson et al. [35]	USA	2	297
Arruda et al. [29]	Brazil	3	327
Bowen et al. [45]	UK	1	300
Franco et al. [30]	Brazil	5	337
Hill and FitzPatrick [46]	UK	1	122
Schneider et al. [25]	Central African Republic; Gambia; Kenya; Madagascar; Australia, UK, USA; French Polynesia; Hong Kong; Mongolia; Indonesia; Sri Lanka; Yemen	16	881
Antoniadi et al. [47]	Greece	1	160
Friedman et al. [61]	Israel	1	401
Mack et al. [34]	USA	4	84
Mutchinick et al. [36]	Mexico	1	250
Zuo et al. [62]	Korea, Japan	2	239
Chango et al. [48]	France	1	169
Pollak et al. [63]	Israel	8	897
Yu et al. [64]	China	1	200
Murakami et al. [65]	Japan	1	816
Mynett-Johnson et al. [49]	Ireland	1	115
Rady et al. [37]	USA	4	507
Rosenberg et al. [27]	Ghana; Israel; Japan	3	444
Sadewa et al. [66]	Indonesia, Japan	2	312
Al-Habboubi et al. [67]	Bahrain, Lebanon	2	560
Bailey et al. [38]	USA	1	185
Chowdary et al. [39]	USA	1	172
Esfahani et al. [40]	USA	1	433
Wilcken et al. [26]	Australia; Italy; Spain; France; Netherland; Finland; Hungary; Russia; Canada; USA; Mexico; China, Israel	24	7130
Zijno et al. [50]	Italy	1	172
Almawi et al. [68]	Lebanon	7	1178
Angeline et al. [69]	India	1	20
Lovricevic et al. [51]	Croatia	1	228
Spiridonova et al. [52]	Yakutia	3	477
Vaughn et al. [41]	USA	1	360
Ameen et al. [28]	Tunisia; Lebanon; Saudi Arabia; Bahrain	4	1234
Golbahar et al. [70]	Iran	1	391
Sazci et al. [53]	Turkey	1	1684
Parle-McDermott et al. [54]	Ireland	1	508
Barbosa et al. [31]	Brazil	1	100
Bhat et al. [71]	India	1	110
Gialeraki et al. [55]	Greece	1	100
Sabbagh et al. [72]	Lebanon	1	205
Saraswathy et al. [73]	India	1	81
Al-Allawi et al. [74]	Iraq	1	150
Algasham et al. [75]	Saudi Arabia	1	270
Mansoor et al. [76]	Pakistan	14	701
Misra et al. [78]	India	1	200

Table 2 continued

Author	Country	No. of population studies	Sample size
Ozarda et al. [56]	Turkey	1	402
Tsai et al. [42]	USA	1	1689
Zappacosta et al. [57]	Italy	1	104
Bagheri et al. [77]	Iran	1	108
Gra et al. [58]	Russia	1	352
Ghodke et al. [79]	India	1	144
Murry et al. [80]	India	2	112
Ramos et al. [43]	Mexico	1	150
Sukla and Raman [81]	India	4	1426
Sachdeva et al. [82]	India	1	350
Saraswathy et al. [83]	India	24	1142
Stur et al. [32]	Brazil	2	200
Amela et al. [59]	Bosnia	1	206
Dajani et al. [84]	Jordan	2	192
Seremak-Mrozikiewicz et al. [60]	Poland	1	1326
Yang et al. [85]	China	1	14,405
Romero-Sánchez et al. [33]	Colombia	1	152
Present study (2016)	India	1	1000

TT genotype was 797 (80 %), 192 (19 %) and 11 (1 %) respectively (Table 1). The number of C and T alleles was 1786 (89 %) and 214 (11 %) respectively. The population was in Hardy–Weinberg equilibrium ($p = 0.88$). Out of 1000 samples, 528 were male and 472 were female. In male samples the number of CC, CT and TT genotype 416 (79 %), 103 (19 %) and 9 (2 %) respectively. The number of C and T alleles was 935 (89 %) and 121 (11 %) respectively (Table 1). In female samples the number of CC, CT and TT genotype 381 (81 %), 89 (18 %) and 2 (0.04 %) respectively. The number of C and T alleles was 851 (90 %) and 93 (10 %) respectively.

Meta-analysis

Characteristic of Eligible Studies

With our initial search strategy, total 6124 articles were retrieved. After exclusion of reviews, meta-analysis, case–control studies, letter to editor, editorials, comments and observational studies etc., 62 articles (investigated 178 populations) with 47,592 samples were found suitable for the inclusion in the present meta-analysis (including present study) (Fig. 2). In included 62 articles—two articles were published by Australian scientists and they analyzed C677T polymorphism in three populations [25, 26] and 3 articles were published from African region, investigated total seven populations [25, 27, 28]. Five articles with twelve populations were published from South America [29–33], and 12 articles with 27 populations were published from North America [25, 26, 34–43], 5 articles with twelve populations from South America [29–33]. From Europe 19 articles were published which investigated 31 populations [25, 26, 44–60]. Highest number of articles were published from Asian continent i.e. 30 studies, in which total 98 populations were investigated [25–28, 61–85, present study]. These studies include 47,592 samples. The highest sample size was 14,405 in the study of Yang et al. [85] and lowest sample size was 20 in the study of Angeline et al. [69] (Table 2).

Meta-analysis

The meta-analysis showed that the global frequency of T allele was 24.0 % by adopting random effect model (95 % CI 21.7–26.5, $p < 0.001$; $I^2 = 98.74$ %) and 26.0 % (95 % CI 25.8–26.3, $p < 0.001$) by fixed effect model. The frequency of TT genotype was 7.7 % (95 % CI 6.5–8.9, $p < 0.001$; $I^2 = 96.25$ %) by random effect model and 6.5 (95 % CI 6.3–6.7, $p < 0.001$) by fixed effect model (Table 3). Between studies heterogeneity was high so random effect model was adopted.

Sub-group Analysis

In sub-group meta-analysis, the frequency of T allele was 10.3 % (95 % CI 3.8–16.8, $p = 0.002$; $I^2 = 92.79$ %) and frequency of TT genotype was 2.4 % (95 % CI −0.00 to

Table 3 Summary estimates for the prevalence proportion (PP) of *MTHFR* C677T in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric

Population	FE estimate (95 % CI)	RE estimate (95 % CI)	I^2	p (Q)
All				
T	26.0 (25.8–26.3), <0.001	24.0 (21.7–26.5), <0.001	98.74	<0.001
C	74.0 (73.7–74.2), <0.001	75.9 (73.5–78.3), <0.001	98.74	<0.001
TT	6.5 (6.3–6.7), <0.001	7.7 (6.5–8.9), <0.001	96.25	<0.001
CT	33.2 (30.8–35.6), <0.001	33.2 (30.8–35.6), <0.001	96.49	<0.001
CC	50.7 (50.3–51.1), <0.001	58.9 (55.4–62.4), <0.001	98.41	<0.001
African				
T	10.0 (8.4–11.7), <0.001	10.3 (3.8–16.8), 0.002	92.79	<0.001
C	90.0 (88.3–91.6), <0.001	89.7 (83.2–96.2), <0.001	92.79	<0.001
TT	1.3 (0.3–2.4), 0.01	2.4 (–0.0–4.8), <0.001	69.43	0.006
CT	18.3 (6.5–30.0), 0.002	18.3 (6.5–30.0), <0.001	89.03	<0.001
CC	77.3 (73.7–80.9), <0.001	80.3 (65.0–95.5), <0.001	93.57	<0.001
American (North)				
T	28.4 (27.6–29.2), <0.001	31.2 (25.7–36.7), <0.001	97.58	<0.001
C	71.6 (70.8–72.4), <0.001	68.8 (63.3–74.3), <0.001	97.58	<0.001
TT	5.3 (4.8–5.9), <0.001	11.9 (8.8–14.9), <0.001	95.6	<0.001
CT	37.5 (33.5–41.4), <0.001	37.5 (33.5–41.4), <0.001	87.66	<0.001
CC	48.7 (47.5–49.9), <0.001	49.8 (41.9–57.7), <0.001	97.36	<0.001
American (South)				
T	23.0 (21.3–24.6), <0.001	27.8 (19.8–35.8), <0.001	95.58	<0.001
C	77.0 (75.4–78.7), <0.001	72.2 (64.2–80.2), <0.001	95.58	<0.001
TT	3.4 (2.4–4.4), <0.001	7.6 (4.5–10.7), <0.001	85.2	<0.001
CT	37.9 (29.0–46.9), <0.001	37.9 (29.0–46.9), <0.001	90.83	<0.001
CC	55.0 (52.4–57.7), <0.001	53.0 (40.9–65.2), <0.001	95.14	<0.001
Asian				
T	24.6 (24.3–25.0), <0.001	19.7 (16.0–23.4), <0.001	99.13	<0.001
C	75.4 (75.0–75.7), <0.001	80.3 (76.6–84.0), <0.001	99.13	<0.001
TT	6.8 (6.5–7.1), <0.001	5.5 (3.7–7.2), <0.001	97.22	<0.001
CT	28.6 (24.8–32.4), <0.001	28.6 (24.8–32.4), <0.001	97.6	<0.001
CC	52.3 (51.7–52.8), <0.001	65.9 (60.2–71.6), <0.001	98.93	<0.001
Australian				
T	16.3 (14.0–18.6), <0.001	20.5 (2.6–38.4), 0.02	98.1	<0.001
C	83.7 (81.4–86.0), <0.001	79.5 (61.6–97.4), <0.001	98.1	<0.001
TT	2.5 (1.1–3.9), <0.001	5.9 (–0.5–12.3), 0.071	91.12	<0.001
CT	28.8 (6.6–51.0), 0.01	28.8 (6.6–51.0), 0.01	96.53	<0.001
CC	67.2 (63.3–71.2), <0.001	65.0 (36.6–93.4), <0.001	97.82	<0.001
European				
T	33.0 (32.4–33.6), <0.001	34.1 (31.9–36.3), <0.001	91.08	<0.001
C	67.0 (66.4–67.6), <0.001	65.9 (63.7–68.1), <0.001	91.08	<0.001
TT	9.7 (9.2–10.2), <0.001	11.6 (9.9–13.3), <0.001	87.33	<0.001
CT	44.3 (42.7–45.8), <0.001	44.3 (42.7–45.8), <0.001	57.83	<0.001
CC	44.0 (43.1–45.0), <0.001	43.6 (40.8–46.3), <0.001	87.77	<0.001

4.8, $p < 0.001$; $I^2 = 69.43$ %) in Africa (Table 3; Fig. 3a), the frequency of T allele was 20.5 % (95 % CI 2.6–38.4, $p = 0.02$; $I^2 = 98.1$ %) and frequency of TT genotype was 5.9 % (95 % CI −0.5 to 12.3, $p < 0.071$; $I^2 = 91.12$ %) in Australia (Table 3; Fig. 3b), the frequency of T allele was 27.8 % (95 % CI 19.8–35.8, $p < 0.001$; $I^2 = 95.58$ %) and frequency of TT genotype was 7.6 % (95 % CI 4.5–10.7, $p < 0.001$; $I^2 = 85.2$ %) in South America (Table 3;

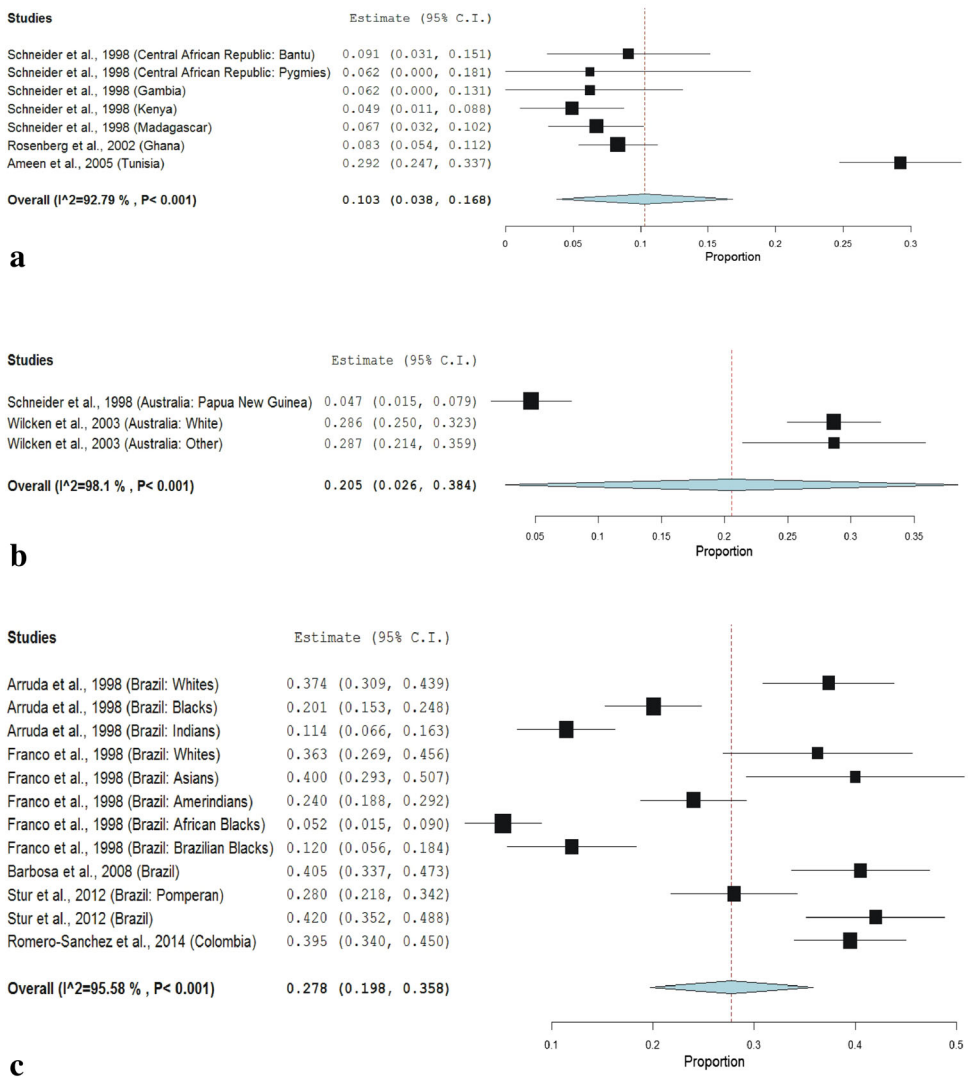

Fig. 3 **a** Random effect forest plot of T allele in African population; **b** random effect forest plot of T allele in Australian population; **c** random effect forest plot of T allele in South American population

Fig. 3c), the frequency of T allele was 34.1 % (95 % CI 31.9–36.3, $p < 0.001$; $I^2 = 91.08$ %) and frequency of TT genotype was 11.6 % (95 % CI 9.9–13.3, $p < 0.001$; $I^2 = 87.33$ %) in Europe (Table 3; Fig. 4a), the frequency of T allele was 31.2 % (95 % CI 25.7–36.7, $p < 0.001$; $I^2 = 97.58$ %) and frequency of TT genotype was 11.9 % (95 % CI 8.8–14.9, $p < 0.001$; $I^2 = 95.6$ %) in North America (Table 3; Fig. 4b), the frequency of T allele was 19.7 % (95 % CI 16.0–23.4, $p < 0.001$; $I^2 = 99.13$ %) and the frequency of TT genotype was 5.5 % (95 % CI 3.7–7.2, $p < 0.001$; $I^2 = 97.22$ %) in Asia (Table 3; Fig. 4c). Heterogeneity was high so random effect model was adopted.

Country-specific prevalence estimates for T allele is presented in Fig. 5a. Visual inspection of the map, revealed the highest prevalence of T allele in China, Mexico and some part of Europe. Same trend are also seen for the TT genotype (Fig. 5b).

Discussion

The objectives of present study were to determine (1) the frequency of *MTHFR* C677T polymorphism in Eastern UP population, and (2) the worldwide prevalence of C677T polymorphism by conducting a meta-analysis. *MTHFR* C677T has a high degree of heterogeneity in its world distribution. T allele is less prevalent in African population, its incidence is intermediate among Asian populations, and it is more prevalent in European and American populations. In present study, the prevalence of the mutant T allele was found as 11 % in the Eastern UP population (1 % TT homozygous) which is similar to that reported earlier from other Indian population. The main strength of this study is that this is the largest study (1000 samples) conducted on healthy individuals from Eastern U P population. The present study has some limitations like-only distribution of genotype in healthy subjects was reported and not presenting an association report.

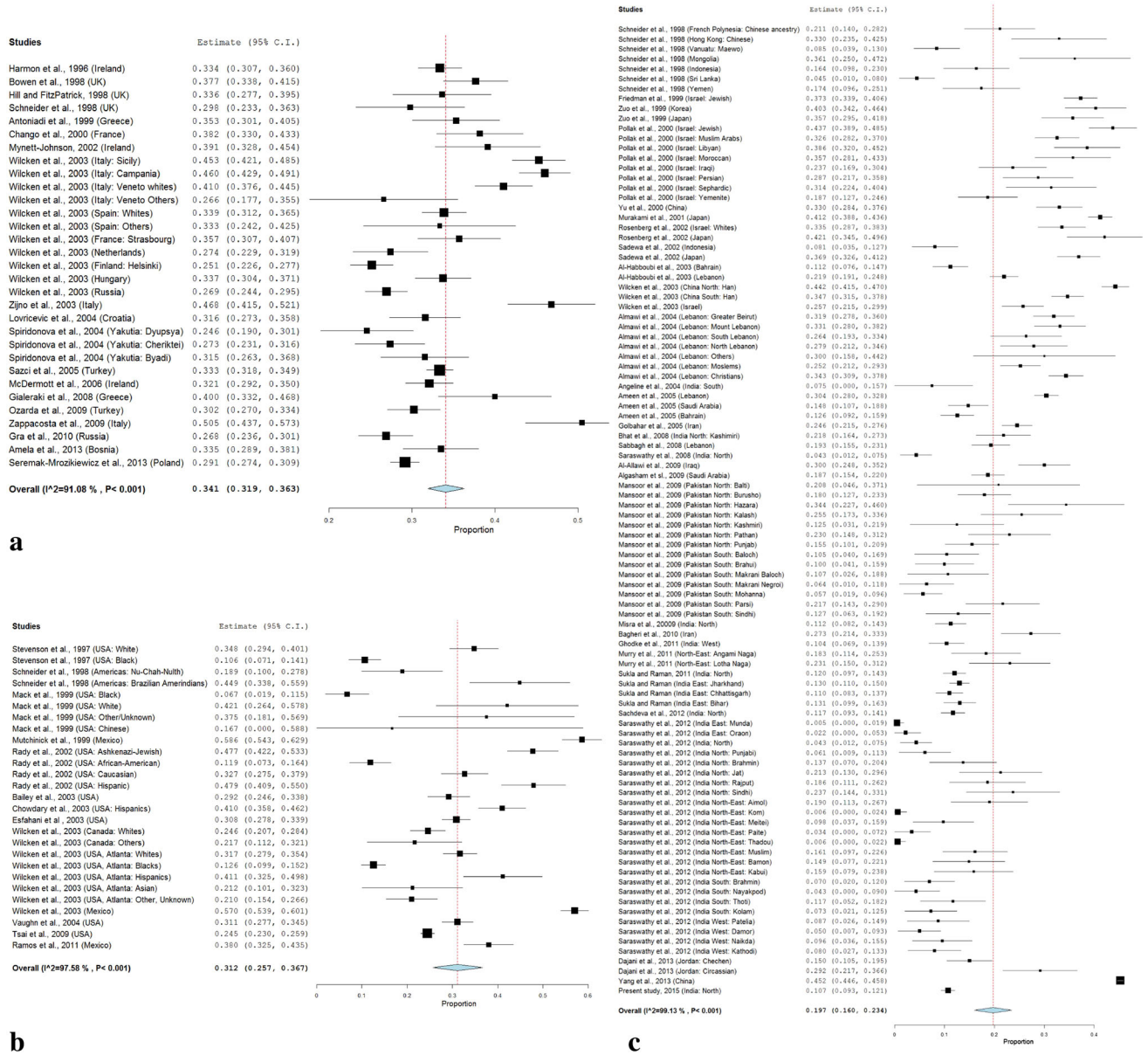

Fig. 4 **a** Random effect forest plot of T allele in European population; **b** random effect forest plot of T allele in North American population; **c** random effect forest plot of T allele in Asian population

In meta-analysis, global frequency of T allele was found 24.0 %. In subgroup analysis the frequency of T allele was 10.3 % in Africans, 31.2 % in North Americans, 27.8 % in South Americans, 19.7 % in Asians, 20.5 % in Australians and 34.1 % in Europeans. The global frequency of TT genotype was 7.7 %. In subgroup analysis, the frequency of TT genotype was 2.4 % in Africans, 11.9 % in North Americans, 7.6 % in South Americans, 5.5 % in Asians, 5.9 % in Australians and 11.6 % in Europeans. The frequency of both the T allele and TT genotype was lowest in Africans and highest in Europeans. Among Asian studies the frequency of this gene polymorphism was found higher in East Asian countries (44.7 % in China, 40.3 % in Korea, 39.9 % in Japan) than South Asian countries (11.4 % in India, 16 % in Pakistan, 4.5 % in Sri Lanka. The high frequency of T allele in East Asian countries might be due to folate and vitamin B12 rich non-vegetarian food habits of the population. Similarly, in South Asian countries highest frequency of T allele is found in Pakistan where majority of the population is non-vegetarian.

Meta-analysis is a powerful tool for analyzing cumulative data of studies where the individual sample sizes are small. During past decade several meta-analyses were published assessing *MTHFR* C677T gene polymorphism as risk factor for various diseases/disorders like Down syndrome [86, 87], cleft lip and palate [88], cardiovascular

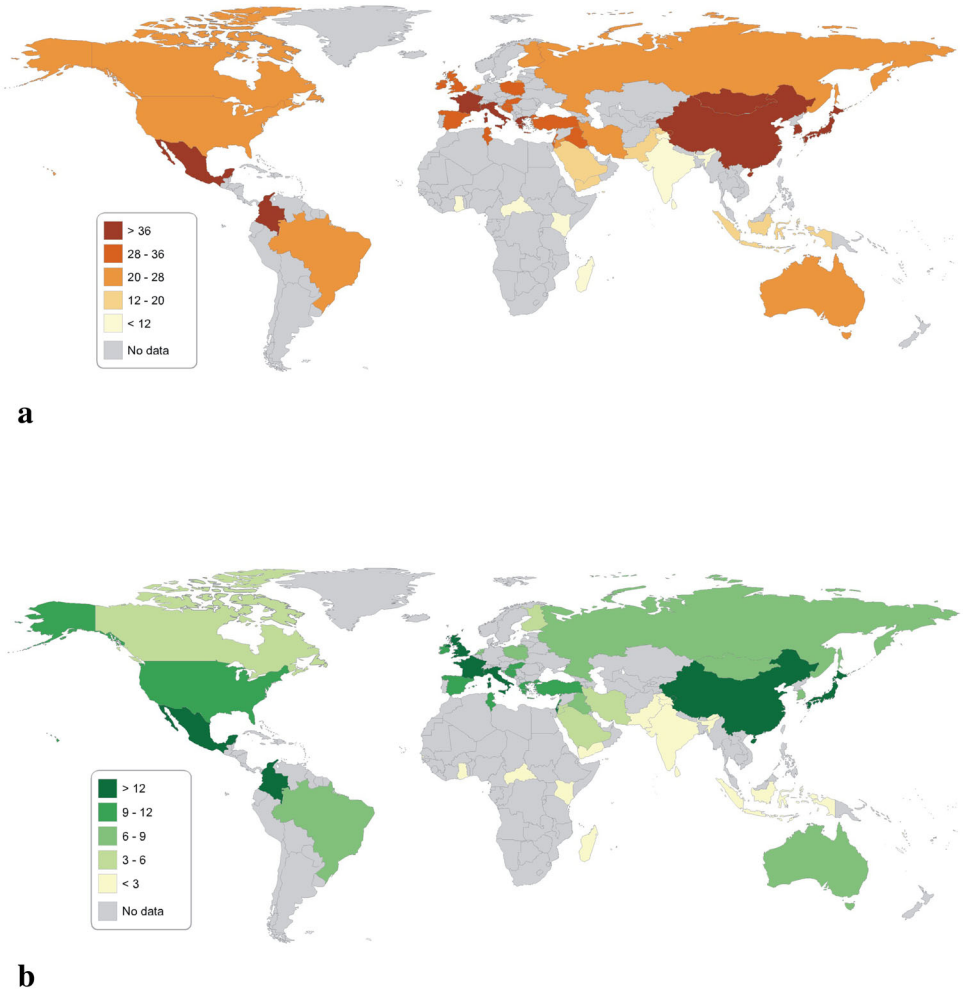

Fig. 5 **a** Worldwide distribution of T allele; **b** worldwide distribution of TT genotype

disease [89], diabetes [90], psychiatric disorder [91], neural tube defects [92], cancer [93] etc.

The meta-analysis has a number of strengths—(1) this is the first meta-analysis on the prevalence of *MTHFR* C677T gene polymorphism and (2) absence of publication bias. However, there are also some limitations—(1) we did not include case–control studies, (2) some studies might have not been included in this meta-analysis due to limitations of searched databases and (3) presence of high heterogeneity.

In conclusion, the frequency of the *MTHFR* C677T gene polymorphism discriminates the Indian population from other ethnic groups like Africans or Caucasians. Overall, our study showed a varied distribution of *MTHFR* C677T allele in various ethnic groups residing in diverse geographical regions of the world. The reason for the varied distribution seems to be directed not only by the environmental effect (specially folate and B12 rich/deficient diet) but also due to diversity in the origin and relatedness of various ethnic groups residing in the world. The aim of the study was to show countrywide prevalence of this highly important gene variant. Since *MTHFR* C677T polymorphism has been found to be associated with different diseases, the data would be very useful in regional health management programs and will facilitate predicting population-based risk factors for a number of congenital and other anomalies associated with *MTHFR* polymorphism.

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Compliance with Ethical Standards

Conflict of interest None.

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