Update meta-analysis from biomedical literature about MTHFR'polymorphisms and the CML' risk

Associations: polymorphismes du MTHFR et leucémie myéloïde chronique. Méta analyse de la littérature biomédicale

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Abstract

Background : The MTHFR gene polymorphisms are closely related to the chronic myeloid leukemia (CML). Case-control studies have associated the MTHFR polymorphisms and susceptibility to CML but the results were not conclusive.

Aim : To assess this association through an update meta-analysis.

Methods : A descriptive and qualitative study was conducted among students in the 6th year of the faculty during the academic year 2020/2021. The data were collected through a questionnaire written in french evaluating the teaching methods. A focus group of ten persons was led to understand better student's opinions.

Results : Totally, 17 and 12 case-control studies including CML cases and controls were enrolled in the meta-analysis respectively for C677T and A1298C polymorphism and CML risk. A poor association between the C677T (T vs C ; OR= 1,28; IC95%= [1,01;1,63]; p=0,04) and the one not significant between the A1298C (C vs A ; OR= 1,52; IC95%= [0,92; 2,51]; p= 0,1) polymorphisms and the CML risk for overall population were found.

Conclusion : The results of this meta-analysis suggested no significant association between C677T and A1298C polymorphisms and CML risk leading to consider other factors such us folic acid intake, gene-gene and gene- environment interactions.

Key words : Methylene tetrahydrofolatereductase, myeloid chronic leukemia, polymorphism, Risk.

Résumé

Introduction : Les polymorphismes du gène MTHFR sont impliqués dans la survenue de la leucémie myéloïde chronique (LMC). Des études cas-témoins ont évalué l'association entre ces polymorphismes et le risque de la LMC dans différents pays mais les résultats ont été discordants.

But : Analyser la relation entre les polymorphismes du MTHFR et le risque de la LMC via une méta-analyse actualisée

Méthodes : IUne recherche électronique sur la banque de données « Pubmed » a été conduite par deux investigateurs indépendants pour la sélection des articles cas-témoins publiés avant Juin 2018. L'analyse statistique a été faite selon les critères de l'éthnicité et le modèle génétique. Le odds ratio avec son intervalle de confiance (IC95%) est calculé à partir des données de la distribution génétique. Des tests d'hétérogénéité et des tests pour la détermination des biais de publication ont été utilisés.

Résultats : Les études cas-témoins incluant des cas de LMC et des sujets contrôles ont été inscrites pour le polymorphisme C677T et A1298C au nombre de 17 et 12, respectivement.

Une association faible entre le polymorphisme C677T (T vs C ; OR= 1,28; IC95%= [1,01;1,63]; p=0,04), absente entre le polymorphisme A1298C (C vs A ; OR= 1,52; IC95%= [0,92; 2,51]; p= 0,1) et le risque de LMC ont été décrites dans cette méta-analyse.

Conclusion: La faible ou l'absence d'association entre les polymorphismes C677T et A1298C et le risque de survenue de la LMC suggère la considération d'autres facteurs comme la consommation de l'acide folique, les interactions entre gène-gène et gène-environnement.

Mots-clefs : Méthylène tétrahydrofolate réductase, Leucémie myéloïde chronique, polymorphisme, risque.

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INTRODUCTION

The cause of human cancer is a consequence of complex interplay between genetic predisposition and environmental factors. Susceptibility to different types of leukemia seems to be related to polymorphisms in multiple genes and various genetic events during blood cell development(1). Chronic myelogenous leukemia (CML), an example treated in this meta-analysis, is a myeloproliferative disease characterized by the reciprocal translocation t (9; 22) (q34; q11) and bcr-abl fusion transcript(2). It's a common malignancy of hematopoietic stem cells distinguished by an abnormal accumulation of white blood cells in the bone marrow that interferes with the normal production of blood cells. Since the introduction of Imatinib in 2000, the annual mortality in CML has decreased from 10%-20% down to 1%-2% (3,4). Clinical and biological aspects of CML are well investigated, but the factors that cause individuals ' susceptibilities to CML are still not fully understood(3). Assessment of causes of CML may be beneficial for clinical management and prevention.

CML are likely to be affected by the metabolic fate of folic acid(5). Folate methylation plays an essential role in DNA synthesis and methylation processes(6). Folate deficiency have been associated with hypomethylation and uracil mis-incorporation into DNA during replication, increasing the risk of chromosomal aberrations and facilitating the onset of oncogenic processes(7). Folate metabolism requires the optimal activity of various enzymes(8).

5, 10 methylene tetrahydrofolate reductase (MTHFR) is one of the important enzymes of the folate cycles(9). It irreversibly reduces 5, 10 methylene tetrahydrofolate to 5 methyl tetrahydrofolate, the primary form of serum folate and carbon donor for the remethylation of homocysteine to methionine(10).

The MTHFR gene is located on the short arm of chromosome 1(1p36.3).

There are two commonly occurring polymorphisms in the MTHR gene: C677T and A1298C. C677T occurs in exon 4 and results in alanine to valine substitution at codon 222, while a second common polymorphism, A1298C in exon 7, results in a glutamate to alanine substitution at codon 429(11).

To date, several studies performed in different countries have assessed the association between MTHFR' polymorphisms and susceptibility to malignant hemopathies like acute lymphoblastic leukemia (12) and CML. But, the results were inconclusive and conflicting (13,14). Moreover, the previous meta-analyses had limited sample sizes and so the statistical study still not powerful to demonstrate a significant association (15-17). Therefore, we carried out our meta-analysis which will be the most exhaustive in order to determine the relationship between the MTHFR polymorphisms and the risk of development of CML according to the models and the ethnicity.

METHODES

Search strategy

A comprehensive search that investigated the association between the MTHFR C677T or A1298C genetic variants and the risk of adult CML published before June 2018 was conducted in PubMed electronic databases. The following combined descriptor terms were used: («Methylenetetrahydrofolate reductase» OR « MTHFR ») AND (« Chronic myeloid leukemia»). The search included only journal articles. All references cited in the studies were extensively reviewed to identify additional published articles.

Studies selection

Published studies before June 2018; were selected in the analysis according to these following inclusion criteria: (1) case-control study; (2) study evaluating the association between C677T and A1298C polymorphisms and susceptibility to CML; and (3) study presenting available data on the distribution of MTHFR gene polymorphisms in cases and in control groups which are sufficient for calculating odds ratio. For multiple studies using the same groups of patients or controls, the study with the largest sample size was included in the meta-analysis. We excluded articles which are reviews and not publications. Data of Meta-analyses which are also excluded will be used for comparison and discussion later.

As a whole, this meta-analysis was carried out according to the preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA).

Data extraction

Two independent investigators checked each report and extracted and tabulated the following data from eligible studies: name of the first author, year of publication, country of origin, ethnicity of the study population, numbers and genotype distributions of cases and controls.

Statistical analysis

The meta-analysis, using the software http://bioinfo.genyo.es/metagenyo/ (18), examined different genetic comparison models namely allele contrast model (« T vs. C » or « C vs. A »), recessive model (« CC+CT vs. TT » or « AA+AC vs. CC »), dominant model (« TT+TC vs. CC » or « CC+AC vs. AA »), homozygote model (« TT vs. CC » or « CC vs. AA ») and heterozygote model (« CT vs. CC » or « AC vs. AA ») for estimation of the association between MTHFR C677T and/or A1298C polymorphisms and CML risk, respectively.

The genotype distribution was assessed for Hardy-Weinberg Equilibrium (HWE) to check study quality, P(HWE)< 0,05 showed statistical significance and so control genotype might not be in HWE and should be excluded from the meta-analysis (18).

The odds ratio (OR) and 95% confidence interval (CI) were calculated using data for genotype distribution. These association tests results evaluated the association' strength between MTHFR polymorphisms and CML risks.

If the value 1 was not in the range of CI, it was considered that there is an increased relative risk in one group compared with the other (18).

The heterogeneity was determined by calculating I^2 metric statistic. $I^2<25\%$, $25\%<I^2<50\%$ and $I^2>50\%$ were interpreted as low, moderate, and high degrees of heterogeneity, respectively (19). When no heterogeneity was found with p >0, 05 or $I^2 < 50\%$, a fixed effect model was chosen to estimate the pooled ORs with their corresponding 95%CIs. Otherwise, a random-effects model was used (20).

Forest plots were provided by the meta-analysis to estimate the global result of all studies.

Funnel plot, a graphical test, was used to check if publication bias exists or not. So asymmetry of the funnel plot suggested a possible publication bias. The Egger's test could be used ; p<0,05 was considered as a potential statistical publication bias(21).

Subgroup analysis also was performed according to the ethnicities.

Sensitivity analyses were conducted to examine whether the individual study influenced the pooled results.

RESULTATS

Search of published reports

A flow chart depicting the study selection process is shown in figure1.

26 articles were identified based on various combinations of the keywords listed in the Methods and focused on the relationship between MTHFR gene SNPs and the risk of CML. On these articles, 9 were reviews and 3 were meta-analyses. Therefore, only 17 studies qualified for inclusion in this meta-analysis.

Study characteristics

Characteristics of the 17 articles included in the meta-analysis were shown in Table 1 and 2. The studies were conducted in various populations of different ethnicities, as Asian (6 studies), Caucasian (5 studies), Mixed (5 studies) and one on African population for MTHFR C677T polymorphism. However, the A1298C polymorphism was distributed in 4, 2, 1 and 5 studies for Asian, Caucasian, African and Mixed population respectively.

Only one study (Hur s' study (22)) was removed, since the distributions were not in accordance with the HWE, for the C677T polymorphism. Likewise, the distribution of the A1298C genotypes in controls were not in accordance with the WHE in two studies: Vahid(23) and Lordelo (24) reports.

1362 cases and 4357 controls were found in these studies for MTHFR C677T polymorphism. The allele and variants genotype frequency of each study were listed in table II. In the case group, the range of T allele frequencies was from 16 to 45%. It changed from 17 to 36% for Asian population, from 23 to 33% for Caucasian population and from 16 to 29% for mixed population. The T allele frequency was 45% for African population. In the control group of C677T polymorphism, the allele T frequencies varied from 14 to 38%.

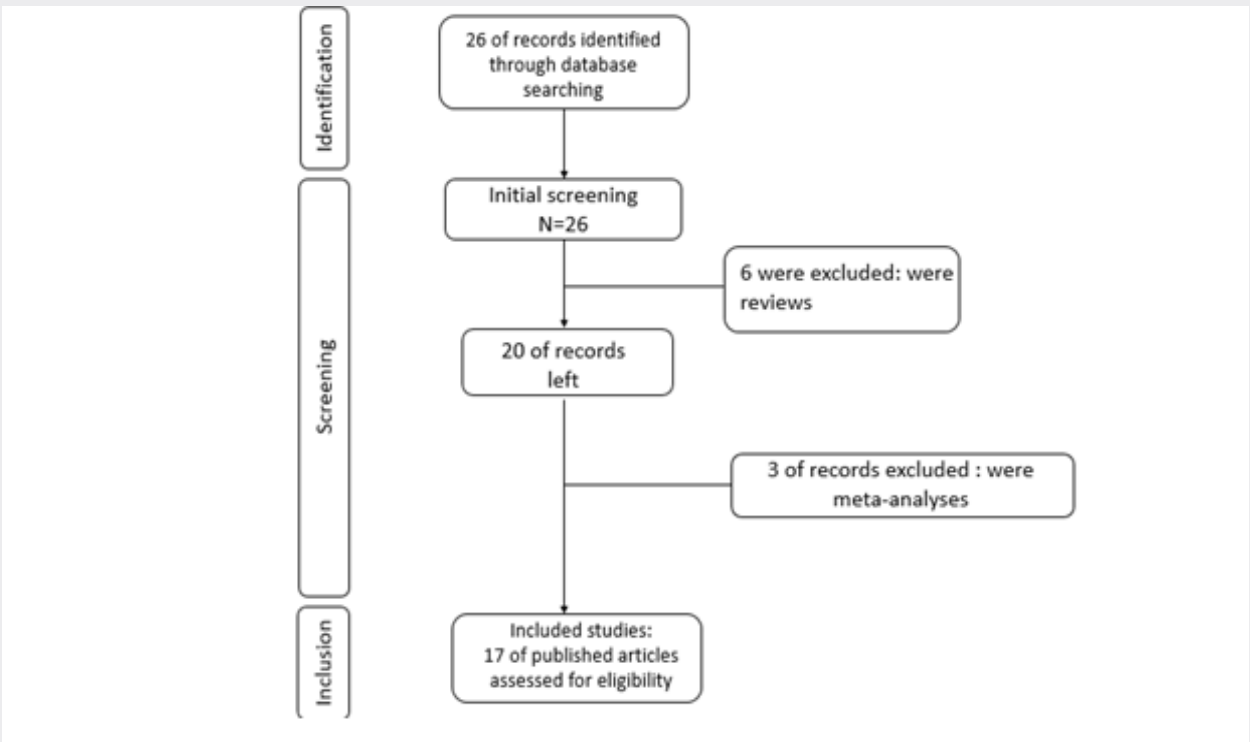

Figure. 1. Flow diagram for study inclusion in the meta-analysis and reasons for study exclusion

Table 1. Study characteriscs : Publication year ; ethnicity ; polymorphisms studied; number and mean age of both cases and controls

First author	Publication year	Ethnicity (country)	Polymorphisms studied	Cases		Controles	
				Number	Mean Age (years)	Number	Mean Age (years)
Deligezer 2003(25)	2003	Caucasian (Turkey)	C677T	131 (F/M= 70/61)	45,9	161 (F/M= 112/48)	39
Hur 2006(22)	2006	Asian (Korea)	C677T, A1298C	40 (F/M= 13/27)	50	200 (F/M= 72/128	34
Chen 2006(6)	2006	Asian (China)	C677T	7 (NA)	NA	157 (NA)	NA
Moon 2007(26)	2007	Asian (Korea)	C677T, A1298C	115 (F/M= 40/75)	43,8	434 (F/ M=238/19)	41,4
Barbosa(7)	2008	Mixed (Brasil)	C677T, A1298C	67 (F/M= 30/36)	44	100 (F/M=47/53)	29
Kim 2009(27)	2009	Asian (Korea)	C677T, A1298C	149 (F/M= 55/94)	50,4	1700 (F/M= 879/821)	52,2
Ismail 2008(28)	2009	Mixed (Jordan)	C677T, A1298C	149 (NA)	NA	170 (NA)	NA
Vahid 2010(23)	2010	Mixed (Iran)	C677T, A1298C	38 (F/M= 19/19)	45	97(F/M= 50/47)	44,8
Jankovic 2011(29)	2011	Caucasian (Serbia)	C677T	43 (NA)	NA	26 (NA)	NA
Lordelo 2012(24)	2012	Caucasian	C677T, A1298C	41 (NA)	NA	155 (NA)	NA
Lordelo 2012(24)	2012	Mixed (Brasil)	C677T, A1298C	64 (NA)	NA	118 (NA)	NA
Hussein 2012(30)	2012	Asian (India)	C677T	43 (NA)	39,5	251 (NA)	41,5
Jakoljevic 2013(31)	2012	Caucasian (Serbia)	C677T	52 (F/M= 24/28)	NA	53 (F/M= 24/30)	NA
Dorgham 2014(32)	2014	African (Algeria)	C677T, A1298C	90 (NA)	45,9	100 (NA)	47,3
Banescu 2014(33)	2014	Caucasian (Romania)	C677T, A1298C	151 (F/M= 65/86)	51	305 (F/M= 179/126)	47
Rabab M Aly 2014(14)	2014	Mixed (Egypt)	C677T, A1298C	85 (F/M= 40/45)	46,7	100 (F/M= 49/51)	48,2
Korshied 2014(13)	2014	MIxed (Egypt)	C677T, A1298C	97 (F/M= 51/46)	NA	130 (F/M= 68/62)	NA

F: female; M: male; NA: not available.

Table 2. Distribution of MTHFR C677T and A1298C genotypes in cases and controls and calculation of P value.

Study	Distribution of C677T MTHFR genotype(n)						HWE P value	Distribution of A1298 MTHFR genotype(n)						HWE P value
	Case			Contol				Case			Control			
	CC	CT	TT	CC	CT	TT		AA	AC	CC	AA	AC	CC	
Deligezer 2003 (25)	72	50	9	74	73	14	0,5006							
Hur 2006(22)	13	17	10	80	80	40	0,0184	31	7	2	116	78	6	0,0944
Chen 2006(6)	2	2	3	72	66	19	0,522							
Moon 2007(26)	43	45	27	144	196	94	0,0779	74	33	8	307	120	7	0,2189
Barbosa 2008(7)	46	19	2	65	29	6	0,2701	41	23	3	63	32	5	0,7221
Kim 2009(27)	54	72	26	540	863	297	0,1326	97	49	5	1147	500	53	0,8678
Ismail 2008(28)	63	67	19	94	66	10	0,722	59	68	22	76	81	13	0,1724
Vahid 2010(23)	24	11	3	56	37	4	0,4872	12	19	7	39	36	22	0,0211
Jankovic 2011(29)	17	21	5	6	16	4	0,2247							
Lordelo 2012(24)	15	21	5	74	66	15	0,9594	26	15	0	68	79	8	0,0132
Lordelo 2012(24)	31	26	7	66	48	4	0,1787	35	28	1	51	64	3	0,0844
Hussein 2012(30)	28	8	7	180	61	10	0,106							
Jakoljevic 2013(31)	8	29	5	13	33	7	0,057							
Dorgham 2014(32)	8	35	40	48	38	3	0,1649	27	31	26	50	42	5	0,3082
Banescu 2014(33)	58	68	25	154	116	35	0,0727	67	68	16	149	119	37	0,0868
Rabab M Aly 2014(14)	30	44	11	45	49	6	0,1192	32	38	15	40	51	9	0 ,199
Korshied 2014(13)	41	45	11	65	52	13	0,5872	54	37	6	55	65	10	0,1206

P HWE: P value for Hardy-weinberg equilibrium

In the same way, a total of 1086 cases and 3609 controls were enrolled in these studies for MTHFR A1298C polymorphism. For the case group, the C allele frequencies varied from 11 to 34%. For African population, the C allele frequency was 34%. It changed from 11 to 34%for Asian population, from 18 to 28% for Caucasian population and from 19 to 31% for mixed population. While in the control group the rate of C allele frequency varied from 15 to 30%.

Meta- analysis results

The meta-analysis of association studies were summarized in table 3. By examining the heterogeneity tests 'results, we took into consideration the random effect model since the p value<0,05 and I^2>50% for the C677T and A1298C polymorphisms, except one heterozygous model of the A1298C polymorphism.

Overall, we have found a poor association between CML risk and the C677T polymorphism under the allele contrast model (Figure. 2 A); recessive model (TT vs. TC+CC) and homozygous model (TT vs. CC). For the dominant model (CC vs. TT+TC) and the heterozygous model (TC vs. CC), the association is not significant.

According to the ethnicity, a significant association between CML risk and C677T variant in African population was described under all models (table 3a).

Moreover, in the overall population, we have found no significant association between the A1298C polymorphism and the CML risk, respectively for the allele contrast, the recessive and the homozygous model (Figure. 2B).

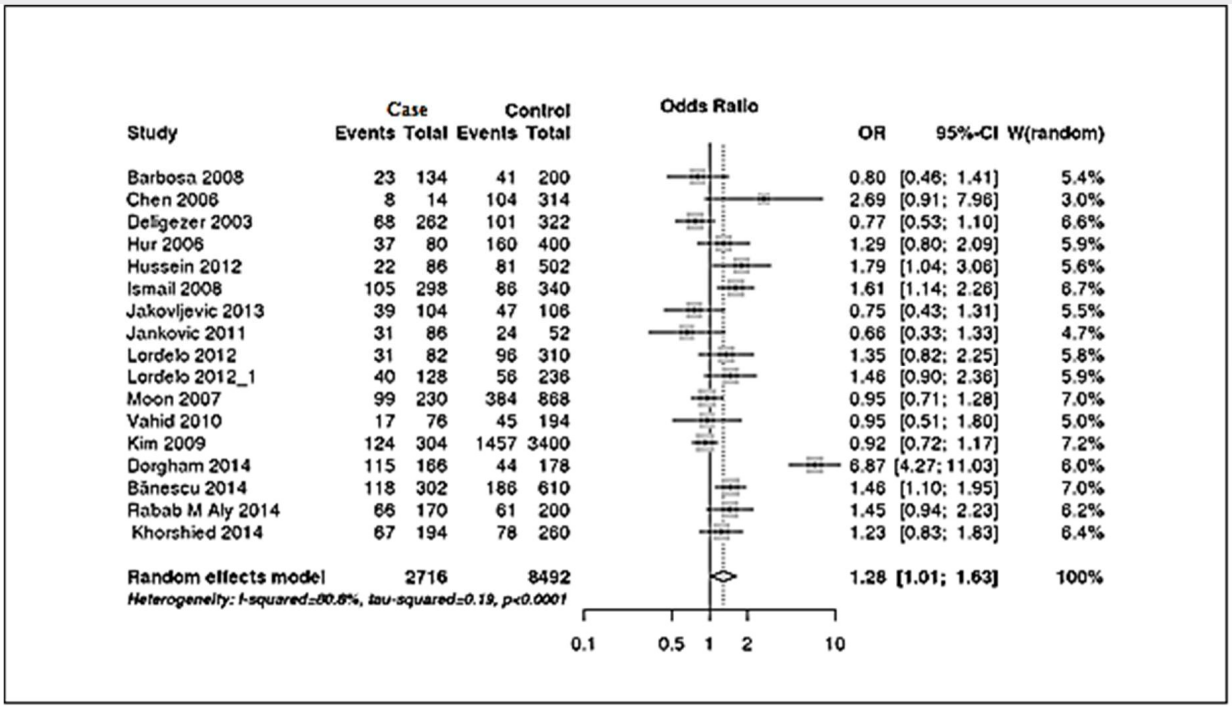

Figure 2 A. Forest plot of C677T polymorphisms and CML under the allele contrast model

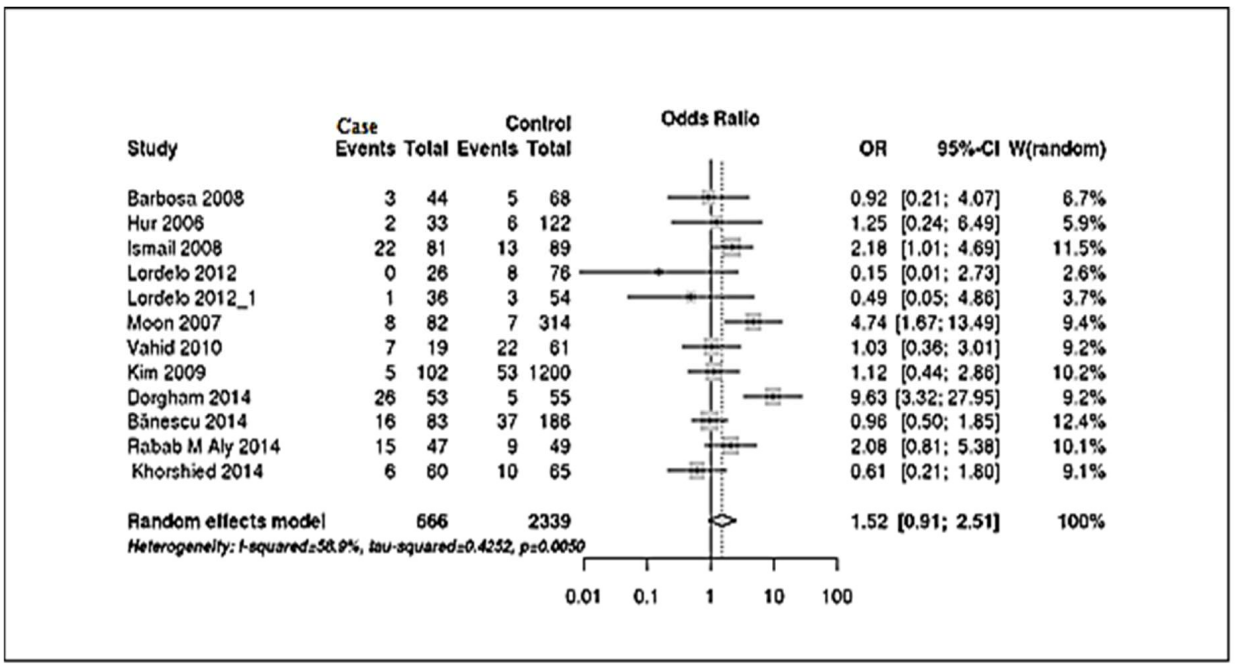

Figure 2. Forest plot of C677T and A1298C polymorphisms under different models

Subgroup analysis by ethnicity showed a magnitude effect in African population under all models except those heterozygote model (CA vs. AA) whose association was not significant (OR= 1, 37, 95%CI=0, 7071-2,6423, p=0,3527) (table 3b).

Table 3 b. Odds ratio (OR), heterogeneity results and publication bias for the genetic contrasts of MTHFR gene A1298C polymorphisms for CML risks.

Table 3 a. Odds ratio (OR), heterogeneity results and publication bias for the genetic contrasts of MTHFR gene C677T polymorphisms for CML risks.

Model C677T	Ethnicity	Number	Heterogeneity 's tests		Association 's tests			Publication bias P-value Egger's test)
			I²	P-value	OR	CI	P-value	
Allele contrast (Tvs C)	Overall	17	80,82%	0,0001	1,28	[1.01; 1.63]	0,039721	0,4453
	African	1	NA	NA	6,87	[4.27; 11.03]	0	NA
	Asian	6	44,68%	0,1076	1,04	[0.89; 1.22]	0,58702	0,0513
	Caucasian	5	66,6%	0,0175	0,98	[0.69; 1.39]	0,935452	0,3034
	Mixed	5	13,94%	0,3254	1,35	[1.12; 1.63]	0,001918	0,1257
Recessive model (TT vs TC+CC)	Overall	17	63,8%	0,0002	1,67	[1.16; 2.41]	0,005973	0,1311
	African	1	NA	NA	26,66	[7.8; 91.14]	1,64E-07	NA
	Asian	6	56,57%	0,0421	1,60	[0.97; 2.63]	1,64E-07	0,0364
	Caucasian	5	0%	0,5732	1,14	[0.77; 1.68]	0,063045	0,1149
	Mixed	5	24,07%	0,2609	1,78	[1.13; 2.8]	0,508805	0,6376
Dominant model (CC vs TT+TC)	Overall	17	69,79%	0,0001	1,23	[0.94; 1.6]	0,012638	0,4733
	African	1	NA	NA	10,97	[4.74; 25.42]	0,127175	NA
	Asian	6	0	0,5513	0,94	[0.75; 1.18]	2,25E-08	0,0904
	Caucasian	5	68,69%	0,0124	0,95	[0.57; 1.58]	0,586297	0,3761
	Mixed	5	0%	0,5631	1,38	[1.08; 1.77]	0,842135	0,105
Homozygote (TT vs CC)	Overall	17	73,57%	0,0001	1,79	[1.13; 2.83]	0,009783	0,1821
	African	1	NA	NA	80	[19.89; 321.7]	0,01326	NA
	Asian	6	56,79%	0,0411	1,53	[0.89; 2.65]	7E-10	0,0403
	Caucasian	5	44,69%	0,1242	1,19	[0.78; 1.8]	0,126373	0,1361
	Mixed	5	28,73%	0,23	2,05	[1.28; 3.28]	0,419753	0,5168
Heterozygote (TT vs CC)	Overall	17	51,02%	0,0082	1,10	[0.88; 1.38]	0,397935	0,8322
	African	1	NA	NA	5,53	[2.29; 13.29]	0,000136	NA
	Asian	6	0%	0,8978	0,84	[0.66; 1.08]	0,171749	0,5167
	Caucasian	5	61,23%	0,0354	0,95	[0.59; 1.53]	0,845969	0,4038
	Mixed	5	0%	0,8226	1,29	[1.001; 1.68]	0,048527	0,0384

I2 : degree of heterogenity ; OR : Odds Ratio ; IC: confidence interval

Publication's bias

Egger's test was applied to assess possible publication bias of the included studies. For C677T polymorphism, Table 3a showed publication bias for Asian population under recessive model and homozygote model (TT vs. CC) and for mixed population under heterozygote model (TC vs. CC). The A1298C polymorphism didn't show any publication bias according to Egger's test represented on table 3b.

Begg's Funnel plots was used showing a symmetrical shape for the two polymorphisms (Figure.3).

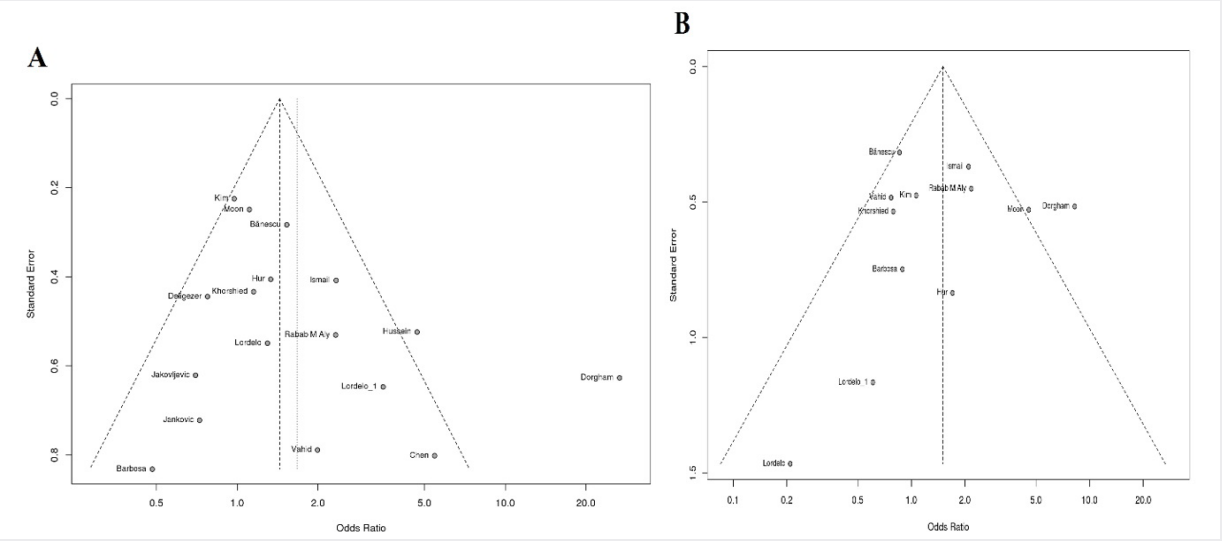

Figure 3. Funnel plot analysis of potential publication biais. A: Funnel plots analysis for publication biais between MTHFR C677T polymorphism and CML ; B : Funnel plots analysis for publication biais between MTHFR A1298C polymorphism and CML.

Table 3 b. Odds ratio (OR), heterogeneity results and publication bias for the genetic contrasts of MTHFR gene A1298C polymorphisms for CML risks.

Model A1298C	Ethnicity	Number	Heterogeneity 's tests		Association 's tests			Publication bias
			I^2	P-value	OR	CI	P-value	P-value Egger's test)
Allele contrast (C vs A)	Overall	12	70,61%	0,0001	1,06	[0.85; 1.33]	0,610745	0,2087
	African	1	NA	NA	2,66	[1.72; 4.13]	1,17E-05	NA
	Asian	4	53,73%	0,0903	1,08	[0.79; 1.49]	0,616595	0,3436
	Caucasian	2	78,63%	0,0305	0,77	[0.37; 1.59]	0,488287	NA
	Mixed	5	52,04%	0,0799	0,99	[0.75; 1.31]	0,951556	0,3622
Recessive model (CC vs AC+AA)	Overall	12	58,74%	0,0052	1,51	[0.93; 2.45]	0,097038	0,744
	African	1	NA	NA	8,25	[2.99; 22.69]	4,38E-05	NA
	Asian	4	56,31%	0,0763	1,54	[0.67; 3.52]	0,305413	0,7443
	Caucasian	2	0%	0,3463	0,80	[0.44; 1.48]	0,486417	NA
	Mixed	5	0%	0,4084	1,53	[0.97; 2.43]	0,067319	0,1337
Dominant model (AA vs CC+AC)	Overall	12	60,14%	0,0037	0,99	[0.78; 1.27]	0,971503	0,1982
	African	1	NA	NA	2,24	[1.22; 4.12]	0,008968	NA
	Asian	4	60,22%	0,0565	1,04	[0.68; 1.61]	0,846408	0,5194
	Caucasian	2	82,04%	0,0183	0,77	[0.29; 1.99]	0,590829	NA
	Mixed	5	38,73%	0,163	0,91	[0.71; 1.16]	0,435625	0,5895
Homozygote (CC vs AA)	Overall	12	58,86%	0,005	1,52	[0.92; 2.51]	0,106747	0,5131
	African	1	NA	NA	9,63	[3.32; 27.95]	3,09E-05	NA
	Asian	4	44,7%	0,1432	1,65	[0.95; 2.87]	0,073228	0,944
	Caucasian	2	32,95%	0,222	0,88	[0.46; 1.66]	0,691687	NA
	Mixed	5	25%	0,2548	1,43	[0.88; 2.32]	0,143511	0,1763
Heterozygote (CA vs AA)	Overall	12	46,02%	0,0404	0,94	[0.75; 1.17]	0,585394	0,133
	African	1	NA	NA	1,37	[0.71; 2.64]	0,352779	NA
	Asian	4	63,73%	0,0407	0,99	[0.61; 1.61]	0,980956	0,5831
	Caucasian	2	79,93%	0,0256	0,83	[0.33; 2.08]	0,694699	NA
	Mixed	5	8,51%	0,358	0,84	[0.65; 1.09]	0,197205	0,7727

Sensitivity analysis

Sensitivity analyses of both MTHFR C677T and A1298C indicated that no study has significantly influenced the pooled ORs.

DISCUSSION

In the current meta-analysis, the relationship was explored between the polymorphisms of MTHFR and CML involving 1362 cases and 4357 controls for the C677T and 1086 cases and 3609 controls for the A1298C variant.

In the overall population, the prevalence of T allele (for the C677T) and the C allele (for the A1298C) ranged from 16 to 45% and from 11 to 34%, respectively. These frequencies were largely presented in the African population. This finding could explain the significant association that we have found, among other reasons, between the two polymorphisms and the CML risk in the African population.

In fact, a poor association was described between the C677T MTHFR polymorphism and CML risk, with the allele contrast model, the recessive and the homozygous model. These results were not consistent with a previous published meta-analysis by Li et al (17).

The stratification analysis by ethnicities showed a significant association for African population, suggesting a possible role of ethnic differences in the genetic background and environmental factors regarding the C677T polymorphisms and the risk of CML, especially since the T allele frequency varied by ethnicities.

Concerning the A1298C polymorphism, no association between for the allele contrast, the recessive and the homozygous models in the overall population was described. Subgroup analysis by ethnicity showed a magnitude effect in African population under all models except that heterozygote model (CA vs. AA) whose association was not significant. These results were not consistent with the last meta-analysis (17).

Previously, many studies indicated the association between the polymorphisms of the MTHFR gene and CML with inconclusive results. The inconsistent conclusions are due to many reasons leading to the low statistical power. They may be also the result of fundamental differences and heterogeneity between studies like the selection of controls, age distribution and life style factors.

Heterogeneity between studies is a critical problem which must be conducted. To avoid the potential heterogeneity, carefully publication search, strict studies inclusion criteria, precise data extraction, and strict statistical analysis were performed in this meta-analysis. First of all, the distributions of the C677T genotypes in controls were not in accordance with the HWE in one study(22). In the same way, the distribution of the A1298C genotypes in controls were not in accordance with the WHE in two studies (23,24). So these reports whose P (HWE) < 0, 05 might not be in HWE should be excluded from the meta-analysis. Moreover, high heterogeneities emerged in comparisons between MTHFR C677T and A1298C polymorphisms in overall, Asian and Caucasian populations under different genetic models.

Heterogeneities cannot be avoided and may result from selection of the control groups, study design, ethnicity differences and lifestyle factors.

Substantial publication bias was found for the C677T polymorphism in Asian population under recessive model, homozygote model (TT vs. CC), and in Mixed population under heterozygote model (TC vs. CC). The A1298C polymorphism didn't show any publication bias according to Egger's test. So there is always a certain degree of publication bias, since only published studies were included in this meta-analysis. Non-significant or negative results may be unpublished.

Likewise, the results of sensitivity analysis showed that no individual study influenced the pooled ORs, indicating the results of this meta-analysis are stable.

It should be noted that the use of only "Pubmed" database may skew our results. So, adding indexed publications in other biomedical databases such as Scopus or Web of Science may prevent publications bias and improve our study'conclusion.

Considering the limitations of this meta-analysis, the results should be interpreted with caution.

CONCLUSION

In conclusion, this meta-analysis suggested poor and no association between C677Tand A1298C polymorphisms respectively, and CML risk. However, due to the limitations of this study, these results should be interpreted with caution and still require future large-scale studies to confirm their accuracy. Moreover, considering that CML is a complex disease with a multifactorial etiology, the development of adult CML might be associated with other factors such us folic acid intake, gene-gene and gene-environment interactions in order to provide more conclusive evidence regarding the genetic susceptibility to adult CML.

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