

Folate Metabolism and Genetic Variant in Down Syndrome: A Meta-Analysis

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Abstract

Objectives: Studies investigating the association between gene polymorphisms involved in homocysteine/folate metabolism and Down syndrome (DS) have reported contradictory or inconclusive results. A meta-analysis of 25 studies on association between MTHFR and MTRR polymorphism and DS including 1,934/2,081 cases/controls for MTHFR C677T polymorphism, 1,404/1,632 cases/control for MTHFR A1298C polymorphism and 859/1,132 cases/control for MTRR A66G polymorphism was carried out.

Study design: Studies were identified by searching the PubMed database for relevant articles published. Case-control studies were chosen, and odds ratio (OR) with confidence interval (CI) were used to assess the strength of association.

Results: The overall results suggested that the variant genotypes MTHFR C677T were associated with DS risk (homozygote, TT vs. CC: OR=2.991; 95% CI: 1.321-3.558; P=0.001 and co dominant model, CT vs. CC: OR=1.1616 (1.216-1.845; P=0.0001). The result of the variant genotypes MTHFR A1298C showed its association with the DS risk (homozygote, AA vs. CC: OR=1.428; 95% CI: 1.016-1.849; P=0.0067). In the stratified analysis, results obtained in variant genotype of MTHFR C677T A66G had increased risk of DS in Caucasian subjects in codominant and dominant model while the increased risk was found in dominant models for Brazilian and Asian subjects. Again, for MTHFR A1298C variant, increased risk was found in Caucasian subject in co-dominant, dominant and recessive models and in co-dominant model for Brazilian population. The results also show that in A66G variant of MTRR had increased risk of DS in both Caucasian and Brazilian subjects in dominant model.

Conclusion: This meta-analysis supports the idea that MTHFR C677T and MTHFR A1298C genotype is associated with increased risk for DS.

Keywords: Down syndrome; Folate metabolism; Gene polymorphisms; Genotype

Introduction

Down syndrome (DS) is one of the commonest disorders with huge medical and social cost. The major cause of DS is the presence of three copies of genes located on chromosome 21. This change is attributed due to abnormal segregation of during meiosis with maternal non disjunction in around 90% of the cases primarily during meiosis I in the maturing oocyte, before conception [1].

The relationships between non disjunction and abnormal folate metabolism have gained interest recent years. The abnormal folate and methyl metabolism lead to DNA hypomethylation and abnormal chromosomal segregation leading to DNA breaks and aberrant chromosome segregation [2,3].

Studies have shown that MTHFR (methylenetetrahydrofolate reductase) and MTRR (methionine synthase reductase) are the two gene identified as one of the risk factors of DS which are involved in folate metabolism [4-10].

Case-control studies that have investigated the association between DS and MTHFR C677T and A1298C and MTRR A66G polymorphisms have provided inconclusive results. This may be because each study involved small numbers of cases and controls due to which enough information was not available to demonstrate association. In order to shed some light on these inconclusive results, as well as to decrease the uncertainty in the effect size of the risk, a meta-analysis is performed in this article relating the MTHFR C677T, MTHFR A1298C and MTRR A66G gene polymorphisms with DS.

Materials and Methods

Selection criteria

Studies were selected from the PubMed published before 2014 using the suitable keywords. Case reports, editorials and review articles were excluded from this study. Case-control studies that determined the distributions of the MTHFR C677T, MTHFR A1298C and MTRR A66G genotypes in case and in a control of were eligible for inclusion in the meta-analysis.

Data extraction

The following information was extracted from each study: first author, journal, year of publication, "race" of study population, matching, genotyping method, and the number of cases and controls for the MTHFR C677T, MTHFR A1298C and MTRR A66G genotypes. In our studies, ethnicities were classified as Caucasians, Asians and Brazilians.

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Statistical analysis

The association between MTHFR C677T, A1298C and MTRR A66G gene polymorphisms and DS risk was assessed by using the co dominant (677CT vs. CC, 677TT vs. CC; 1298AC vs. AA, 1298CC vs. AA; AG vs. AA, GG vs. AA), the dominant (677CT+TT vs. CC; 1298AC+CC vs. AA; AG+GG vs. AA) and the recessive (677TT vs. CC+CT; 1298CC vs. AA+AC; GG vs. AA+AG) models.

The strength of association of the MTHFR and MTRR gene polymorphisms with DS was measured by the ORs together with the 95% CIs. The Chi-square test was first used to assess whether the distribution of genotypes among controls conformed to the Hardy-Weinberg equilibrium (HWE), with P<0.05 considered a departure from HWE.

Results

Selection of studies

In this meta-analysis, we have identified 25 studies on association

between MTHFR and MTRR polymorphism and DS including 1, 934/2,081/ cases/controls for MTHFR C677T polymorphism, 1,404/1,632/ cases/control for MTHFR A1298C polymorphism and 859 /1,132/cases/control for MTRR A66G polymorphism .For MTHFR C677T polymorphism 18 studies was considered in which 10 studies was from Caucasian population, 2 from Brazil and 4 from Asia. For MTHFR A1298C polymorphism, out of 12 studies, 7 studies were form Caucasian population and 2 and 3 from Brazil and Asia respectively. For MTRR A66G polymorphism, we have included only 7 Inc which 4 from Caucasian population, 2 from Brazil and only 1 from Asia (Table 1 and 2).

Meta analysis

The meta-analysis of the 18 populations demonstrated that MTHFR C677T was associated with increased DS risk under the homozygote (TT vs. CC: OR=2.991; 95% CI: 1.321-3.558; P=0.001) (Table 3) and co -dominant model (CT vs. CC: OR=1.1616 (1.216-1.845; P=0.0001) (Table 3).

Ethnicity	Country	Author	Year	N	Cases/Controls for C677T			N	Cases/Controls for A1298C			Ref
					CC	CT	TT		AA	AC	CC	
Caucasian	USA	Hobbs et al	2000	157/140	51/67	84/59	22/14					14
	Italy	Stuppia et al.	2002	64/112	20/27	32/62	12/23	-	-	-	-	24
	Ireland	O'Leary	2002	192/41	18/90	21/84	2/18					25
	Turkey	Bodurologu	2004	152/91	86/58	55/30	11/3	152/91	44/21	97/10	11/0	26
	Italy	Scala et al.	2006	94/264	31/74	39/125	24/57	94/264	38/128	38/108	17/25	27
	Egypt	Meguid et al.	2008	42/48	20/33	17/12	5/3	42/48	8/18	20/29	14/1	28
	Italy	Coppedde et al.	2009	94/113	25/40	52/55	17/18	69/101	37/46	29/48	6/7	8
	Croatia	Vranekovic et al.	2010	111/141	49/66	49/64	13/11	111/141	48/63	56/68	7/10	-
	Jordan	Sadiq et al.	2011	53/29	23/23	27/5	3/1	53/29	24/10	29/18	0/1	30
	Croatia	Bozovic et al.	2011	112/221	46/101	55/97	11/23	112/221	55/101	52/98	5/22	29
Brazilian	Brazil	Brandalize et al.	2009	239/197	94/86	113/93	32/18	239/197	143/113	84/76	12/8	31
	Brazil	Santos et al.	2008	103/108	51/49	43/47	9/12	103/108	58/57	40/49	5/2	32
Asian	India	Rai et al.	2006	149/165	97/124	40/39	12/2	89/70	28/28	39/37	22/5	33
	India	Kohli et al.	2008	103/109	74/71	29/32	6/0					34
	India	Cyril et al.	2009	36/60	33/60	3/0	0	36/60	14/26	19/21	3/13	35
	Present study	Ambreen et al.	2014	233/242	183/169	40/44	10/29	304/302	216/212	71/75	17/15	-

Table 1: Characteristics of studies included in the meta- analysis for MTHFR gene polymorphisms C667T and A1298°C [14-23,26-30].

Ethnicity	Country	Author	Year	N	Cases/Controls for MTRR A66G			Ref
					AA	AG	GG	
Caucasian	USA	Hobbs et al.	2000	145/139	26/39	64/68	55/32	14
	Ireland	O'Leary et al.	2002	48/192	1/35	23/101	24/56	25
	USA	Chango et al.	2005	119/120	5/6	66/72	48/42	36
	Italy	Scala et al.	2006	94/264	28/69	46/131	19/57	27
Brazilian	Brazil	Da Silva et al.	2006	154/158	37/45	92/87	25/26	37
	Brazil	Brandalize et al.	2010	239/197	42/42	137/111	60/44	31
Asian	India	Ambreen et al	2014	60/62	2/1	48/59	10/2	

Table 2: Characteristics of studies included in the meta- analysis for MTRR A66G gene polymorphism [14,16,21,24-26,30].

C677T	TT vs. CC		CT vs. CC		TT vs. CC+CT		TT+CT vs. CC	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Total	2.991 (1.321,3.558)	0.0001	1.616 (1.216,1.845)	0.0001	0.6235 (0.5671,0.7474)	<0.0001	0.7528 (0.8115,0.8503)	0.0001
Caucasian	1.709 (1.083 ,1.209)	<0.0001	0.6506 (0.7610,08841)	<0.0001	2.084 (1.084,2.574)	<0.0001	1.167 (1.003,1.354)	0.0409
Brazilian	0.3419 (0.8648,1.489)	0.1365	0.3419 (0.7404)	0.0002	0.4171 (0.9009)	0.2282	2.925 (1.305,6.031)	0.0029
Asian	0.6790 (0.8198,1.004)	0.0557	0.6298 (0.7404,0.915)	0.0002	0.8249 (0.9442,1.211)	0.3249	1.563 (1.120,1.970)	0.0001

Table 3: ORs and 95% CI for Down syndrome and the MTHFR C677T polymorphism under different genetic models.

The meta-analysis of the 12 populations demonstrated that MTHFR A1298C was associated with increased DS risk under the homozygote (AA vs. CC: OR=1.428; 95% CI: 1.016-1.849; P=0.0067) (Table 4). However, MTRR A66G was not associated with significantly increased risk of DS.

The association of MTHFR (C667T and A1298C polymorphism) and MTRR A66G polymorphism with DS was further stratified by ethnicity. For MTHFR C677T, the positive association was driven by Caucasian codominate model (TT vs. CC: OR=1.709 (1.083, 1.209; P=<0.0001) and in Caucasian dominant and recessive model (TT+CT vs. CC: OR=1.167 (1.003-1.354, P=0.0409 and TT vs. CC+CT: OR= 2.0841(1.084-2.574; P=<0.0001 respectively). The positive association was also driven by Brazilian dominant model (TT+CT vs. CC: OR=2.925 (1.305-6.031; P=0.0029) and also in Asian dominant model

(TT+CT vs. CC: OR=1.563 (1.120-1.970; P=0.001) (Table 3).

For MTHFR A1298C polymorphism, the positive association was observed in Caucasian co dominant model (AA vs. CC: 1.583 (0.9264-1.560; P=0.3759). Similarly, the positive association was also observed in Caucasian and Brazilian codominant model AC vs. AA (OR=1.280 (1.015-1.591; P=0.0257) and OR= 1.192 (0.942-1.644; P=0.2845). The positive association was also driven by Caucasian population in recessive model (CC vs. AA+AC: OR=1.803 (1.03, 2.509; P=0.0004) (Table 4).

For MTRR A66G polymorphism, the association was observed in both Caucasian and Brazilian population of dominant model (AG+GG vs. AA: (OR=1.533(1.021-2.129; P=0.014”) and (OR= 1.1417(0.999-2.006; P=0.0487) respectively) (Table 5) (Figures 1-3).

A1298C	AA vs. CC		AC vs. AA		CC vs. AA+AC		AC+CC vs. AA	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Total	1.428 (1.016,1.849)	0.0067	0.9649 (0.9208,1.122)	0.6432	0.6894 (0.5686,0.8849)	0.6481	1.039 (0.9496,1.198)	0.6012
Caucasian	1.583 (0.9264,1.560)	0.3759	1.280 (1.015,1.591)	0.0257	1.803 (1.033,2.509)	0.0004	1.111 (0.9591,1.138)	0.3185
Brazilian	0.6955 (0.9264,1.560)	0.3759	1.192 (0.942,1.644)	0.2845	0.6481 (0.9519,1.455)	0.2827	1.025 (0.8575,1.350)	0.8615
Asian	0.8891 (0.9304,1.470)	0.6464	1.00 (0.9058,1.345)	1.00	0.8891 (0.9519,1.455)	0.6399	0.8834 (0.7791,1.2071)	0.4360

Table 4: ORs and 95% CI for Down syndrome and the MTHFR A1298C polymorphism under different genetic models.

A66G	AA vs. GG		AG vs. AA		GG vs. AA+AG		AG+GG vs. AA	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Total	0.5801 (0.6271,0.7640)	<0.0001	0.7862 (0.6967,0.995)	0.0493	0.6947 (0.8680,0.8548)	0.0006	0.7169 (0.6323,0.9031)	0.0046
Caucasian	0.6852 (0.778,0.9655)	0.0073	0.328 (0.987,1.87)	0.1067	0.6367 (0.7979,0.8281)	0.0007	1.533 (1.021,2.129)	0.0104
Brazilian	0.4914 (0.6022,0.7868)	0.0029	0.7851 (0.6488,1.124)	0.1863	0.5849 (0.8507,0.8667)	0.0071	1.417 (0.999,2.006)	0.0487
Asian	0.40 (0.0649,6.853)	0.5183	0.7851 (0.6488,1.124)	0.1863	0.1667 (0.762,0.7965)	0.0127	0.4754 (0.9283,5.388)	0.539

Table 5: ORs and 95% CI for Down syndrome and the MTRR A66G polymorphism under different genetic models.

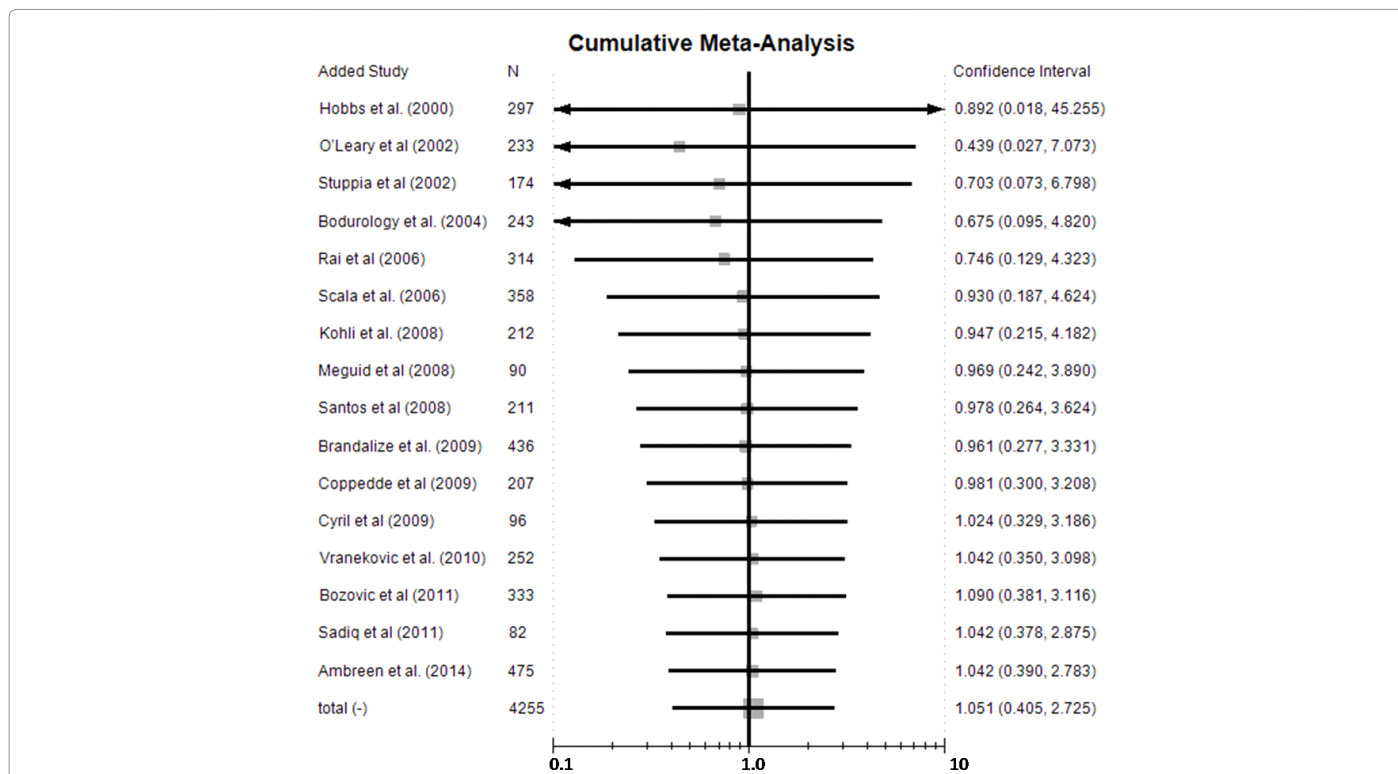


Figure 1: Forest plot of ORs showing TT+CT when compared to the CC genotype. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight [14-23,26-30].

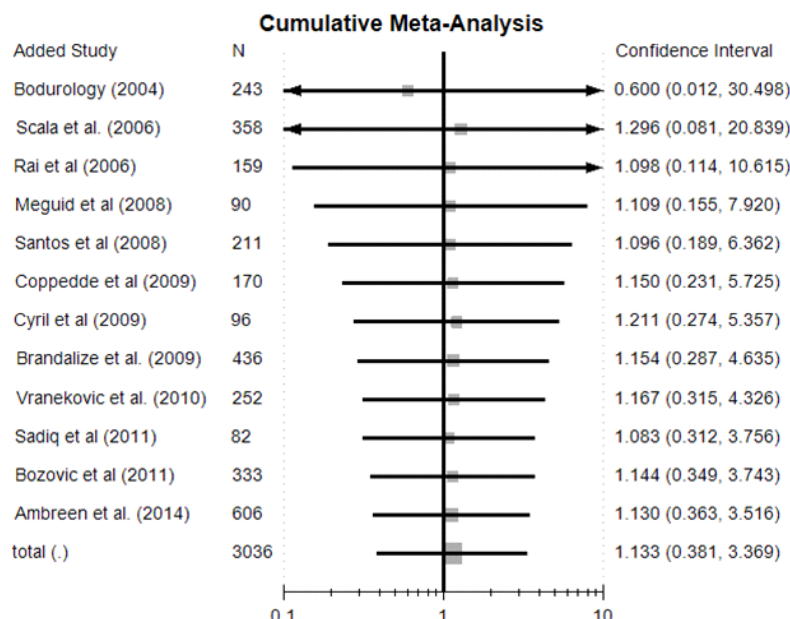


Figure 2: Forest plot of ORs showing AA+AC when compared to the CC genotype. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight [15-23,28-30].

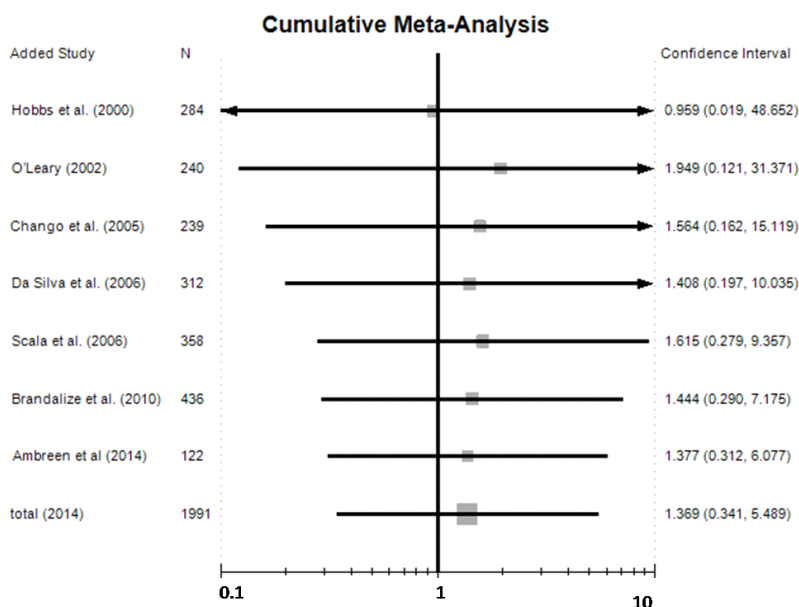


Figure 3: Forest plot of ORs showing AA+AG when compared to the GG genotype. The squares and horizontal lines correspond to the study-specific OR and 95% CI. The area of the squares reflects the study-specific weight [14,16,21,24-26,30].

Discussions

Folate is an important vitamin that contributes to cell division and growth and is therefore of particular importance during infancy and pregnancy. Folate deficiency during conception and early pregnancy has been associated with slow growth, anemia, weight loss, digestive disorders, and neural tube defects. It has been suggested that certain polymorphism present on folate metabolizing genes can increase a risk of conceive baby with DS.

James et al in 1999 have reported a folate metabolizing gene,

MTHFR as one of the risk factor for DS babies and have also suggested that a significant increase in plasma homocysteine levels exists in mothers of DS babies [9,11].

MTHFR gene contains 11 exons, located on chromosome 1p36.3 which encodes for an enzyme methylenetetrahydrofolate reductase (MTHFR), a key enzyme in folate and homocysteine metabolism. MTHFR is composed of an N-terminal catalytic and a C-terminal regulatory domain [12]. It catalyzes the biologically irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group for the remethylation of homocysteine

to methionine [13]. Several single nucleotide polymorphisms are associated with MTHFR including C677T and A1298C which can affect folate and total homocysteine status. The C677T lies at the catalytic domain and the A1298C position is at the regulatory domain.

The MTHFR C677T, which involves a cytosine (C) to thymine (T) substitution at position 677 which changes amino acid from alanine to valine in the enzyme. The C677T increases thermolability of MTHFR and causes impaired folate binding and reduced activity of the MTHFR enzyme [14]. The MTHFR A1298C is due to adenine(A) to cytosine (C) transversion at nucleotide 1298, which produces a glutamate to alanine substitution. The A1298C polymorphism in the MTHFR gene has also been associated with decreased enzyme activity [4,5].

MTRR gene is located at 5p15.31 which maintains the methionine synthase enzyme at an active stage for the remethylation of homocysteine to methionine [6]. Methionine synthase reductase regenerates a functional methionine synthase via reductive methylation. Single nucleotide polymorphism in MTRR gene like A66G is found to affect the homocystein levels in humans [7,8]. The A66G polymorphism in the MTRR gene causes the substitution of isoleucine with the methionine at codon 22 [9]. This polymorphism might be a genetic risk factor for DS since the methionine synthase reaction is important in maintaining normal folate metabolism and DNA methylation.

The meta-analysis examined the MTHFR and MTRR gene polymorphism and their relationship to risk of DS. The frequency of 677T and 1298C allele was found to be significantly higher among DS children than the other groups indicating that MTHFR C677T and MTHFR A1298C polymorphism in DS children would be expected to play an important role in bringing about DS. Some studies reported significantly increased prevalence of MTHFR C677T polymorphism as important risk factors for DS babies while in contrast some studies had also reported insignificant association [15-18]. For MTHFR A1298C polymorphism, much more contradictory reports have been presented [16,17,19-25]. Differences in ethnicity may be one reason for controversy and high intake of food folate may neutralize the metabolic impact of MTHFR polymorphism [26].

The T and C allele variant of MTHFR C677T and A1298C respectively may increase the thermolability of MTHFR gene thereby leading to production of abrupt homocysteine levels causing hypomethylation. Hypomethylation leads to abnormal chromosomal segregation which in turn leads to the risk of elevated trisomic fetuses. However this mechanism of abrupt homocysteine causing DNA methylation needs further elucidation and other risk factors must be studied properly on a larger case-control study which is the main limitations of the study.

The pooled results indicate the homozygous and heterozygous variant of MTHFR C677T polymorphism exerted a risk on DS development (OR=2.991, 95% CI: 1.321-3.558 and OR=1.616, 95% CI: 1.216-1.845, respectively). However, when the analysis was performed by ethnicity, this was not observed in all subgroups except for Caucasian population (OR=1.709; 95% CI: 1.083-1.209). Similarly, when the study was stratified by ethnicity for dominant model, all three sub group showed increased risk of DS but these effect were reduced overall population of DS (OR=0.7528, 95% CI=0.8115-0.8503).

Again, the pooled results of MTHFR A1298C polymorphism of homozygous variant exerted a risk on DS development (OR=1.428, 95% CI: 1.016-1.849). However, when the analysis was performed by ethnicity, this was not observed in either of the subgroups.

In MTRR A66G polymorphism, reduced risk was observed in all

variant but when this risk was stratified in dominant variant, the risk was observed in both Caucasian and Brazilian subgroups (AG+GG vs. AA; OR=1.555, 95% CI: 1.021-2.219 and OR=1.417, 95% CI: 0.999-2.006, respectively) yet this did not produce any overall effect on dominant model. Further well designed large scale case-control studies might be required to validate the association in different population having the risk of DS.

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