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Association of Vitamin D Receptor Polymorphisms and Type 1 Diabetes Susceptibility in children: A Meta-Analysis

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Abstract

Background: There have been studies focused on *FokI*, *Bsm*I, *Apa*I and *Taq*I polymorphisms of the vitamin D receptor(VDR) gene and susceptibility to type 1 diabetes mellitus with controversial results.

Methods: This present study is a meta-analysis investigating association between FokI, ApaI, TaqI and BsmI polymorphisms of VDR gene and type 1 DM in children. A literature search was performed using Medline, EMBASE, Cochrane and Pubmed. Any study was considered eligible for inclusion if at least one of *Fok*I, *Apa*I, *Taq*I and *Bsm*I polymorphisms was determined, outcome was type I DM at pediatric age.

Results: A total of 9 studies comprising 1053 patients and 1017 controls met the study inclusion criteria. The pooled odds ratios (ORs) of the *Fok*I, *Apa*I, *Taq*I and *Bsm*I polymorphisms were combined and calculated. Forest plots and funnel plots of the OR value distributions were drawn. Our meta-analysis has demonstrated statistically significant associations between DM1 and VDR genotypes, *Bsm*IBB($P < 0.05$), *Bsm*IBb, ($P < 0.05$), *Bsm*Ibb($P < 0.05$), *Taq*ITT($P< 0.05$) and *Taq*Itt ($P < 0.05$) in children.

Conclusion: The results indicated that *Bsm*IBB, *Bsm*IBb and *Taq*Itt polymorphisms were associated with an increased risk of type 1DM, while *Bsm*Ibb and *Taq*ITT had protective effect for type1 DM in children.

Keywords:VDR, diabetes mellitus, polymorphisms, meta-analysis

Introduction

Type I Diabetes (DM1) is a complex disease characterized by the autoimmune destruction of pancreatic β cells. Vitamin D is an immune regulatory hormone that exerts its effects through highly polymorphic VDR that belongs to steroid-receptor superfamily and it is expressed in many cell types such as lymphocytes and antigen presenting cells (APCs) (1). During last decade VDR gene polymorphisms have been shown to be associated with autoimmune pathologies (2). Vitamin D seems to downregulate type 1 helper (Th-1) cells, by decreasing their proliferation and inhibiting production of cytokines such as IL-2, TNF-α and interferon-γ (3,4). For many years the strongest genetic contribution to DM1 susceptibility had been attributed to the presence of human leukocyte antigen region (HLA) on chromosome 6 (5,6). Recently, single nucleotide polymorphisms (SNPs) in the VDR gene have been investigated namely *Fok*I *F>f* (rs10735810), *Bsm*I *B>b* (rs1544410), *Apa*I *A>a*

(rs7975232), and *Taq*I t *T>t* (rs731236). Allele F of the *Fok*I SNP creates an alternative ATG initiation codon in exon 2 leading to a three amino acid longer VDR protein. The *Apa*I, *Bsm*I and *Taq*I polymorphisms take place near the 3' end of the VDR gene; *Bsm*I and *Apa*I SNPs are located in intron 8, and the *Taq*I is a silent SNP in exon 9. Several studies with small data sets that suggested an association between these SNPs and type 1DM had inconsistent results (7-10). These inconclusive results had been attributed to ethnic diversities among populations or due to the environmental factors involved in type 1 DM pathogenesis. *Bsm*I is strongly linked with 3 poly(A) microsatellite repeat in the 3'untranslated region which may influence VDR mRNA stability (11). *Bsm*I, *Apa*I and *Taq*I polymorphisms were also designated as polymorphisms without functional effects in several studies (12,13). There are some discrepancies in the literature with small data sets, regarding the relative importance of polymorphisms of VDR genes *Apa*I,*Bsm*I, *Fok*I and *Taq*I. In our meta-analysis we included a total of 9 studies comprising 1053 patients and 1017 controls that indicated statistically significant associations between DM1 and VDR genotypes, *Bsm*IBB($P < 0.05$), *Bsm*IBb, ($P < 0.05$), *Bsm*Ibb($P < 0.05$), *Taq*ITT($P< 0.05$) and *Taq*Itt ($P < 0.05$) in children. This present meta-analysis involves a large data set to investigate the associations between type1 DM and VDR gene polymorphisms *Apa*I, *Bsm*I, *Fok*I and *Taq*I, acordingly demonstrate more reliable statistical results to rule out genotype- phenotype correlation of type 1 DM in children.

Methods

Search strategy criteria.

For meta-analysis, all published studies evaluating associations between type 1 DM and *Fok* I, *Apa* I, *Taq* I and *Bsm* I polymorphisms that are investigated in patients diagnosed as DM1 at pediatric age are included. A literature search for the MeSH terms ‘’type 1 Diabetes mellitus ’’ or ‘’DM 1’’ was performed by O.A , D.G and M.S. Medline, Cochrane and Pubmed abstracts were reviewed for relevance. No language and date of study restriction were applied to search strategy. Search to include the eligible studies ended on 05,14,2016. Any study was considered to be eligible for inclusion if it met the following criteria: 1)the publication was an association study of thr case control type, 2) at least one of the FokI, ApaI, TaqI and BsmI polymorphism was determined, 3) the outcome was DM in children and 3) there was at least one unrelated control group.

Data extraction.

Study selection and data extraction were performed independently by three authors (O.A, D.G and M.S) based on a customized database for extraction. For each study, the following information was collected: first author, year and location of the study, average age at the time of diagnosis, ethnicity, number of participants, number of cases and controls, and number of the genotypes in cases and controls. The disagreements were resolved between the reviewers by consensus. For quality assessment six domains were assessed. Those were representativeness of classes, representativeness of the controls, ascertainment of DM1, genotypic examination and association of assessment. The primary outcome considered in the meta-analysis was the association between DM1 and the presence of FokI, *Apa*I, *Taq*I or *Bsm*I polymorphism at pediatric age. For the primary analysis and to allow appropriate

comparison of all studies, cases and controls were classified based on *Fok*I, *Apa*I, *Taq*I and *Bsm*I genotypes.

Statistical analysis.

The odds ratios (OR) with 95% confidence intervals, representativeness of controls, ascertainment of DM1, ascertainment of controls, genotypic examination and association assessments were done. The primary outcome considered in the meta-analysis was the association between DM1 and the presence of FokI, ApaI, TaqI or BsmI polymorphisms. MedCalc Software Acacialaan 22, 8400 (Ostend, Belgium) was used to perform meta-analysis. The odds ratios (OR) of the genetic polymorphisms were combined and calculated, and the funnel plots were drawn. All of the four studied SNPs (*FokI, ApaI, TaqI* and *BsmI*) were diallelic, we calculated summary odds ratios incorporating both within and between study variation using a random effects model proposed by DerSimonian and Laird (14).

Results

Our search yielded a total of 50 references. After screening the titles and abstracts, 41 studies were excluded because they were not considered relevant to the study topic, leaving 9 potentially eligible studies (Fig.1) (15-23).

Figure 1. PRISMA Flow Diagram for identification and selection of studies.

Table 1. Characteristics features of studies included in the meta-analysis of *Apa*I, *Bsm* I, *Fok* I, *Taq* I polymorphisms in the vitamin D receptor gene.

In the 9 published papers included in the meta-analysis, *Apa*I, *Bsm*I, *Fok*I, and *Taq*I polymorphisms were investigated in pediatric population as case control studies (Table 1).

Eight studies on the ApaI-Type 1 diabetes association recruited 921 cases/patients and 1033 controls while seven studies on the BsmI polymorphism recruited 866 cases and 983 controls. For the Fok I polymorphism, five studies included 465 cases and 569 controls, while eight studies on the TagI polymorphism included 921 cases and 1033 controls. Individual and pooled odds ratio estimates of four single nucleotide polymorphisims in the vitamin receptor gene, p values testing Hardy-Weinberg proportion, test for heterogeneity (Tables 2-5) and funnels plots (Figures 2-4), are documented for BsmI, and TaqI, respectively.

Of the articles included in the study, investigators of all studies included in the meta-analysis spesifically looked for presence of autoantibodies to diagnose type 1 diabetes and all fulfilled World Health Organization and the American Diabetes Association criteria (24).

Selection of controls varied accross studies. Groups of controlls included healthy blood donors and unrelated children.

ApaI,BsmI, FokI and TaqI polymorphisms and the risk for type 1 diabetes

For ApaI-AA, the odds ratio ranged from 0.476 to 2.051 (Table 2a). The random-effects model yielded a pooled odds ratio of 1.081 (95 percent confidence interval (CI): 0.755, 1.547). There was indication of heterogeneitiy (p=0.0221).

For ApaI-Aa, the odds ratio ranged from 0.435 to 1.517 (Table 2b). The random-effects model yielded a pooled odds ratio of 0.866 (95 percent confidence interval (CI): 0.664, 1.131). There was indication of heterogeneity (p=0.0469).

For ApaI-aa, the odds ratio ranged from 0.641 to 2.647 (Table 2c). The fixed- effects model yielded a pooled odds ratio of 0.956 (95 percent confidence interval (CI): 0.772, 1.182). There was indication of homogeneity (p=0.1280). In view of these estimates, there is no evidence that any of the three allels alone is associated with type 1 diabetes.

For BsmI-BB, the odds ratio ranged from 0.460 to 6.458(Table 3a). The fixed- effects model yielded a pooled odds ratio of 1.397 (95 percent confidence interval (CI): 1.034, 1.888, p=0.030). There was indication of homogeneity (p=0.4531).

For BsmI-Bb, the odds ratio ranged from 0.598 to 5.210 (Table 3b). The random-effects model yielded a pooled odds ratio of 1.534 (95 percent confidence interval (CI): 1.001, 2.350, p=0.049). There was indication of heterogeneitiy (p=0.0014).

For BsmI-bb, the odds ratio ranged from 0.242 to 1.407 (Table 3c). The random-effects model yielded a pooled odds ratio of 0.624 (95 percent confidence interval (CI): 0.418, 0.933, p=0.022). There was indication of hetorogeneity (p=0.0075).

Forest plots are shown in figures 2a-c for BsmI BB, Bb and bb alleles respectively. Individual and pooled odds ratio estimates for the BsmI allels are represented as squares and diamonds. In view of these estimates, there is evidence that BsmI-Bb or BsmI-bb alone is associated with type 1 diabetes.

For FokI-FF, the odds ratio ranged from 0.485 to 1.636 (Table 4a). The fixed- effects model yielded a pooled odds ratio of 1.057 (95 percent confidence interval (CI): 0.817, 1.367, p=0.675). There was indication of homogeneity (p=0.1443).

For FokI-Ff, the odds ratio ranged from 0.645 to 1.222 (Table 4b). The fixed- effects model yielded a pooled odds ratio of 0.724 (95 percent confidence interval (CI): 0.564, 0.929, p=0.011). There was indication of homogeneity (p=0.4324).

For FokI-ff the odds ratio ranged from 0.128 to 2.558 (Table 4c). The random- effects model yielded a pooled odds ratio of 1.159 (95 percent confidence interval (CI): 0.573, 2.344, p=0.681). There was indication of heterogeneity (p=0.0384).

For TagI-TT, the odds ratio ranged from 0.203 to 1.181 (Table 5a). The random-effects model yielded a pooled odds ratio of 0.644 (95 percent confidence interval (CI): 0.440, 0.942, p=0.023). There was indication of heterogeneity (p=0.0044).

For TaqI-Tt the odds ratio ranged from 0.580 to 2.983 (Table 5b). The random-effects model yielded a pooled odds ratio of 1.062 (95 percent confidence interval (CI): 0.785, 1.438, p=0.697). There was some indication of heterogeneity (p=0.0536).

For TaqI-tt the odds ratio ranged from 0.524 to 3.586 (Table 5c). The fixed- effects model yielded a pooled odds ratio of 1.655 (95 percent confidence interval (CI): 1.677, 2.295, p=0.001). There was indication of heterogeneity (p=0.3261).

Forest plots are shown in figures 3a-b for TaqI TT and tt alleles respectively. Individual and pooled odds ratio estimates for the TaqI allels are represented as squares and diamonds. In view of these estimates, there is evidence that TaqI-TT and TaqI-tt alone is associated with type 1 diabetes.

Discussion

There are a number of reports on *Fok*I, *Apa*I, *Taq*I and *Bsm*I polymorphisms of the VDR gene in diabetic patients, there have not been conclusive evidence that any of these polymorphisms has causative association with type 1 DM in children. In a 2006 meta-analysis that focused on VDR polymorphisms, FokI, ApaI, TaqI, BsmI and type 1 DM association included mainly adult samples and did not reveal any spesific association (25). However out of 19 published papers included in this meta-analysis, authors of only five papers spesifically looked for the presence of autoantibodies to distinguish type 1 diabetes from type 2. In other 14 studies included in this meta–analysis, investigators used only one criteria (e.g., ketosis, early requirement of insulin). This may be one of the main reasons for different statistical results other than ethnic diversities when compared to our results. In our opinion, DM1 is mainly a disease of pediatric age and considering the qualitative assessment of study inclusion criteria, choosing studies where diagnosis is at pediatric age with age matching control samples, and/or taking American Diabetes Association criteria would end up with more reliable meta-analysis results. In our study, mean age of control samples are in pediatric range. In another meta-analysis involving Chinese adult samples, authors concluded that BsmI polymorphisms in the VDR region would increase the risk of type 1 DM in East Asians (26). In the study of Zhang J, Asian Samples with BsmI polymorphism was found to have a significant association with increased risk of type 1 DM (27). The study of Qin WH, demonstrated a significant relationship among BsmI B allele and BB genotype and increased risk for type 1 DM in Asians while this study included Latino and African adult samples and authors also found another spesific association with BsmIbb genotype and type 1DM in overall populations (28). The novel finding in our study was the presence of an increased risk of type 1 DM in carriers of *Bsm*IBB, *Bsm*IBb and *Taq*Itt polymorphisms and decreased risk of type

1DM in children with *Bsm*Ibb and *Taq*ITT polymorphisms.There are GWAS studies that widen our approach to vitamin D receptor polymorphisms and DM1.

Meta-analysis may be more reliable when evaluating genotype frequencies in certain diseases because in a way it may reduce the effect of biased sampling or nonrandom mating in individual study population. Results of studies so far, regarding VDR polymorphisms and DM1 susceptibility are conflicting. In the study of Garcia et al, an association was found between BsmI polymorphism and DM1 (15). The frequency of genotype bb was found significantly lower in the cases than controls. Among five prevalent haplotypes BAT has been found statistically more frequent in study group in the same study. Among genotype combinations AabbTT was found higher in controls. In our study population genotype combination frequencies were not assessed because of unavailable data and this may be one of the limitations of our study.

In the study of San-Pedro JI , an association of an haplotype ‘’fBAt’’ and risk of type 1 DM in Basque population has been identified (16). In the study of Skrabic V, BBAAtt genotype combination was found to be associated with type 1DM in Dalmatian population of southern Croatia, with the ‘’tt’’ genotype preferentially presented in the affected individuals (17). This was also noticed in previous studies that focused on association of VDR gene polymorphisms with increased susceptibility to T1DM in Taiwanese, Japanese, South Asian (Indian) and German populations (29-32). *Taq*I polymorphism among type 1 DM patients and control subjects differed significantly, with the VDR tt genotype occuring more frequently in T1DM patients. No difference was noticed in the genotype frequencies of BsmI and ApaI polymorphisms in cases and controls. We evaluated *Taq*Itt polymorphism frequency and demostrated a significant increase in diabetic children in our study. In the study of Zemunik et al, some evidence of associtionof Tru91 –BsmI haplotype and type 1

DM in population of South Croatia was found (17). In our meta-analysis we have included two studies from Croatia. One of the limitations of this sudy was its sample size was small, it only included nine studies and the power of this study is not high. In the study of Panierakis et al, homogenious southern European population with low incidence of type 1 DM was included in the study group and they found an association of T1DM and *Fok*I, B*sm*I, *Apa*I and *Taq*I polymorphisms. In this study, *Fok*IFF genotype and F allele and *Bsm*IBB genotype and B allele were less frequent in individuals with T1DM (21). In the same study *Apa*IAA genotype and A allele, as well as *Taq*I TT genotype and T allele were more frequent in individuals. Greear et al, also studied the association of *Taq*1, *Fok*1, *Apa*1 and type1 DM and found no significant difference in distribution of VDR polymorphisms in diabetic patients while diabetic patients had significantly decreased levels of vitamin D levels than healthy controls (22). In the study of Cheon CK et al, the frequency of bb and TT genotype have been found significantly increased among carriers demonstrating a protective effect in Korean subjects (23). Gyorffy et al, suggested a strong linkage disequilibrium between the ‘’b’’ and ‘’a’’ alleles in his study. The close loci of these polymorphisms might be an explanation for the stability of linkage, but the background of these combinations need further investigation (19). In the same study, a strong association have been found between carrier state of the ‘b’+’a’+’u’ alleles and presence of type 1 DM in females. There are other reports as well that point out to a gender spesific association and consequence of gene polymorphism (33). A number of studies have shown that patients with 1DM have low levels of vitamin D although other studies have conflicting results (34, 35, 36). In 2010, GWAS study in approximately 30 000 individuals from European descent identifies variants at four loci that were associated with 25(OH)D levels: GC rs2282679, dhcr7 RS 12785878,

CYP2R1(37). A second GWAS of 25(OH)D levels confirmed the findings with GC, DHCR7, and CYP2R1 (38). These variants are located within or near genes involved in vitamin D transport (GC), cholesterol synthesis (DHCR7),and hydroxilation (CYP2R1 and CTP24A1) (37). Cooper et al recently tested genetic variants influencing 25(OH)D metabolism for an association with both circulating 25(OH)D metabolism for an association with both circulating 25(OH)D concentrations and T1D. They replicated the associations found in the GWAS of the four vitamin D metabolism genes (GC,DHCR7, CYP2R1, and CYP24A1) with 25(OH)D in control subjects and found that CYP27B1, DHCR7, and CYP2R1 were associated with type 1 diabetes(39). The Fok1 polymorphism of the VDR, which increases the transcriptional activity of VDR, has been suggested as an influencing factor for susceptiblity to T1DM. It affects insulin secretion and sensitivity and has been found to be a susceptibility factor for development of diabetic retinopathy (40, 41). Also vitamin D binding protein gene polymorphisms were found to be associated with diabetes-associated antibody insulinoma antigen 2 and with T1DM (42). In the study of Greear et al, low vitamin D levels in the diabetic children have been attributed to inflammatory or other pathologic processes, mainly as a consequence of the diasease rather than being a risk factor, as previously stated in DAISY study (43). In the study of Chang et al, the allele frequency of the BsmI differed between Taiwaneese patients and controls significantly (20). There are some limitations of this meta-analysis. The power of this study should further be increased by additional studies, and this meta-analysis involves only nine studies. Some of the studies contained small number of cases and background of the patients varied across included studies. However our meta-analysis employed a random effects model designed to encounter these variations and found significant effect of polymorphisms on type 1DM susceptibility.

As a conclusion our meta-analysis of accesible published data has demostrated statistically significant association between BsmIBB, BsmIBb, BsmIbb, *Taq*ITT and Taqtt polymorphisms and susceptibility to type 1DM in children however influence of vitamin D receptor gene polymorphisms on susceptibility to type 1 diabetes deserves further investigations. Meta-analysis include larger data sets and acordingly may demonstrate more reliable statistical results to rule out genotype- phenotype correlations of diseases.

Authors declare that they don't have any financial relationship or conflict of interest regarding the study.

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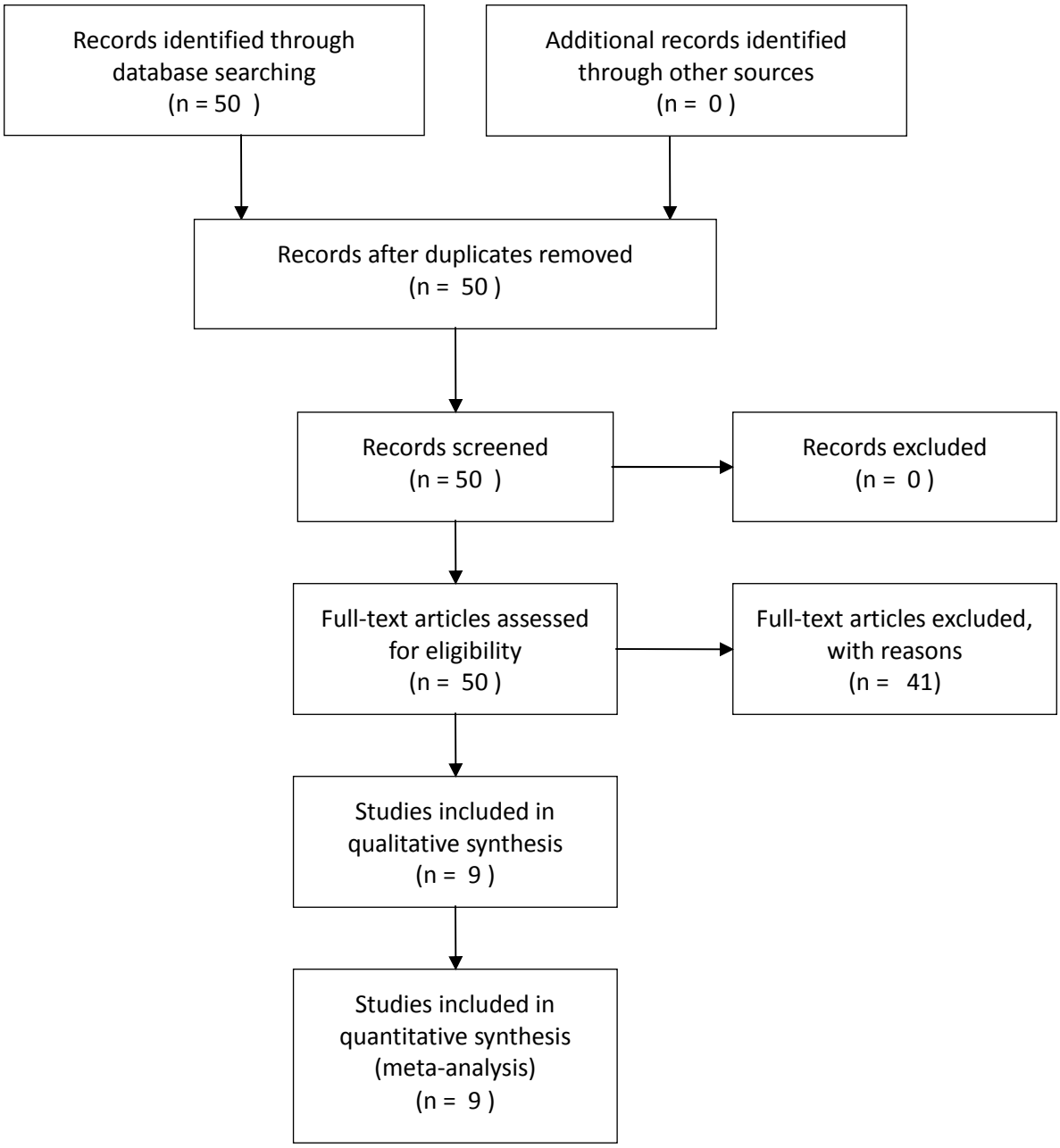
Figure 1. Flow chart for identification and selection of studies.

Figure 2a-c . Forest Plots showing individual and pooled odds ratio estimates of *BsmI BB, BsmI Bb, BsmIbb* polymorphisms respectively.

Figure 3a-b. Forest Plots showing individual and pooled odds ratios of TaqITT, and TaqItt polymorphisms respectively.

PRISMA 2009 Flow Diagram



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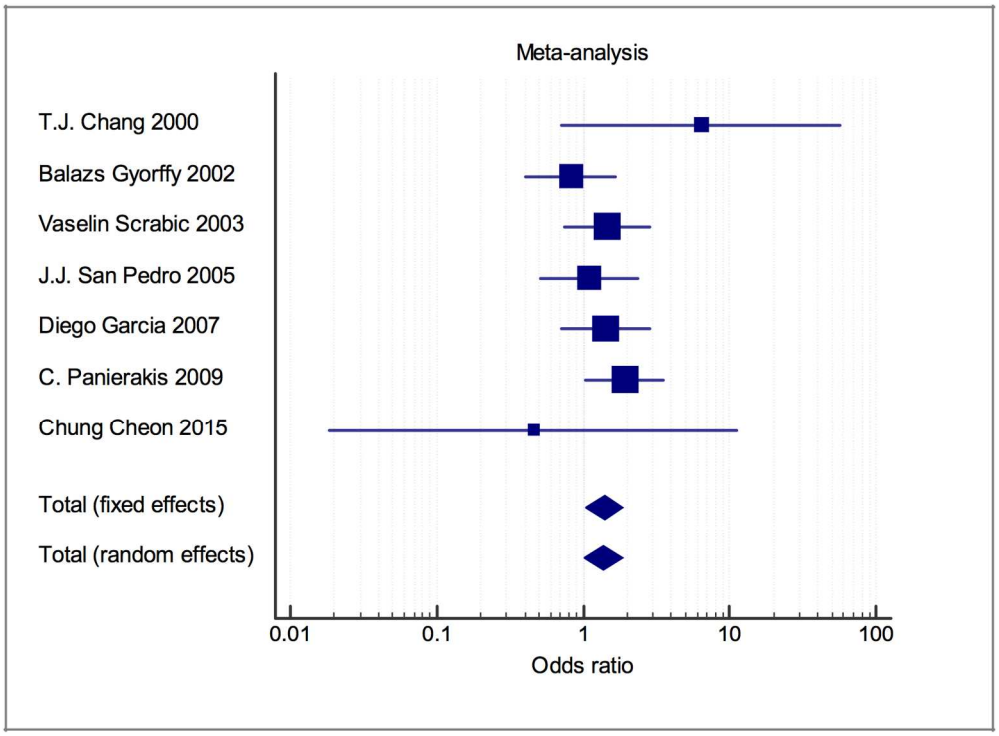
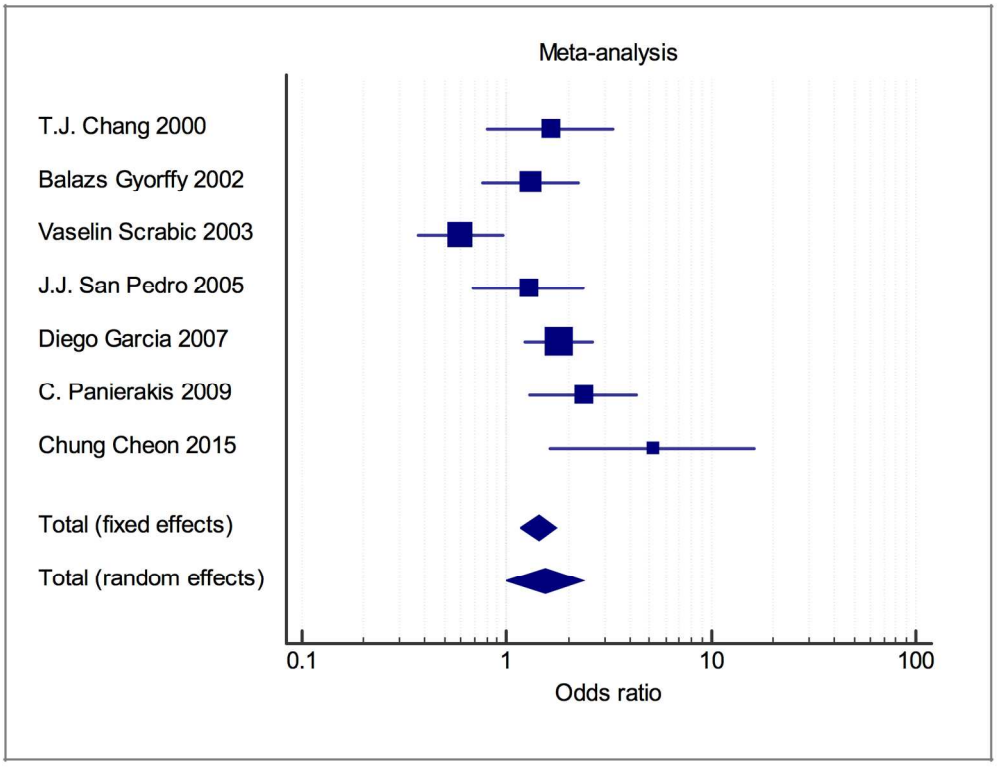


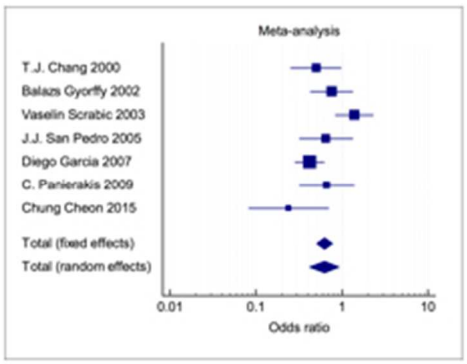
Forest plot showing individual and pooled odds ratio estimate of BsmIBB polymorphism

491x363mm (96 x 96 DPI)



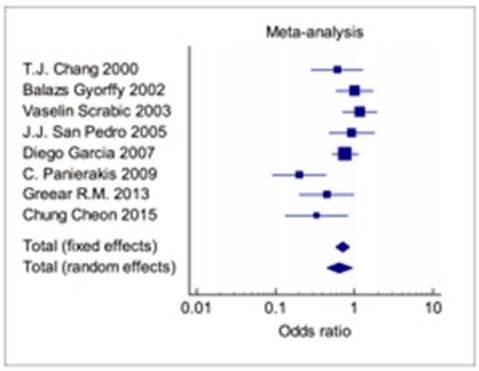
Forest plot showing individual and pooled odds ratio estimate of BsmIBb polymorphism

513x394mm (96 x 96 DPI)



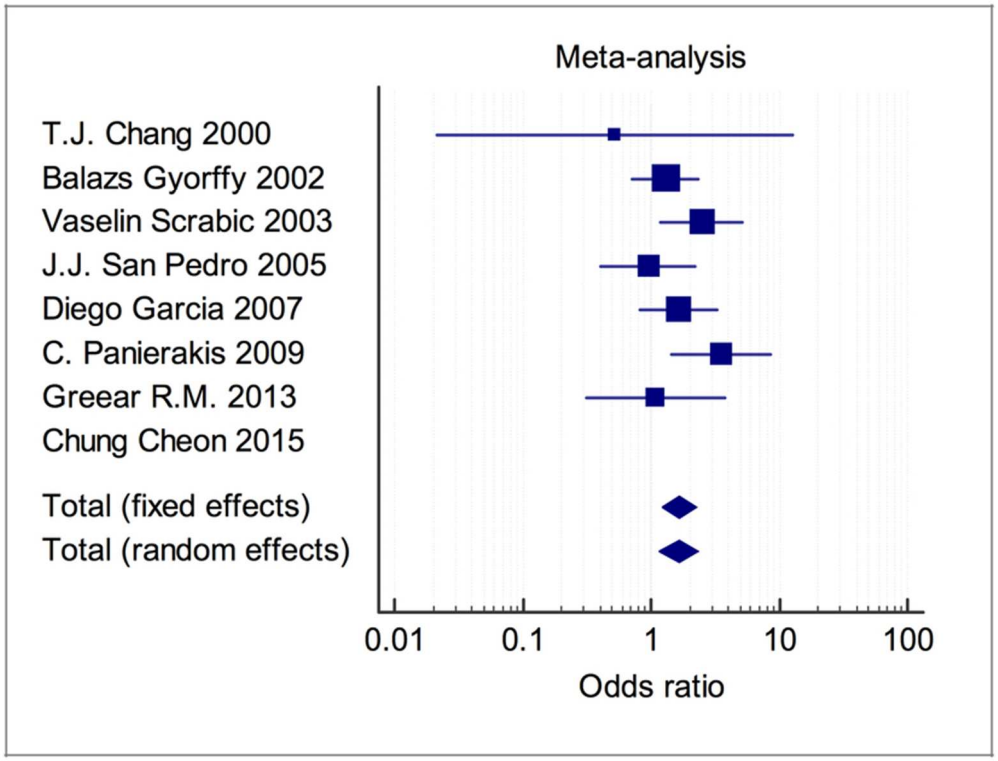
Forest plot showing individual and pooled odds ratio estimate of BsmIbb polymorphism

61x47mm (96 x 96 DPI)



Forest plot showing individual and pooled odds ratio estimate of Taq TT polymorphism

84x64mm (72 x 72 DPI)



Forest plot showing individual and pooled odds ratio estimates of Taq tt polymorphism

70x53mm (300 x 300 DPI)

Table 1. Characteristics features of studies included in the meta-analysis of *Apa*I, *Bsm* I, *Fok* I, *Taq* I polymorphisms in the vitamin D receptor gene.

FIRST AUTHOR	YEAR	REGION	MEAN AGE OF CASES/DIAGNOSIS	CASES	SOURCE OF CONTROLS	MEAN AGE OF CONTROLS	CONTROLS
Diego Garcia	2007	Santiago-Chile	9.3 +/- 4.2 years	216	unrelated children	10.3 +/- 2.5	203
J.I.San Pedro	2005	Bilbao-Spain	14.5 +/- 9.9 years	71	healty blood donors		88
Tatijana Semunik	2005	Split-Croatia	8.6 +/- 4.3 years	132	unrelated children	8.2 +/- 4.9	232
Vaselin Scrabic	2003	Split-Croatia	8.6 +/- 4.3 years	134	unrelated children	8.24 +/- 4.9	132
Balazs Gyorffy	2002	Budapest-Hungary	5.8 +/- 3.2	107	healty blood donors		103
Tien-Jyung Chang	2000	Han Chinese-Taiwan	8.8 +/- 5.6	157	healty subjects		248
Charalambos Panierakis	2009	Crete-Greece	children	100	unrelated children		96
Greear RM	2013	Brisbane-Australia	15 years<	55	healty subjects	15 years<	50
Chong-Kun Cheon	2015	Pusan-South Korea	10.28 +/- 3.23	81	healty children	9.98 +/- 3.56	113

Table 2a-c. *P* values testing Hardy-Weinberg proportion and test for heterogeneity of studies included in the meta-analysis for *ApaI* AA, ApaI Aa and ApaI aa polymorphisms respectively. (df:degree of freedom, Q:Heterogeneity in meta analysis).

Table 2a. ApaI-AA

Study	Intervention	Controls	Odds ratio	95% CI	z	P
T.J. Chang 2000	16/157	13/248	2.051	0.958 to 4.391		
Balazs Gyorffy 2002	33/107	23/103	1.551	0.835 to 2.881		
Vaselin Scrabic 2003	66/134	51/132	1.542	0.947 to 2.509		
J.J. San Pedro 2005	15/71	28/88	0.574	0.278 to 1.185		
Diego Garcia 2007	54/216	43/203	1.240	0.786 to 1.957		
C. Panierakis 2009	23/100	37/96	0.476	0.256 to 0.886		
Greear R.M. 2013	15/55	12/50	1.187	0.493 to 2.861		
Chung Cheon 2015	5/81	9/113	0.760	0.245 to 2.359		
Total (fixed effects)	227/921	216/1033	1.113	0.893 to 1.388	0.954	0.340
Total (random effects)	227/921	216/1033	1.081	0.755 to 1.547	0.425	0.671
Q	16.3471					
DF	7					
Significance level	P = 0.0221					
I^2 (inconsistency)	57.18%					
95% CI for I^2	5.93 to 80.51					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.142857 P=0.5484(low power)

Egger:bias= -1.369742 (95% CI=-6.646958 to 3.907474) P=0.5488

Harbord-Egger:bias= -1.179831(92.5% CI=-5.949539 to 3.589877) P=0.6138

Table 2b. ApaI-Aa

Study	Intervention	Controls	Odds ratio	95% CI	z	P
T.J. Chang 2000	76/157	105/248	1.278	0.855 to 1.910		
Balazs Gyorffy 2002	45/107	54/103	0.659	0.382 to 1.136		
Vaselin Scrabic 2003	52/134	66/132	0.634	0.390 to 1.032		
J.J. San Pedro 2005	37/71	43/88	1.139	0.609 to 2.129		
Diego Garcia 2007	115/216	125/203	0.710	0.481 to 1.048		
C. Panierakis 2009	58/100	57/96	0.945	0.535 to 1.669		
Greear R.M. 2013	24/55	32/50	0.435	0.198 to 0.956		
Chung Cheon 2015	32/81	34/113	1.517	0.833 to 2.765		
Total (fixed effects)	439/921	516/1033	0.873	0.729 to 1.046	-1.473	0.141
Total (random effects)	439/921	516/1033	0.866	0.664 to 1.131	-1.054	0.292
Q	14.2512					
DF	7					
Significance level	P = 0.0469					
I^2 (inconsistency)	50.88%					
95% CI for I^2	0.00 to 78.02					

Bias indicators

Begg-Mazumdar:Kendall's Tau=0 P=0.9049 (low power)

Egger:bias= -0.928241 (95% CI=-7.113964 to 5.257482) P=0.7261

Harbord-Egger:bias= -0.806491(92.5% CI=-6.321591 to 4.708608) P=0.7638

Table 2c. ApaI-aa

Study	Intervention	Controls	Odds ratio	95% CI	z	P
.J. Chang 2000	65/157	130/248	0.641	0.428 to 0.960		
Balazs Gyorffy 2002	27/107	26/103	1.000	0.536 to 1.863		
Vaselin Scrabic 2003	16/134	15/132	1.058	0.500 to 2.238		
J.J. San Pedro 2005	19/71	17/88	1.526	0.724 to 3.217		
Diego Garcia 2007	44/216	35/203	1.228	0.751 to 2.009		
C. Panierakis 2009	15/100	6/96	2.647	0.982 to 7.139		
Greear R.M. 2013	11/55	11/50	0.886	0.346 to 2.270		
Chung Cheon 2015	44/81	70/113	0.731	0.409 to 1.304		
Total (fixed effects)	241/921	310/1033	0.956	0.772 to 1.182	-0.419	0.675
Total (random effects)	241/921	310/1033	1.005	0.754 to 1.339	0.0349	0.972
Q	11.2531					
DF	7					
Significance level	P = 0.1280					
I^2 (inconsistency)	37.79%					
95% CI for I^2	0.00 to 72.53					

Bias indicators

Begg-Mazumdar:Kendall's Tau= 0.428571 P=0.1789(low power)

Egger:bias=2.766246 (95% CI=-0.351565 to 5.884058) P=0.073

Harbord-Egger:bias=2.78392(92.5% CI=-0.118976 to 5.686817) P=0.0847

Table 3a-c. *P* values testing Hardy-Weinberg proportion and test for heterogeneity of studies included in the meta-analysis for *BsmI BB, BsmI Bb, BsmIbb* polymorphisms respectively. (df:degree of freedom, Q:Heterogeneity in meta analysis).

Table 3a. BsmI-BB

Study	Intervention	Controls	Odds ratio	95% CI	z	P
T.J. Chang 2000	4/157	1/248	6.458	0.715 to 58.314		
Balazs Gyorffy 2002	17/107	19/103	0.835	0.407 to 1.713		
Vaselin Scrabic 2003	24/134	17/132	1.476	0.752 to 2.896		
J.J. San Pedro 2005	15/71	17/88	1.119	0.514 to 2.435		
Diego Garcia 2007	21/216	14/203	1.454	0.718 to 2.943		
C. Panierakis 2009	38/100	23/96	1.945	1.048 to 3.611		
Chung Cheon 2015	0/81	1/113	0.460	0.0185 to 11.440		
Total (fixed effects)	119/866	92/983	1.397	1.034 to 1.888	2.174	0.030
Total (random effects)	119/866	92/983	1.386	1.021 to 1.880	2.092	0.036
Q	5.7387					
DF	6					
Significance level	P = 0.4531					
I^2 (inconsistency)	0.00%					
95% CI for I^2	0.00 to 69.98					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.333333 P=0.2389(low power)

Egger:bias=0.081309 (95% CI=-2.562044 to 2.724662) P=0.94

Harbord-Egger:bias=0.057432(92.5% CI=-2.538737 to 2.653601) P=0.9624

Table 3b.BsmI-Bb

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Chang 2000	16/157	16/248	1.645	0.798 to 3.393		
Balazs Gyorffy 2002	53/107	44/103	1.316	0.764 to 2.268		
Vaselin Scrabic 2003	58/134	74/132	0.598	0.368 to 0.971		
J.J. San Pedro 2005	40/71	44/88	1.290	0.688 to 2.418		
Diego Garcia 2007	110/216	74/203	1.809	1.224 to 2.674		
C. Panierakis 2009	43/100	23/96	2.394	1.296 to 4.422		
Chung Cheon 2015	13/81	4/113	5.210	1.632 to 16.633		
Total (fixed effects)	333/866	279/983	1.430	1.160 to 1.762	3.347	0.001
Total (random effects)	333/866	279/983	1.534	1.001 to 2.350	1.966	0.049
Q	21.6238					
DF	6					
Significance level	P = 0.0014					
I^2 (inconsistency)	72.25%					
95% CI for I^2	40.02 to 87.16					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.333333 P=0.3813(low power)

Egger:bias=2.518064 (95% CI=-4.133965 to 9.170093) P=0.3752

Harbord-Egger:bias=2.595692(92.5% CI=-3.668787 to 8.860172) P=0.3955

Table 3c. BsmI-bb

Study	Intervention	Controls	Odds ratio	95% CI	z	P
J. Chang 2000	137/157	231/248	0.504	0.255 to 0.995		
Balazs Gyorffy 2002	35/107	40/103	0.766	0.435 to 1.348		
Vaselin Scrabic 2003	52/134	41/132	1.407	0.848 to 2.336		
J.J. San Pedro 2005	16/71	27/88	0.657	0.321 to 1.347		
Diego Garcia 2007	77/216	115/203	0.424	0.286 to 0.628		
C. Panierakis 2009	15/100	20/96	0.671	0.321 to 1.402		
Chung Cheon 2015	68/81	108/113	0.242	0.0826 to 0.710		
Total (fixed effects)	400/866	582/983	0.632	0.508 to 0.786	-4.114	<0.001
Total (random effects)	400/866	582/983	0.624	0.418 to 0.933	-2.298	0.022
Q	17.5208					
DF	6					
Significance level	P = 0.0075					
I^2 (inconsistency)	65.76%					
95% CI for I^2	23.26 to 84.72					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.142857 P=0.5619(low power)

Egger:bias= -0.656941 (95% CI=-7.053883 to 5.74) P=0.8023

Harbord-Egger:bias=0.561504(92.5% CI=-6.312289 to 5.189281) P=0.8354

Table 4a-c. *P* values testing Hardy-Weinberg proportion and test for heterogeneity of studies included in the meta-analysis for *FokI FF, FokIFf and FokIff* polymorphisms respectively. (df:degree of freedom, Q:Heterogeneity in meta analysis).

Table 4a. Fok-FF

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Balazs Gyorffy 2002	36/107	29/103	1.294	0.719 to 2.328		
Tatijana Semunik 2005	42/132	73/232	1.016	0.642 to 1.609		
J.J. San Pedro 2005	31/71	41/88	0.888	0.474 to 1.666		
C. Panierakis 2009	64/100	50/96	1.636	0.923 to 2.898		
Greear R.M. 2013	21/55	28/50	0.485	0.223 to 1.058		
Total (fixed effects)	194/465	221/569	1.057	0.817 to 1.367	0.420	0.675
Total (random effects)	194/465	221/569	1.036	0.733 to 1.465	0.202	0.840
Q	6.8448					
DF	4					
Significance level	P = 0.1443					
I^2 (inconsistency)	41.56%					
95% CI for I^2	0.00 to 78.48					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.6 P=0.00833(low power)

Egger:bias= -3.653382 (95% CI=-14.60785 to 7.301086) P=0.3664

Harbord-Egger:bias=-3.796013(92.5% CI=-13.262673 to 5.670648) P=0.3612

Table 4b. Fok-Ff

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Balazs Gyorffy 2002	49/107	56/103	0.709	0.412 to 1.221		
Tatijana Semunik 2005	63/132	136/232	0.645	0.419 to 0.991		
J.J. San Pedro 2005	35/71	39/88	1.222	0.652 to 2.287		
C. Panierakis 2009	31/100	43/96	0.554	0.309 to 0.993		
Greear R.M. 2013	21/55	22/50	0.786	0.361 to 1.714		
Total (fixed effects)	199/465	296/569	0.724	0.564 to 0.929	-2.538	0.011
Total (random effects)	199/465	296/569	0.723	0.563 to 0.929	-2.535	0.011
Q	3.8098					
DF	4					
Significance level	P = 0.4324					
I^2 (inconsistency)	0.00%					
95% CI for I^2	0.00 to 79.45					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.4 P=0.4833(low power)

Egger:bias=1.95378 (95% CI=5.613844 to 9.521404) P=0.4715

Harbord-Egger:bias=2.01803(92.5% CI=-4.313428 to 8.349488) P=0.4557

Table 4c.Fok-ff

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Balazs Gyorffy 2002	22/107	18/103	1.222	0.612 to 2.441		
Tatijana Semunik 2005	29/132	23/232	2.558	1.410 to 4.643		
J.J. San Pedro 2005	5/71	8/88	0.758	0.237 to 2.426		
C. Panierakis 2009	1/100	7/96	0.128	0.0155 to 1.064		
Greear R.M. 2013	7/55	5/50	1.312	0.388 to 4.435		
Total (fixed effects)	64/465	61/569	1.374	0.943 to 2.003	1.656	0.098
Total (random effects)	64/465	61/569	1.159	0.573 to 2.344	0.411	0.681
Q	10.1246					
DF	4					
Significance level	P = 0.0384					
I^2 (inconsistency)	60.49%					
95% CI for I^2	0.00 to 85.20					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.6 P=0.0833(low power)

Egger:bias= -3.173487 (95% CI=-6.536026 to 0.189052) P=0.575

Harbord-Egger:bias= -4.109035(92.5% CI=-8.5417147 to 0.323644) P=0.0889

Table 5a-c. *P* values testing Hardy-Weinberg proportion and test for heterogeneity of studies included in the meta-analysis for TaqITT, TaqITt and TaqItt polymorphisms respectively. (df:degree of freedom, Q:Heterogeneity in meta analysis).

Table 5a.TaqTT

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Chang 2000	142/157	233/248	0.609	0.289 to 1.284		
Balazs Gyorffy 2002	44/107	42/103	1.014	0.585 to 1.759		
Vaselin Scrabic 2003	54/134	48/132	1.181	0.720 to 1.938		
J.J. San Pedro 2005	24/71	31/88	0.939	0.486 to 1.813		
Diego Garcia 2007	115/216	121/203	0.772	0.524 to 1.137		
C. Panierakis 2009	10/100	34/96	0.203	0.0933 to 0.440		
Greear R.M. 2013	18/55	26/50	0.449	0.204 to 0.990		
Chung Cheon 2015	66/81	105/113	0.335	0.135 to 0.834		
Total (fixed effects)	473/921	640/1033	0.713	0.580 to 0.876	-3.213	0.001
Total (random effects)	473/921	640/1033	0.644	0.440 to 0.942	-2.270	0.023
Q	20.6282					
DF	7					
Significance level	P = 0.0044					
I^2 (inconsistency)	66.07%					
95% CI for I^2	28.03 to 84.00					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.642857 P=0.0141(low power)

Egger:bias= -3.773452 (95% CI=-8.197852 to 0.650947) P=0.0819

Harbord-Egger:bias= -3.522136(92.5% CI=-8.048273 to 1.004) P=0.1452

Table 5b.TaqTt

Study	Intervention	Controls	Odds ratio	95% CI	z	P
Chang 2000	15/157	14/248	1.766	0.828 to 3.766		
Balazs Gyorffy 2002	28/107	33/103	0.752	0.414 to 1.367		
Vaselin Scrabic 2003	55/134	72/132	0.580	0.357 to 0.943		
J.J. San Pedro 2005	36/71	43/88	1.076	0.576 to 2.012		
Diego Garcia 2007	79/216	69/203	1.120	0.750 to 1.673		
C. Panierakis 2009	64/100	59/96	1.115	0.625 to 1.990		
Greear R.M. 2013	26/55	24/50	0.971	0.451 to 2.091		
Chung Cheon 2015	15/81	8/113	2.983	1.199 to 7.423		
Total (fixed effects)	318/921	322/1033	1.017	0.829 to 1.248	0.165	0.869
Total (random effects)	318/921	322/1033	1.062	0.785 to 1.438	0.390	0.697
Q	13.8658					
DF	7					
Significance level	P = 0.0536					
I^2 (inconsistency)	49.52%					
95% CI for I^2	0.00 to 77.47					

Bias indicators

Begg-Mazumdar:Kendall's Tau= 0.047619 P>0.9999 (low power)

Egger:bias=1.307065(95% CI=-4.196619 to 6.810748) P=0.5682

Harbord-Egger:bias=1.410342(92.5% CI=-3.284733 to 6.105417) P=0.5305

Table 5c.Taq-tt

Study	Intervention	Controls	Odds ratio	95% CI	z	P
T.J. Chang 2000	0/157	1/248	0.524	0.0212 to 12.939		
Balazs Gyorffy 2002	33/107	26/103	1.321	0.721 to 2.418		
Vaselin Scrabic 2003	25/134	11/132	2.523	1.186 to 5.367		
J.J. San Pedro 2005	11/71	14/88	0.969	0.410 to 2.290		
Diego Garcia 2007	22/216	13/203	1.657	0.811 to 3.386		
C. Panierakis 2009	22/100	7/96	3.586	1.453 to 8.849		
Greear R.M. 2013	6/55	5/50	1.102	0.314 to 3.862		
Chung Cheon 2015	0/81	0/113	-			
Total (fixed effects)	119/921	77/1033	1.677	1.225 to 2.295	3.230	0.001
Total (random effects)	119/921	77/1033	1.655	1.163 to 2.356	2.798	0.005
Q	6.9433					
DF	6					
Significance level	P = 0.3261					
I^2 (inconsistency)	13.59%					
95% CI for I^2	0.00 to 75.19					

Bias indicators

Begg-Mazumdar:Kendall's Tau=-0.047619 P>0.9999 (low power)

Egger:bias=-6.680722 (95% CI=-3.88097 to 2.519525) P=0.608

Harbord-Egger:bias=-1.122847(92.5% CI=-3.907446 to 1.661601) P=0.4074