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Meta-analysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity

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Abstract

Objective—Methotrexate is an effective therapy for Rheumatoid Arthritis (RA) but is also associated with toxicity. Pharmacogenetics is the systematic evaluation of the role of genetic differences in the efficacy and toxicity of therapeutic interventions. Because the results of small pharmacogenetic studies are often misleading, we undertook a meta-analysis of published studies to determine the role of polymorphisms in the therapeutic efficacy and toxicity of methotrexate.

Methods—A search of PUBMED produced 55 publications which were then reviewed for relevance to methotrexate toxicity and efficacy in patients with RA. To ensure that no studies were missed, each polymorphism found was then entered as an independent search string and all results were again reviewed.

Results—Only 2 polymorphisms (C677T and A1298C in methylenetetrahydrofolate reductase, MTHFR, total: 8 studies) relevant to methotrexate metabolism and efficacy had sufficient data to perform a meta-analysis of their association with toxicity; there was no polymorphism with sufficient data to perform a meta-analysis of efficacy. In a fixed effects model, the C677T polymorphism was associated with increased toxicity (OR 1.71, CI 1.32 – 2.21, p<0.001). The A1298C polymorphism was not associated with increased toxicity (OR 1.12, CI 0.79 – 1.6, p=0.626).

Conclusions—As pharmacogenetics evolves, more data are needed to assess the role of various polymorphisms for drug efficacy and toxicity. These results illustrate the paucity of reliable pharmacogenetic data on a commonly used anti-rheumatic drug and the potential role of pharmacogenetics in tailoring drug therapy for an individual patient.

Key Terms

Methotrexate; Polymorphism; Toxicity

Introduction

Rheumatoid Arthritis (RA) is among the best studied chronic inflammatory diseases. One of the most effective therapies for RA, and the ‘anchor’ of many therapeutic regimens, is Methotrexate (MTX). Reports of the use of aminopterin (MTX) to treat RA date back to 1951, and the initial clinical trials showing the efficacy of MTX date back to the mid 1980s[1–5]. While highly effective, it is also associated with toxicity, including worsening of nodulosis, pneumonitis, neurologic toxicity, gastrointestinal complications including nausea, vomiting and diarrhea, transaminitis, hematologic abnormalities, rash, stomatitis, and alopecia[6–14].

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MTX is a structural analogue of folic acid[15]. It enters cells via solute carrier family 19 member 1 (SLC19A1), the reduced folate carrier, and then needs to be activated by a gamma-glutamyl hydrolase (GGH) to a polyglutamated form. This blocks the enzyme dihydrofolate reductase (DHFR), inhibiting purine metabolism, as well as impairing protein synthesis by blocking the conversion of other amino acids. In addition, the polyglutamated MTX can also interfere with thymidylate synthetase (TYMS) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (also called ATIC). Lastly, the polyglutamated MTX can interfere with methylenetetrahydrofolate reductase (MTHFR), causing elevated homocysteine levels and toxicity[15].

The sequencing of the human genome, and the understanding of the potential function of single nucleotide polymorphisms (SNPs) of appropriate genes, have provided a vast amount of data to study associations between toxicity or efficacy of different medications, a burgeoning field known as pharmacogenetics. As the technology to study SNPs has advanced, reducing costs, more studies of SNPs related to specific medications have been published. However, many of these studies are small and of limited utility.

The results of pharmacogenetic studies are often both conflicting and difficult to understand. A 2002 study examining genetic associations found that of 166 putative associations that had been studied 3 or more time between a SNP and disease susceptibility, only 6 could be reproduced consistently[16].

Because the results of small pharmacogenetic studies are often misleading we undertook a meta-analysis of published studies to determine the role of polymorphisms in MTHFR, an enzyme affected by methotrexate and its metabolites, in the therapeutic efficacy and toxicity of methotrexate.

Materials and Methods

We searched PUBMED using keywords Methotrexate, Arthritis, and either SNP (single nucleotide polymorphism) or polymorphism. Fifty five articles were identified, and each was individually reviewed for relevance to efficacy of treatment of rheumatoid arthritis or toxicity. Over 20 different polymorphisms were identified that impacted either efficacy or toxicity (Table 1). Because several of the efficacy trials had widely disparate definitions of efficacy, it was the opinion of the authors that an adequate meta-analysis could not be done on that literature. Only two SNPs were identified with three or more articles published with sufficient data on toxicity; MTHFR C677T and MTHFR A1298C (Table 2).

Meta-analysis performed on those studies examining toxicity, using both random effects model and fixed effects models. There was insufficient data on populations to know if appropriate haplotype stratification had been done in each study to know which model was appropriate

All analyses were done using Comprehensive Meta Analysis Version 2.2.046.

Results

Of the fifty five studies identified in the literature, eight were identified that discussed the C677T polymorphism[17–24]. Of those eight, five also discussed the A1298C polymorphism[18, 20–22, 24]. Table 1 shows a list of all polymorphisms identified with studies documenting impact on efficacy, toxicity, or both[17–33]. Table 2 shows the details of the studies in this analysis. Figure 1, Figure 2 and Figure 3 show the funnel plot of the effects of the studies.

Of the eight studies that assessed the C677T polymorphism, either homozygous or heterozygous, only three showed a significant increase in toxicity with the use of MTX [19,

[21, 23]. Two others also showed an increase in toxicity, though not significant[18, 22]. The other three studies showed a possible decrease in toxicity, though again not approaching significance[17, 20, 24]. When assessed together, and weighting for the relative sizes of the different studies, assuming a fixed effects model, there is a significant, though small, increase in toxicity (OR 1.71, CI 1.32 – 2.21, $p < 0.001$). Assuming a random effects model, however, the confidence interval crosses the null hypothesis (OR 1.60, CI 0.90 – 2.86, $p = 0.11$).

Of the five studies that assessed the A1298C polymorphism, again either homozygous or heterozygous, only one showed a significant increase in toxicity[24]. Three of the remaining studies showed almost no impact at all[20–22], and the fourth showed a possible decrease in toxicity[18], approaching but not reaching significance in a fixed effects model (OR 1.12, CI 0.79 – 1.6, $p = .53$). A random effects model showed similar results (OR 1.04, CI 0.6 – 1.81, $p = 0.88$).

All studies used any toxicity as an endpoint. As such, a mild elevation in LFTs or stomatitis was treated the same as nausea or as LFT elevations greater than 3 times the upper limit of normal. In addition, almost all studies did not discriminate between whether patients had only one copy of the polymorphism or two copies of the polymorphism.

Discussion

The primary findings of this investigation are the increased OR of Methotrexate toxicity used to treat Rheumatoid Arthritis associated with the C677T polymorphism in a fixed effects model. There was no association between the A1298C polymorphism and toxicity.

This meta-analysis illustrates the paucity of data about the pharmacogenetics of one of the most commonly used DMARDs. The C677T and A1298C polymorphisms are just two of over a dozen polymorphism reported in the MTHFR gene; of those 12, only 7 have been associated with efficacy or toxicity in RA[34]. The C677T polymorphism was first described in the mid-1990s, leading to decreased activity of the MTHFR enzyme; the homozygous variant has about 30% of the function of the wild type[35, 36]. The heterozygous variant has about 60% of the function of the wild type. The A1298C polymorphism was first discovered in 1998; the homozygous variant has about 60% of the function wild type[37, 38].

In attempting to draw a collective conclusion from the individual trials, it is important to comment on the strengths and weaknesses of each. The first article assessing the connection between the C677T polymorphism and toxicity, published by van Ede in 2001, focused on discontinuation due to toxicity or elevation of LFTs[23]. In addition, patients filled out a ‘standard toxicity questionnaire’ to assess other side effects. The primary purpose of this study was actually to assess the impact of folic acid and folinic acid supplementation on MTX efficacy and toxicity in RA patients, and was performed in a prospective manner, and this analysis only used a random subset of patients from that original study. This study is confounded somewhat by the variable use of folic acid supplementation among the RA patients – 1/3 of patients received placebo, 1/3 received daily folic acid, and 1/3 received folinic acid weekly. While this study’s strengths include a thorough statistical analysis, including defined patient numbers needed for adequate power, toxicity in this study was defined as discontinuation. Many patients suffer from side effects insufficient to warrant discontinuation, and most of the other studies did not discriminate between more minor and more significant toxicities in their analyses.

Urano and colleagues assessed the role of both the C677T and A1298C polymorphisms[22]. There is no discussion of numbers needed for adequate power of this cross-sectional analysis, and there is no description of how these patients were chosen from the outpatient clinics population in Tokyo. In addition, patients in this study did not receive doses of MTX higher

than 12.5mg, markedly different from conventional therapy elsewhere. The authors also do not discriminate between transaminitis and less severe side effects, such as stomatitis or alopecia. The authors do note that no patients in their study had both the 677T and 1298C haplotype. Urano's group published a second paper on MTX polymorphisms several years later, this time with Taniguchi as the lead auther[21]. The purpose of this study was to validate their previous work. The design was retrospective, with patients chosen randomly from their outpatient clinic population at the Institute of Rheumatology, Tokyo Women's Medical University. This study also examined both polymorphisms. Again, there is no discussion of power. In addition, less than 1/3 of the patients in this study received folic acid supplementation, and greater than half of patients received 6mg or less of MTX. Toxicities and adverse events are not clearly defined in this study beyond a definition of transaminitis.

Kumagai et al., another group based in Japan, studied both polymorphisms. This was a prospective analysis, with the primary purpose of assessing the impact of several polymorphisms. The authors do not state where the patients were recruited from. They also do not discuss how many patients they needed for adequate power[20]. The authors also had a maximum dose of 12mg of MTX in this study. While toxicities are broken down by frequency, the authors use the aggregate of all adverse events, not discriminating between minor and more significant side effects. Unlike most of the other studies, this one does discriminate between heterozygous and homozygous genotypes and rate of adverse events.

Berkun and colleagues also studied both polymorphisms[18]. This is a prospective study, with 93 consecutive RA patients recruited from three different rheumatology outpatient clinics in Israel. As opposed to the previous studies, the definition of toxicity is more clearly described. However, the authors use a composite 'side effects' result, and do not discuss severe versus mild effects. Methotrexate doses are a little higher in this population, with an average dose just under 12mg weekly. In addition, patients in this analysis received an average dose of over 5mg of folate supplementation daily.

Aggarwal et al. analyzed only the C677T polymorphism. This is a retrospective study selecting patients randomly from an outpatient clinic in Lucknow, India. All patients in this study received folic acid supplementation, and MTX doses were similar to Berkun's study. Toxicity was better defined in this study; the authors broke down rates of toxicity for specific genotypes as any, hematologic, hepatic, gastrointestinal, and pulmonary. Only one other study in this analysis provided similar data on toxicity.

Kim and colleagues also only studied the C677T polymorphism. Of the eight studies, this prospective study in Seoul, South Korea, was by far the largest. The mean MTX dose is similar to the previous two studies, 11.6mg weekly, and all patients received daily folic acid supplementation. Toxicities were well defined by the authors, and they note which patients required temporary versus permanent withdrawal. The authors also provide data on specific toxicities related to genotype.

Lastly, Wessels et al. assessed toxicity related to both C677T and A1298C polymorphisms. These patients were a subcohort of the BeSt trial. All patients received folic acid supplementation, but average MTX dose is not noted. Toxicity is well defined, and the authors present data on specific toxicities for each genotype, and the authors also discriminate between the heterozygous and homozygous genotype.

An additional variable that may have clinical impact is the time from initiation or titration of MTX dose to onset of adverse effects. This clearly would be an important component in assessing the risk of medication and for patient counseling. However, the data presented in the articles in this analysis did not include sufficient data to assess whether the presence or absence of the above SNPs impacted time to adverse event.

Another potential issue in studying pharmacogenetics is the impact of multiple SNPs on the efficacy or toxicity of a drug. While a single SNP may not have significance alone, the combination of several SNPs for a given protein may lead to significant changes in function that either increase or decrease toxicity or efficacy or both. To date, no study has been published assessing the presence and impact of both C677T and A1298C in patients with rheumatoid arthritis. Other studies have found a correlation between the presence of both SNPs and outcome, including increased frequency of neural tube defects (NTDs), and patients heterozygous for both SNPs have significantly decreased MTHFR activity, compared to patients with only one SNP, and the expected increase in homocysteine levels as well.[37]

In analyzing the data presented here it is unclear whether the fixed or random effects model is the most appropriate analytic model as the frequency of the respective SNPs in various populations has not been fully explored among all RA patients. In Caucasians and Asians, 12 to 15% of individuals are homozygous TT and as many as 50% are heterozygous for the C677T polymorphism [39, 40]. The C677T polymorphism has a frequency of about 35% in North America[36, 41, 42]. For the A1298C polymorphism, the homozygous CC polymorphism among Caucasians, was present in 7–12% of the population, and the allelic frequency was about 33% [37, 43, 44]. Nonetheless it is likely that, regardless of penetrance of the polymorphism, the clinical impact that it has would be the same regardless of where the study was performed or the frequency of the polymorphism within each study population, so the fixed-effects model, which demonstrated a clear and significant association between the C677T polymorphism and methotrexate toxicity, may be more applicable. It is also notable that none of the studies in this analysis discuss the racial or ethnic background of their study participants. As the rate of the different SNPs may be different in different ethnic groups, this data would be useful to help further understand the impact of a given SNP, and the utility of studying different SNPs in different patient populations.

The strengths of this study include the size of the analysis, with over 1400 patients for the C677T analysis, and over 660 for the A1298C analysis. In addition, the relative merits of each study are discussed, with a focus on the differences in both the treatments and toxicity analyses of the different studies. This analysis has limitations as well. First, there is an inherent heterogeneity to meta-analysis, and there were differences in definition of toxicity, methotrexate dose, and folic acid supplementation among the different studies examined. Second, not all studies discriminated between the heterozygous and homozygous genotype. Because of this, the meta-analysis was performed combining all patients who deviated from the wild type, allowing all studies to be compared in the meta-analysis.

In conclusion, as pharmacogenetics evolves more and larger studies are needed to assess the role of various polymorphisms for drug efficacy and toxicity. However, until larger studies are carried out meta-analysis of pooled data is the best available tool to validate genetic associations with efficacy and toxicity. The results presented here illustrate both the paucity of reliable pharmacogenetic data on a very commonly used anti-rheumatic drug as well as the potential role that pharmacogenetics can play in tailoring drug therapy for an individual patient.

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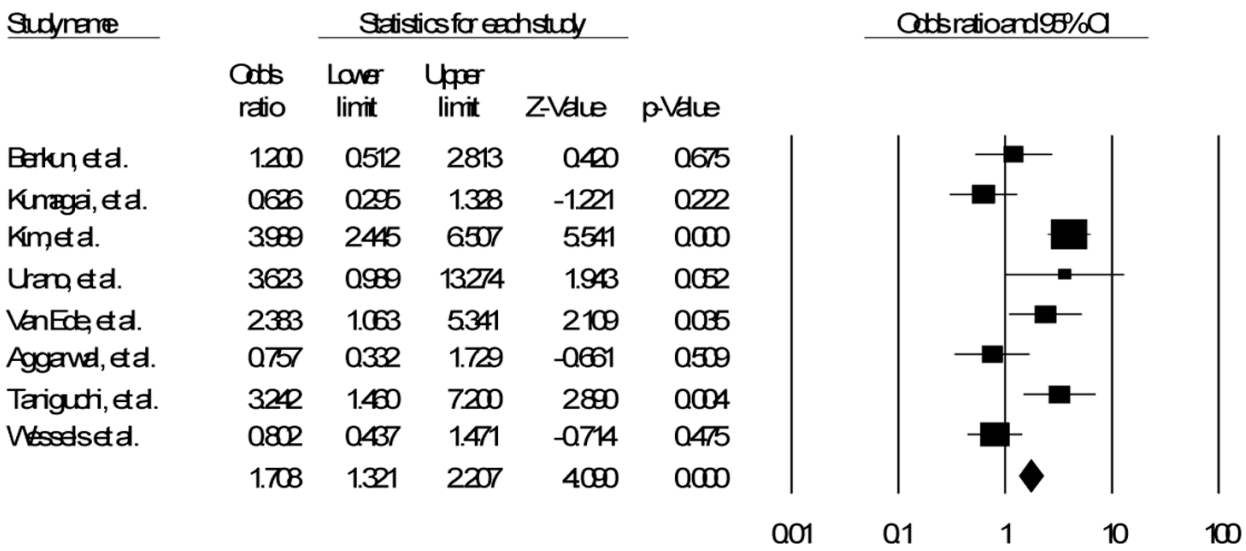

Figure 1.
SNP C677T Fixed Effects Model

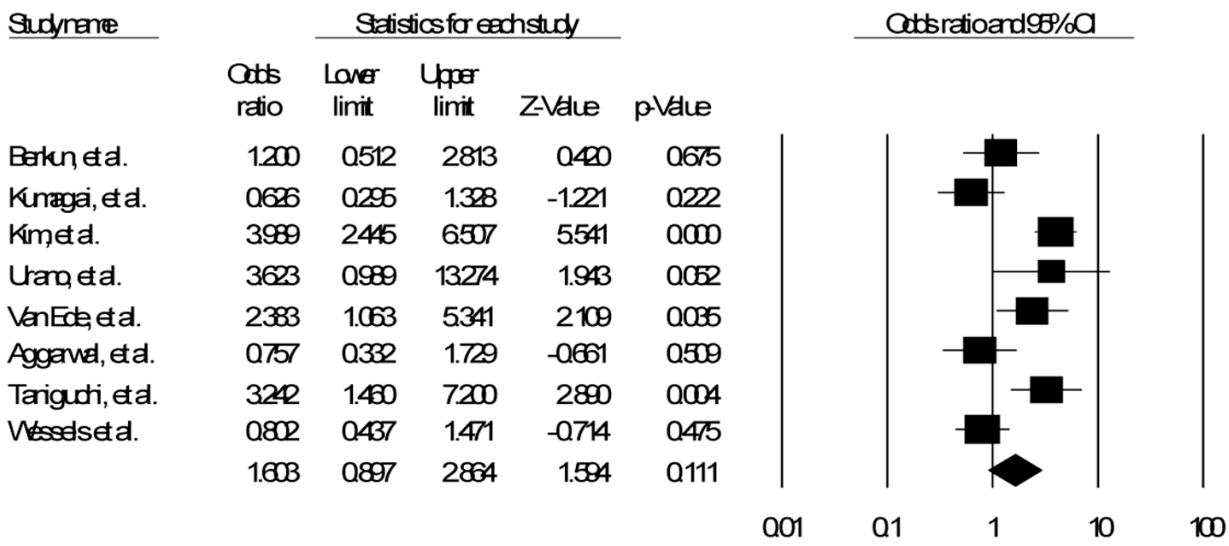

Figure 2.
SNP C677T Random Effects Model

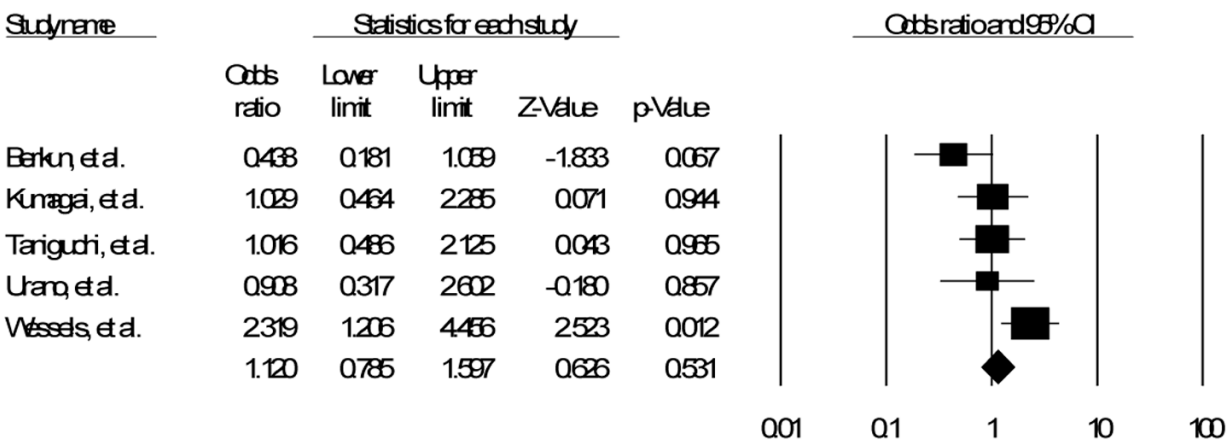

Figure 3.
SNP A1298C Fixed Effects Model

Table 1

All Methotrexate Single Nucleotide Polymorphisms Studied in Rheumatoid Arthritis Efficacy and Toxicity

Polymorphism	Number of articles	Efficacy, Toxicity, or Both
MTHFR C677T	van Ede, 2001[23] Urano 2002[22] Kumagi 2003[20] Berkun 2004[18] Wessels 2006[24] Kim 2006[19] *Dervieux 2006[26] Aggarwal 2006[17] Taniguchi 2007[21] *Kurzawski 2007[29]	Toxicity Both Both Toxicity Both Toxicity Both Both Both Efficacy
MTHFR A1298C	Kumagi 2003[20] Berkun 2004[18] Wessels 2006[24] *Dervieux 2006[26] Taniguchi 2007[21] *Kurzawski 2007[29]	Both Toxicity Both Both Both Efficacy
TYMS 3'UTR	Kumagi 2003[20] Takatori 2006[31]	Both Both
TSER*2*3	*Dervieux 2004[27] *Dervieux 2006[26]	Efficacy Both
RFC1 G80A	*Dervieux 2004[27] *Wessels 2006[24] *Dervieux 2006[26] Takatori 2006[31] Drozdzik 2006[28]	Efficacy Both Both Both Efficacy
ATIC C347G	*Dervieux 2004[27] *Wessels 2006[24] *Dervieux 2006[26] Takatori 2006[31]	Efficacy Both Both Both
ITPA C94A	Wessels 2006[24]	Both
MTXPGs	*Dervieux 2004[27]	Efficacy
DHFR –G473A	Wessels 2006[24]	Both
DHFR G35289A	Wessels 2006[24]	Both
HLA-G 14b	Rizzo 2006[30]	Efficacy
HLA DRB1	Ali 2006[25]	Efficacy
HLADQB1	Ali 2006[25]	Efficacy
MDR1 C3435T	Drodzik 2006[28]	Efficacy
AMPD1 C34T	Wessels 2006[24]	Both
MTR A2756G	Wessels 2006[24]	Both
MS A2756G	*Dervieux 2006[26]	Both
MTRR A66G	Wessels 2006[24] *Dervieux 2006[26]	Both Both
GGH C401T	*Dervieux 2006[26]	Both
GGH C452T	van der Stratten 2007[32]	Both
GGH T16C	van der Stratten 2007[32]	Both
SHMT1 C1420T	Dervieux 2006[26]	Both

Polymorphism	Number of articles	Efficacy, Toxicity, or Both
ABCB1 C3435T	Takatori 2006[31]	Both
FPGS A1994G	van der Stratten 2007[32]	Both
FPGS G114A	van der Stratten 2007[32]	Both

MTHFR Methylenetetrahydrofolate Reductase, TYMS thymidylate synthase, TSER thymidilate synthase enhancer region, RFC1 Reduced Folate Carrier1, ATIC 5 aminoimidazole-4-carboxamide ribonucleotide transformylase, ITPA inosine triphosphate phosphatase, MTXPGs Methotrexate Polyglutamtes, DHFR dihydrofolate reductase, HLA Human Leukocyate Antigen, MDR1 multidrug resistance 1, AMPD1 adenosine monophosphate deaminase 1, MTR methionine synthase, MTRR methionine synthase reductase, GGH gamma glutamyl hydrolase, MS methionine synthase, SHMT1 serine hydroxymethyl transferase 1, ABCB1 ATP binding cassette transporter B1, FPGS folylpoly-gamma-glutamase synthetase

* Insufficient data in article to permit inclusion in meta-analysis

Table 2

Methotrexate Studies Included in Analysis

C677T:						
Authors	**Year**	**CT or TT (#)**	**CC (#)**	**OR**	**CI**	**P value**
Van Ede[23]	2001	114	122	2.383	1.063 – 5.341	0.035
Urano[22]	2002	71	35	3.623	0.989 – 13.274	0.052
Kumagai[20]	2003	69	46	0.626	0.295 – 1.328	0.222
Berkun[18]	2004	48	45	1.200	0.512 – 2.813	0.675
Kim[19]	2006	252	133	3.989	2.445 – 6.507	0.000
Aggarwal[17]	2006	63	87	0.757	0.332 – 1.729	0.509
Wessels[24]	2006	111	89	0.802	0.437 – 1.471	0.475
Taniguchi[21]	2007	90	66	3.242	1.460 – 7.200	0.004
FIXED				1.708	1.321 – 2.207	0.000
RANDOM				1.603	0.897 – 2.864	0.111

A1298C:						
Authors	**Year**	**AC or CC (#)**	**AA (#)**	**OR**	**CI**	**P value**
Urano[22]	2002	32	74	0.908	0.317 – 2.602	0.857
Kumagi[20]	2003	35	80	1.029	0.464 – 2.285	0.944
Berkun[18]	2004	43	50	0.438	0.181 – 1.059	0.067
Wessels[24]	2006	115	83	2.319	1.206 – 4.456	0.012
Taniguchi[21]	2007	32	74	1.016	0.486 – 2.125	0.965
FIXED				0.826	.0541 – 1.260	0.375
RANDOM				0.826	.0541 – 1.260	0.375