Association Between CTLA-4 Gene Polymorphism and Risk of Rheumatoid Arthritis (RA): Evidence from a Meta-analysis Involving 21681 Cases and 23457 Controls.

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Abstract

**Background:** Genetic predisposition was well known to be involved in the pathogenesis of Rheumatoid arthritis (RA). Lots of genetic studies on association between Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) polymorphisms and RA susceptibility had been accumulated in the past few decades, yet reporting inconsistent results. Therefore, we summarized the most-often 3 polymorphisms and performed a meta-analysis to comprehensively evaluate the effect of CTLA-4 gene polymorphisms on RA risk.

**Methods:** Five electronic databases were searched for eligible studies till Oct. 2020. The pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength of association. Stratified analysis was conducted by ethnicity.

**Results:** In total, 42 case-control studies including 21681 cases and 23457 controls were obtained. For rs3087243 polymorphism, significant association was detected in Asians (A vs. G: OR=0.77, 95%CI=0.65-0.90, P=0.001; AA vs. GG: OR=0.67, 95%CI=0.48-0.94, P=0.02) and Caucasians (A vs. G: OR=0.89, 95%CI=0.86-0.93, P<0.00001; AA vs. GG: OR=0.81, 95%CI=0.75-0.88, P<0.00001). For rs231775 polymorphism, significant association was observed in the overall (G vs. A: OR =1.16, 95%CI=1.08-1.25, P<0.0001; GG vs. AA: OR=1.29, 95%CI=1.12-1.50, P=0.0006), in Asians (G vs. A: OR=1.27, 95%CI=1.10-1.47, P=0.001; GG vs. AA: OR=1.58, 95%CI=1.24-2.01, P=0.0002), and not in Caucasians. However, there was no association between rs5742909 polymorphism and RA risk.

**Conclusion:** This meta-analysis confirmed that rs3087243 and rs231775 polymorphism were associated with the risk of RA in both overall population and ethnic-specific analysis, but there was no association between rs5742909 polymorphism and RA risk.

Introduction

Rheumatoid arthritis, one of the most common inflammatory joint diseases in humans, is characterized by inflammation in synovium, destruction of cartilage and bone, generation of autoantibody, and complications of systemic organ [1]. Although RA affects 0.5–1% of the Western populations, the worldwide incidence of RA is increasing with the aging trend of the population [2]. Because of the results of reduced physical function, declined work capacity, decreased quality of life, and increased comorbid risk, RA carries heavy socioeconomic burden [3]. RA is believed to be a consequence of both genetic factors and environmental factors though main etiology has not yet been clearly clarified. In twin studies 50–65% of the risk for developing RA is ascribed to its heritability [4], indicating genetic factors have a strong effect on RA. So far More than one hundred gene loci associated with RA risk have been identified by single nucleotide polymorphisms (SNPs) [5, 6]. Apart from the human leukocyte antigen (HLA) locus, a well-known genetic risk factor for RA, numbers of other susceptibility genes and loci have been characterized [6]. Recently, a growing body of non-HLA genetic predisposition studies have been conducted on the association with the risk of RA [7–9].

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4), one of widely studied non-HLA susceptibility gene of RA, is mainly expressed on the surface of Treg cells and conventional T cells and suppresses self-reactive T cell responses via downregulating ligand availability for the costimulatory receptor CD28 to elicit inhibitory signals [10, 11]. Besides, the polymorphisms of CTLA-4 have already been proved to be candidates of the risk of the common autoimmune diseases at the genetic level [12–15]. As RA is a T cell mediated autoimmune disorder and CTLA-4 play a vital role in regulating T cell function [11, 12, 16], it suggests that CTLA-4 expression or function is most likely associated with the pathogenesis of RA. Single nucleotide polymorphisms in the CTLA-4 gene may contribute to abnormal levels of CTLA-4, and subsequently play a leading part in the susceptibility to RA [12, 17, 18].

Among the identified SNPs in this gene, these three loci of CTLA-4, + 49A/G (rs231775), -318C/T (rs5742909) and CT60 G/A(rs3087243), are most-often studied for the association with the predisposition of RA worldwide [18–20]. However, the
conclusions which previous reports drew are inconsistent and incomprehensive. Although the association of CTLA-4 genetic polymorphisms and the risk of RA has been assessed in several meta-analyses [21–23], some recent studies also described this association in different populations in the past several years [9, 15, 24–27]. Hence these studies should be included to increase statistical power and gain the reliable conclusion. On the other hand, the previous meta-analysis only researched one or two of the above loci, all the three common loci should be included to embody the association comprehensively. In view of these, it is necessary to incorporate the latest research into investigating the association of the three polymorphisms of CTLA-4 with susceptibility to RA. Here we use the latest case-control data to carry out an updated and comprehensive meta-analysis and obtain a more accurate estimation of the effect of the 3 SNPs (+49A/G (rs231775), CT60 G/A(rs3087243) and −318 C/T (rs5742909)) on RA risk.

Methods And Study Designs

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [28].

Search strategy

From the databases PubMed, EMBASE, Web of Science and, the China National Knowledge Infrastructure (CNKI) and Wan Fang data, a comprehensive systematic literature retrieval was conducted to derive all relevant studies published before 10 October, 2020 (the search was constantly updated to submission). The following terms as Medical Subject Heading (MeSH) and free words were applied: “CTLA4 or cytotoxic T lymphocyte antigen-4” and "single nucleotide polymorphism or polymorphism or variant or variation" and “rheumatoid arthritis or RA”. The bibliographic lists of included studies were also browsed for potential related studies. There were no restrictions on language and publication date in this study.

Inclusion and exclusion Criteria

The current meta-analysis used the following inclusion criteria to screen available literatures: 1) case-control study; 2) evaluation of the associations between CTLA-4 (rs231775, rs3087243 and rs5742909) polymorphism and RA risk; 3) with sufficient data for extract odds ratios (ORs) and 95% confidence intervals (CI); (4) with reported allele or genotype numbers or frequencies in cases and control group; 5) with a clear diagnostic criteria. Accordingly, we excluded meaninglessness literatures if they had the following trait: 1) case report, comment, animal studies and conference abstracts; 2) with no detailed allele or genotype data; 3) duplications or no controls.

Data extraction and assess of quality

Two independent investigators respectively conducted a literature search according to the above search strategy, screened each article based on the predesigned inclusion and exclusion criteria, and extracted data from these eligible studies. It would be settled by discussion with the third party when the disagreement between investigators occurred. The following information was collected from every paper: 1) first author's surname, 2) the year of publication, 3) country or region of origin, 4) ethnicity, 5) total numbers of cases and controls, 6) genotype method, 7) diagnostic criteria; 8) polymorphism locus; 9) allele distribution or/and genotype distribution.

The methodological quality of included studies was accessed in light of the Newcastle–Ottawa Scale (NOS) for the evaluation of observational studies [29]. In brief, three broad perspectives were evaluated using the Star system(http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Any divergence between two investigators was solved by discussion until agreement was reached.

Statistical Analysis
The strength of association of rs231775, rs5742909 and rs3087243 SNPs with RA risk was appraised via estimating ORs with their corresponding 95% CIs. For each SNP, the pooled ORs were calculated separately for five gene models (allele model, homozygote model, heterozygote model, dominant model and recessive model). The Z test was used to evaluate the significance of the pooled ORs. \( p < 0.05 \) was judged as statistically significant difference. Statistical Heterogeneity between studies was assessed by Chi square and \( I^2 \) values which range from 0–100%. 25%, 50%, and 75% were regarded as respectively low, moderate, and high level [30, 31]. The random-effect model was employed when the value of \( I^2 \) was more than 50%. If not, the fixed effect model was employed. Hardy–Weinberg equilibrium (HWE) was tested in the control group for all studies by Chi-square test to judge whether the selection bias existed. Potential publication bias was examined by funnel plots. Besides, the current meta-analysis had carried out subgroup analyses by the racial descent to assess the effects of ethnic background.

The above statistical analyses were performed using Review Manager 5.3 software (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen). All the \( P \) values were 2-sided and \( P < 0.05 \) signified statistically significance.

Results

Characteristics of the studies

Based on the predetermined inclusion criteria, 66 eligible case–control studies with 42 articles were enrolled ultimately in the current analysis [8, 9, 13–15, 17–20, 24–27, 32–61]. These publications had a high methodological quality whose NOS stars were more than 6 in general. There were 22 studies with 16394 patients and 17453 controls for rs3087243 SNP [8, 9, 13–15, 18, 19, 26, 44, 45, 47, 51–54, 57, 58, 61], 34 studies with 11452 patients and 12444 controls for rs231775 SNP [9, 14, 17, 19, 20, 24, 25, 32–43, 45–48, 50, 54–56, 59], and 10 studies with 2477 patients and 2941 controls for rs5742909 SNP [14, 20, 27, 33, 38, 41–43, 48, 61]. The references of all enrolled articles were subject to scrutiny and no more ones were available. The process of study selection according to the PRISMA principle was generalized in Fig. 1. Quality assessment of included studies was shown in Table S1. Details of included studies were listed in Table 1. Allele/genotype frequencies were displayed in Table 2.
Table 1
Main characteristics of included studies

| Study               | Year | Country      | Ethnicity     | Numbers | Genotype method | diagnostic criteria | Quality score |
|---------------------|------|--------------|---------------|---------|-----------------|--------------------|---------------|
| Rs3087243(CT60)     |      |              |               |         |                 |                    |               |
| Orozco              | 2004 | Spain        | Caucasian      | 433     | TaqMan          | ACR1987            | 7             |
| Lei                 | 2005 | China        | Asian          | 326     | DGGE            | ACR1987            | 8             |
| Plenge (EIRA)       | 2005 | Sweden       | European       | 1505    | MALDI-TOF       | ACR1987            | 8             |
| Plenge (NARAC)      | 2005 | Sweden       | European       | 828     | MALDI-TOF       | ACR1987            | 8             |
| Zhernakova          | 2005 | Dutch        | Caucasian      | 153     | PCR-RFLP        | ACR1987            | 6             |
| Suppiah             | 2006 | Northern Ireland | Caucasian     | 289     | PCR-RFLP        | ACR1987            | 7             |
| Costenbader         | 2008 | USA          | Caucasian      | 423     | TaqMan          | ACR1987            | 7             |
| Tsukahara           | 2008 | Japan        | Asian          | 1498    | TaqMan          | ACR1987            | 8             |
| Kelley              | 2009 | USA          | African        | 505     | TaqMan          | ACR1987            | 7             |
| Daha                | 2009 | Dutch        | Caucasian      | 867     | Sequenom        | ACR1987            | 7             |
| Barton              | 2009 | UK           | European       | 3669    | TaqMan          | ACR1987            | 8             |
| Walker              | 2009 | Canada       | Caucasian      | 1140    | Sequenom        | ACR1987            | 8             |
| Plant (1)           | 2010 | France       | Caucasian      | 671     | Sequenom        | ACR1987            | 8             |
| Plant (2)           | 2010 | Germany      | Caucasian      | 218     | Sequenom        | ACR1987            | 8             |
| Plant (3)           | 2010 | Greece       | Caucasian      | 268     | Sequenom        | ACR1987            | 8             |
| Plant (4)           | 2010 | UK           | Caucasian      | 1002    | Sequenom        | ACR1987            | 8             |
| Danoy               | 2011 | China        | Asian          | 1035    | Sequenom        | ACR1987            | 7             |
| Torres-Carrillo     | 2013 | Mexico       | Latin American | 200     | PCR-RFLP        | ACR1987            | 8             |
| Luterek-Puszyńska    | 2016 | Poland       | Caucasian      | 422     | TaqMan          | ACR1987            | 7             |
| Schulz              | 2020 | Germany      | Caucasian      | 111     | PCR-RFLP        | ACR2010            | 6             |
| El-Gabalawy         | 2011 | Canada       | Caucasian      | 332     | Sequenom        | ACR1987            | 6             |
| Vernerova           | 2016 | Slovakia     | Caucasian      | 499     | TaqMan          | ACR2010            | 9             |
| Rs231775(49G/A)     |      |              |               |         |                 |                    |               |
| Alfadhli            | 2013 | Kuwait       | Asian          | 114     | PCR-RFLP        | ACR1987            | 6             |
| Barton (I)          | 2000 | Spain        | Caucasian      | 136     | PCR-RFLP        | ACR1987            | 7             |
| Study           | Year | Country | Ethnicity | Numbers | Genotype method | diagnostic criteria | Quality score |
|----------------|------|---------|-----------|---------|-----------------|---------------------|---------------|
| Barton (II)    | 2000 | UK      | Caucasian | 192/96  | PCR–RFLP        | ACR1987             | 7             |
| Benhatchi      | 2011 | Slovakia| Caucasian | 57/51   | PCR–RFLP        | ACR1987             | 6             |
| Elshazli       | 2015 | Egypt   | Caucasian | 112/122 | PCR–RFLP        | ACR1987             | 6             |
| Feng           | 2005 | China   | Asian     | 50/60   | PCR–RFLP        | ACR1987             | 6             |
| Gonzalez-Escribano | 1999 | Spain   | Caucasian | 138/305 | PCR–ARMS        | ACR1987             | 6             |
| Hadj           | 2001 | Tunisia | African   | 60/150  | PCR–RFLP        | ACR1987             | 7             |
| Lee 2002       | 2002 | Korea   | Asian     | 86/86   | PCR–RFLP        | ACR1987             | 6             |
| Lee 2003       | 2003 | China   | Asian     | 186/203 | PCR–RFLP        | ACR1987             | 6             |
| Lei            | 2005 | China   | Asian     | 326/250 | DGGE            | ACR1987             | 8             |
| Liu 2004       | 2004 | Taiwan  | Asian     | 65/81   | PCR–RFLP        | ACR1987             | 6             |
| Barton         | 2004 | UK      | European  | 132/156 | TaqMan          | ACR1987             | 7             |
| Liu 2013       | 2013 | China   | Asian     | 213/303 | PCR–RFLP        | ACR1987             | 7             |
| Luterek-Puszyńska | 2016 | Poland  | Caucasian | 422/338 | TaqMan          | ACR2010             | 7             |
| Matsushita     | 1999 | Japan   | Asian     | 461/150 | PCR-SSCP        | ACR1987             | 7             |
| Milicic        | 2001 | UK      | Caucasian | 421/452 | PCR–RFLP        | ACR1987             | 8             |
| Mitterski      | 2004 | Germany | Caucasian | 284/362 | PCR–RFLP        | ACR1987             | 7             |
| Munoz-Valle    | 2010 | Mexico  | Mexican   | 199/199 | PCR–RFLP        | ACR1987             | 6             |
| Plant (1)      | 2010 | France  | Caucasian | 684/162 | Sequenom        | ACR1987             | 8             |
| Plant (2)      | 2010 | Germany | European  | 220/209 | Sequenom        | ACR1987             | 8             |
| Plant (3)      | 2010 | Greece  | European  | 272/287 | Sequenom        | ACR1987             | 8             |
| Plant (4)      | 2010 | UK      | European  | 1004/2659 | Sequenom      | ACR1987             | 6             |
| Seidl          | 1998 | Germany | Caucasian | 258/456 | RFLP–SSCP      | ACR1987             | 8             |
| Suppiah        | 2006 | UK      | European  | 289/475 | PCR–RFLP        | ACR1987             | 7             |
| Study        | Year | Country | Ethnicity     | Numbers | Genotype method | diagnostic criteria | Quality score |
|--------------|------|---------|---------------|---------|-----------------|---------------------|---------------|
|              |      |         |               | RA      | Con             |                     |               |
| Takeuchi     | 2006 | Japan   | Asian         | 100     | 104             | PCR–RFLP            | ACR1987       | 6             |
| Tang         | 2013 | China   | Asian         | 1489    | 1200            | TaqMan              | ACR1987       | 8             |
| Tsukahara    | 2008 | Japan   | Asian         | 1490    | 448             | TaqMan              | ACR1987       | 8             |
| Kelley       | 2009 | USA     | African       | 505     | 712             | TaqMan              | ACR1987       | 7             |
| Vaidya       | 2002 | UK      | Caucasian     | 123     | 349             | PCR–RFLP            | ACR1987       | 6             |
| Walker       | 2009 | Canada  | Caucasian     | 1140    | 1248            | Sequenom            | ACR1987       | 8             |
| Yanagawa     | 2000 | Japan   | Asian         | 85      | 200             | PCR–RFLP            | ACR1987       | 6             |
| Zhou         | 2007 | China   | Asian         | 39      | 44              | PCR–RFLP            | ACR1987       | 6             |
| Sameem       | 2015 | Pakistani | Asian     | 100     | 100             | PCR–RFLP           | RF test        | 6             |
| **Rs5742909 (318C/T)** |     |         |               |         |                 |                     |               |
| Gonzalez-Escribano | 1999 | Spain   | Caucasian     | 138     | 305             | PCR-ARMS           | ACR1987       | 6             |
| Lee 2002     | 2002 | Korea   | Asian         | 86      | 86              | PCR–RFLP            | ACR1987       | 6             |
| Barton       | 2004 | UK      | European      | 151     | 152             | TaqMan              | ACR1987       | 7             |
| Liu 2004     | 2004 | Tainan  | Asian         | 65      | 81              | PCR–RFLP            | ACR1987       | 6             |
| Mitterski    | 2004 | Germany | Caucasian     | 284     | 362             | PCR–RFLP            | ACR1987       | 7             |
| Takeuchi     | 2006 | Japan   | Asian         | 100     | 104             | PCR–RFLP            | ACR1987       | 6             |
| Walker       | 2009 | Canada  | Caucasian     | 1140    | 1248            | Sequenom            | ACR1987       | 8             |
| Liu 2013     | 2013 | China   | Asian         | 213     | 303             | PCR–RFLP            | ACR1987       | 7             |
| Torres-Carrillo | 2013 | Mexico  | Latin American| 200    | 200             | PCR–RFLP            | ACR1987       | 7             |
| Fattah       | 2017 | Egypt   | Caucasian     | 100     | 100             | PCR–RFLP            | ACR2010       | 6             |
| Study                     | Cases   | Con   | HEW |
|---------------------------|---------|-------|-----|
|                           | MM      | Mm    | mm  | M  | m   | MM   | Mm    | mm   | M  | m   |
| Rs3087243(CT60)           |         |       |     |    |     |       |       |      |    |     |
| Orozco                    | 118     | 198   | 117 | 434| 432 | 98   | 199   | 101  | 395| 401 |
| Lei                       | 33      | 137   | 156 | 203| 449 | 32   | 131   | 87   | 195| 305 |
| Plenge (EIRA)             | 230     | 680   | 595 | 1140|1870 | 145  | 396   | 337  | 686| 1070 |
| Plenge (NARAC)            | 133     | 387   | 308 | 653| 1003| 165  | 426   | 254  | 756| 934 |
| Zhernakova                | NA      | NA    | NA  | 133| 173 | NA   | NA    | NA   | 841| 959 |
| Suppiah                   | NA      | NA    | NA  | 234| 344 | NA   | NA    | NA   | 145| 191 |
| Costenbader               | 82      | 201   | 140 | 365| 481 | 87   | 195   | 138  | 369| 471 |
| Tsukahara                 | 87      | 538   | 873 | 712| 2284| 33   | 163   | 245  | 229| 653 |
| Kelley                    | NA      | NA    | NA  | NA | 505 | NA   | NA    | NA   | 712| NA |
| Daha                      | NA      | NA    | NA  | 729| 1005| NA   | NA    | NA   | 785| 941 |
| Barton                    | 677     | 1760  | 1232| 3114|4224 | 634  | 1523  | 892  | 2791|3307 |
| Walker                    | 207     | 518   | 415 | 932| 1348| 273  | 613   | 362  | 1159 |1337 |
| Plant (1)                 | 131     | 332   | 208 | 594| 748 | 45   | 91    | 41   | 181 | 173 |
| Plant (2)                 | 35      | 105   | 78  | 175| 261 | 35   | 101   | 73   | 171 | 247 |
| Plant (3)                 | 55      | 135   | 78  | 245| 291 | 70   | 145   | 75   | 285 | 295 |
| Plant (4)                 | 204     | 487   | 311 | 895| 1109| 542  | 1344  | 839  | 2428 |3022 |
| Danoy                     | NA      | NA    | NA  | 310| 1760| NA   | NA    | NA   | 681 |2723 |
| Torres-Carrillo           | 31      | 86    | 83  | 148| 252 | 32   | 106   | 62   | 170 | 230 |
| Luterek-Puszyńska         | 53      | 193   | 176 | 299| 545 | 45   | 174   | 119  | 264 | 412 |
| Schuz                     | 13      | 49    | 49  | 75 | 147 | 42   | 124   | 90   | 208 | 304 |
| El-Gabalawy               | 126     | 161   | 45  | 413| 251 | 198  | 226   | 66   | 622 | 358 |
| Vernerova                 | NA      | NA    | NA  | 616| 382 | NA   | NA    | NA   | 1064|1064 |
| Rs231775(49G/A)           |         |       |     |    |     |       |       |      |    |     |
| AlfFadhli                 | 10      | 30    | 74  | 50 | 178 | 14   | 86    | 182  | 114 | 450 |
| Barton (I)                | 14      | 57    | 65  | 85 | 187 | 12   | 70    | 62   | 94  | 194 |

M, major allele; m, minor allele; NA, not available; HWE, Hardy-Weinberg Equilibrium.
| Study                        | Cases | Con | HEW |
|-----------------------------|-------|-----|-----|
|                             | MM    | Mm  | mm  | M  | m  | MM | Mm  | mm  | M | m | YES  |
| Barton (II)                 | 38    | 68  | 162 | 222| 51 | 19 | 26  | 89  | 103| YES  |
| Benhatchi                   | 6     | 45  | 5   | 9  | 9  | 5  | 25  | 35  | 67 | YES  |
| Elshazli                    | 14    | 83  | 141 | 14  | 45 | 71  | 57  | 187| NO   |
| Feng                        | 20    | 9   | 61  | 9  | 32 | 19 | 50  | 70  | YES |
| Gonzalez-Escribano          | 10    | 193 | 30  | 103| 172| 163| 447 | 70  | YES |
| Hadj                        | 23    | 73  | 47  | 68 | 62 | 20 | 198 | 102| YES |
| Lee 2002                    | 41    | 117 | 55  | 49 | 29 | 8  | 127 | 45  | YES |
| Lee 2003                    | 103   | 273 | 99  | 85 | 100| 18 | 270 | 136| YES |
| Lei                         | 148   | 434 | 218 | 86 | 125| 39 | 297 | 203| YES |
| Liu 2004                    | 14    | 70  | 60  | 50 | 10 | 92 | 70  | NO  |     |
| Barton                      | 34    | 123 | 141 | 29 | 68 | 59 | 126 | 186| YES |
| Liu 2013                    | 77    | 265 | 161 | 130| 125| 48 | 385 | 221| YES |
| Luterek-Puszyńska           | 79    | 133 | 368 | 476| 63 | 160| 286 | 390| YES |
| Matsushita                  | 200   | 599 | 323 | 56 | 72 | 22 | 184 | 116| YES |
| Milicic                     | 63    | 349 | 493 | 73 | 213| 166| 359 | 545| YES |
| Miterski                    | NA    | NA  | NA  | 222| 346| NA | NA  | NA  | 269| 455 | NA  |
| Munoz-Valle                 | 42    | 186 | 212 | 82 | 83 | 150| 248 | YES |     |
| Plant (1)                   | 96    | 507 | 861 | 15 | 75 | 72 | 105 | 219| YES |
| Plant (2)                   | 37    | 185 | 255 | 32 | 94 | 83 | 158 | 260| YES |
| Plant (3)                   | 26    | 185 | 359 | 33 | 107| 147| 173 | 401| YES |
| Plant (4)                   | 146   | 743 | 1265| 410| 1255|994 | 2075|3243|YES |
| Seidl                       | 37    | 212 | 304 | 68 | 210| 179| 346 | 568| YES |
| Suppiah                     | 40    | 224 | 354 | 92 | 241| 142| 425 | 525| YES |
| Takeuchi                    | 49    | 137 | 63  | 44 | 49 | 11 | 137 | 71 | YES |
| Tang                        | 652   | 1946| 1032|474 | 535| 191| 1483| 917| YES |
| Tsukahara                   | 636   | 1940| 1040|181 | 194| 73 | 556 | 340| YES |
| Kelley                      | NA    | NA  | NA  | NA | 505| NA | NA  | NA  | 712| NA  |

M, major allele; m, minor allele; NA, not available; HWE, Hardy-Weinberg Equilibrium.
### Efficiency analysis

**Meta-analysis of CTLA-4 CT60(rs3087243) SNP and RA susceptibility**

By analyzing quantitatively allele or genotype distribution of 16394 patients and 17453 controls, a significant association between RA and CTLA-4 CT60(rs3087243) SNP was observed in all genetic comparisons (A vs. G: OR = 0.87, 95% CI = 0.83–0.91, P < 0.00001; AA vs. GG: OR = 0.80, 95% CI = 0.74–0.87, P < 0.00001; AG vs. AA: OR = 0.85, 95% CI = 0.80–0.90, P < 0.0001; AA + AG vs. GG: OR = 0.83, 95% CI = 0.77–0.90, P < 0.0001, and AA vs. AG + GG: OR = 0.88, 95% CI = 0.83–0.94, P = 0.0003) (Table 3, Fig. 2). Among the 22 included studies, 17 studies were performed in Caucasians, 3 were in Asians, 1 was African and 1 was in Latin Americans. Likewise, we carried out a stratified analysis by race to evaluate the ethnicity effects. In Caucasians, a protective role of rs3087243 SNP on RA was detected in all the five genetic comparisons. Similarly, a decreased risk of RA was found among Asians in the allelic comparison (OR = 0.77, 95% CI = 0.65–0.90, P = 0.001) and the homozygote comparison (OR = 0.67, 95% CI = 0.48–0.94, P = 0.02). The heterozygote model and dominant model detected also this correlation in Latin Americans and the allelic comparison detected this correlation in Africans, but it needed more enrolled studies to elevate statistical power because this analysis currently included individually only one study. The outcomes were shown in Table 3. Collectively, Subgroup analyses revealed a significant protective association.
in Caucasians and Asians. When the $p > 50\%$ and $P > 0.1$, the Fix-effect model was used for the synthesis; otherwise, the Random-effect model was used.
Table 3
Results of different comparative genetic models on the association of CTLA-4 SNPs with RA

| genetic model | Population | Cases | Con | association | OR   | 95%CI  | \(P\)-value\(^a\) | Model | \(\bar{\h}\) | \(P\)-value\(^a\) |
|---------------|------------|-------|-----|-------------|------|--------|-------------------|-------|-------------|-------------------|
| Rs308724      |            |       |     |             |      |        |                   |       |             |                   |
| A vs. G       | total      | 16394 | 17453 | 0.87        | 0.83–0.91 | <0.00001 | REM 39 | 0.003 |
|               | Caucasian  | 12830 | 14148 | 0.89        | 0.86–0.93 | <0.00001 | FEM 25 | 0.17  |
|               | Asian      | 2859  | 2393  | 0.77        | 0.65–0.90 | 0.001   | REM 56 | 0.10  |
|               | Latin      | 200   | 200   | 0.79        | 0.60–1.06 | 0.11    | -      | -     |
|               | African    | 505   | 712   | 0.83        | 0.67–1.02 | 0.08    | -      | -     |
| AA vs. GG     | total      | 13046 | 12214 | 0.80        | 0.74–0.87 | <0.00001 | FEM 22 | 0.20  |
|               | Caucasian  | 11022 | 11323 | 0.81        | 0.75–0.88 | <0.00001 | FEM 32 | 0.13  |
|               | Asian      | 1824  | 691   | 0.67        | 0.48–0.94 | 0.02    | FEM 0  | 0.48  |
|               | Latin      | 200   | 200   | 0.72        | 0.40–1.31 | 0.29    | -      | -     |
| AG vs. GG     | total      | 13046 | 12214 | 0.85        | 0.80–0.90 | <0.0001  | FEM 28 | 0.14  |
|               | Caucasian  | 11022 | 11323 | 0.86        | 0.81–0.92 | <0.0001  | FEM 11 | 0.33  |
|               | Asian      | 1824  | 691   | 0.75        | 0.48–1.18 | 0.21    | REM 78 | 0.03  |
|               | Latin      | 200   | 200   | 0.61        | 0.39–0.94 | 0.02    | -      | -     |
| AA + GA vs. GG| total      | 13046 | 12214 | 0.83        | 0.77–0.90 | <0.0001  | REM 46 | 0.02  |
|               | Caucasian  | 11022 | 11323 | 0.85        | 0.78–0.93 | <0.0002  | REM 40 | 0.07  |
|               | Asian      | 1824  | 691   | 0.74        | 0.48–1.12 | 0.15    | REM 77 | 0.04  |
|               | Latin      | 200   | 200   | 0.60        | 0.40–0.90 | 0.01    | -      | -     |
| AA vs. GA + GG| total      | 13046 | 12214 | 0.88        | 0.83–0.94 | 0.0003   | FEM 0  | 0.75  |
|               | Caucasian  | 11022 | 11323 | 0.89        | 0.83–0.95 | 0.0008   | FEM 0  | 0.60  |
|               | Asian      | 1824  | 691   | 0.76        | 0.55–1.06 | 0.10    | FEM 0  | 0.98  |
|               | Latin      | 200   | 200   | 0.96        | 0.56–1.65 | 0.89    | -      | -     |
| Rs231775      | G vs. A    | 11452 | 12444 | 1.16        | 1.08–1.25 | <0.0001  | REM 66 | 0.0001|

OR, odds ratio; CI, confidence interval; FEM, fix-effect model; REM, random-effect model.
| genetic model | Population | Cases | Con | association | heterogeneity |
|---------------|------------|-------|-----|-------------|---------------|
|               |            |       |     | OR         | 95% CI | P-value<sup>a</sup> | Model | I<sup>2</sup> | P-value<sup>a</sup> |
|               | Caucasian  | 5884  | 7872| 1.09  | 1.01–1.19 | 0.04 | REM | 38 | 0.004 |
|               | Asian      | 4804  | 3511| 1.27  | 1.10–1.47 | 0.001 | REM | 71 | < 0.00001 |
|               | African    | 565   | 862 | 1.06  | 0.68–1.65 | 0.81 | REM | 73 | 0.05 |
|               | Latin      | 199   | 199 | 1.45  | 1.09–1.92 | 0.010 | - | - | - |
| GG vs. AA     | total      | 10663 | 11370| 1.29  | 1.12–1.50 | 0.0006 | REM | 54 | 0.0002 |
|               | Caucasian  | 5600  | 7510| 1.11  | 0.94–1.31 | 0.21 | FEM | 25 | 0.17 |
|               | Asian      | 4804  | 3511| 1.58  | 1.24–2.01 | 0.0002 | REM | 51 | 0.01 |
|               | African    | 60    | 150 | 0.68  | 0.28–1.65 | 0.39 | - | - | - |
|               | Latin      | 199   | 199 | 1.24  | 1.09–1.42 | 0.03 | - | - | - |
| GA vs. AA     | total      | 10663 | 11370| 1.19  | 1.07–1.32 | 0.001 | REM | 46 | 0.003 |
|               | Caucasian  | 5600  | 7510| 1.18  | 1.02–1.35 | 0.02 | REM | 59 | 0.001 |
|               | Asian      | 4804  | 3511| 1.20  | 1.05–1.38 | 0.08 | FEM | 3 | 0.42 |
|               | African    | 60    | 150 | 0.87  | 0.36–2.11 | 0.76 | - | - | - |
|               | Latin      | 199   | 199 | 1.88  | 1.20–2.94 | 0.006 | - | - | - |
| GG + GA vs. AA total | 10663   | 11370| 1.24  | 1.11–1.39 | 0.0001 | FEM | 56 | 0.001 |
|               | Caucasian  | 5600  | 7510| 1.17  | 1.02–1.34 | 0.02 | REM | 62 | 0.0006 |
|               | Asian      | 4804  | 3511| 1.33  | 1.17–1.51 | < 0.0001 | FEM | 31 | 0.12 |
|               | African    | 60    | 150 | 0.77  | 0.34–1.76 | 0.53 | - | - | - |
|               | Latin      | 199   | 199 | 1.87  | 1.23–2.85 | 0.003 | - | - | - |
| GG vs. GA + AA total | 10663   | 11370| 1.15  | 1.02–1.30 | 0.02 | REM | 57 | < 0.0001 |
|               | Caucasian  | 5600  | 7510| 1.01  | 0.91–1.12 | 0.80 | FEM | 10 | 0.34 |
|               | Asian      | 4804  | 3511| 1.34  | 1.08–1.65 | 0.008 | REM | 72 | < 0.0001 |
|               | African    | 60    | 150 | 0.75  | 0.41–1.38 | 0.36 | - | - | - |
|               | Latin      | 199   | 199 | 1.30  | 0.79–2.15 | 0.31 | - | - | - |
| Rs5742909     | T vs. C    | total | 2477 | 2941 | 1.21  | 0.93–1.57 | 0.15 | REM | 71 | 0.0003 |
|               | Caucasian  | 1813  | 2167| 1.31  | 0.94–1.84 | 0.11 | REM | 73 | 0.005 |
|               | Asian      | 464   | 574 | 1.05  | 0.56–1.96 | 0.88 | REM | 80 | 0.002 |

OR, odds ratio; CI, confidence interval; FEM, fixed-effect model; REM, random-effect model.
### Meta-analysis of CTLA-4 + 49A/G (rs231775) SNP and RA susceptibility

By quantitative analysis of allele or genotype distribution of 11452 patients and 12444 controls, there is a significant risk association between RA and CTLA-4 + 49A/G (rs231775) SNP. The overall pooled ORs of all the populations were as follows: G vs. A: OR = 1.16, 95% CI = 1.08–1.25, P < 0.0001; GG vs. AA: OR = 1.29, 95% CI = 1.12–1.50, P = 0.0006; GA vs. AA: OR = 1.34, 95% CI = 1.24–1.45, P = 0.0002; GG + GA vs. AA: OR = 1.33, 95% CI = 1.17–1.51, P < 0.0001; GG vs. GA + AA: OR = 1.19, 95% CI = 1.07–1.32, P = 0.001; GG + GA vs. AA: OR = 1.34, 95% CI = 1.17–1.51, P < 0.0001; GG vs. AA: OR = 1.29, 95% CI = 1.12–1.50, P = 0.0006; GA vs. AA: OR = 1.34, 95% CI = 1.24–1.45, P = 0.0002; GG + GA vs. AA: OR = 1.33, 95% CI = 1.17–1.51, P < 0.0001; GG vs. GA + AA: OR = 1.19, 95% CI = 1.07–1.32, P = 0.001. The main results of overall analyses were shown in Table 3. 17 studies were conducted on Caucasians, 14 on Asians, 2 on Africans and 1 on Latin Americans. Subsequently, stratified analysis by ethnicity was conducted to get more clarifications. In the subgroup analysis, a significantly increased risk of RA was observed among the Asian population in all genetic comparisons except heterozygote comparison (G vs. A: OR = 1.29, 95% CI = 1.12–1.50, P = 0.0006; GG vs. AA: OR = 1.29, 95% CI = 1.12–1.50, P = 0.0006; GA vs. AA: OR = 1.34, 95% CI = 1.24–1.45, P = 0.0002; GG + GA vs. AA: OR = 1.33, 95% CI = 1.17–1.51, P < 0.0001; GG vs. AA: OR = 1.29, 95% CI = 1.12–1.50, P = 0.0006; GA vs. AA: OR = 1.34, 95% CI = 1.24–1.45, P = 0.0002; GG + GA vs. AA: OR = 1.33, 95% CI = 1.17–1.51, P < 0.0001; GG vs. GA + AA: OR = 1.19, 95% CI = 1.07–1.32, P = 0.001). In Latin American population, there is a significantly increased risk of RA, but it only included single study and the result might be incredible. Besides, no
association of the rs231775 SNP with RA risk was found among the Caucasian population in all genetic comparisons when the Elshazli’s study [24] was excluded because of its heterogeneity (G vs. A: OR = 1.07, 95% CI = 0.99–1.15, P = 0.08; GG vs. AA: OR = 1.07, 95% CI = 0.92–1.23, P = 0.37; GA vs. AA: OR = 1.15, 95% CI = 1.00–1.31, P = 0.05; GG + GA vs. AA: OR = 1.14, 95% CI = 1.00–1.29, P = 0.05 and GG vs. GA + AA: OR = 1.00, 95% CI = 0.90–1.11, P = 0.98)(Table 3 and Fig. 3). There was no remarkable association between rs231775 SNP and RA in Africans. The results are summarized in Table 3 and Fig. 3. These data with moderate heterogeneity employed the random-effect model for the synthesis.

**Meta-analysis of CTLA-4 318C/T (rs5742909) SNP and RA susceptibility**

Through the pooled analysis of genetic data of 2547 patients and 2791 controls in a total of 11 studies, of which 6 were conduct on Caucasians, 4 on Asians, and 1 on Latin Americans, no significant associations between rs5742909 SNP and RA in the overall pooled results were found among all populations for the allelic and genotypic comparisons (T vs. C: OR = 1.21, 95% CI = 0.93–1.57, P = 0.15; TT vs. CC: OR = 1.71, 95% CI = 1.08–2.73, P = 0.08; TC vs. CC: OR = 1.19, 95% CI = 0.84–1.69, P = 0.33; TT + TC vs. CC: OR = 1.19, 95% CI = 0.84–1.69, P = 0.33 and TT vs. TC + CC: OR = 1.43, 95% CI = 0.90–2.27, P = 0.13)(Table 3, Fig. 4). Meanwhile, the subgroup analysis by ethnicity did not indicate any remarkable associations across all genetic models (Table 3). As the heterogeneity of genetic model existed, random effect model in this part was used to make a reliable result.

**Heterogeneity analysis and publication bias**

To ensure the reliability of the results, we first evaluate the heterogeneity (by $I^2$) and found that heterogeneity existed in some genetic models of rs231775 SNP and rs5742909 SNP (Table 3). In order to minimize heterogeneity, the current Meta-analysis did the following aspects. On the one hand, the random-effect models were exploited in the genetic models with moderate heterogeneity ($I^2 > 50$%). On the other hand, sensitivity analysis was adopted to evaluate the effect of a single study on the pooled ORs by removing each study in turn from the pooled analysis. Although the heterogeneity had not changed obviously, the P values for pooled ORs under allelic comparison, heterozygous comparison and dominant comparison were reversed when the study [24] led by Elshazli R was removed. Therefore, we deleted this study and recalculated the relevant ORs and 95%CIs to harvest a stable and credible outcome (Fig. 3). The funnel plots were used to investigate publication bias and the outlines of the funnel plots appear to be symmetrical (Figs. 5 and 7). For rs231775 SNP (Fig. 6), the asymmetry of the funnel plot was attributed to Zhou et al.’s study which was published in Chinese. HWE estimation indicate that allele or genotype frequencies were deviant from HWE in control group in the Liu et al., Gonzalez-Escribano et al. and Sameem et al.’s studies [25, 33, 42], but the results of synthesis analysis were not substantially inversed. Hence, we don’t remove these studies from the Meta-analysis.

**Discussion**

In the current latest meta-analysis, we first investigated simultaneously and comprehensively the most-studied SNPs of CTLA-4 on RA susceptibility and provided more reliable and robust evidence. From the data integration of 66 studies in 21681 cases and 23457 controls, we found that the rs3087243 SNP was associated with RA risk in Caucasians and Asians, the rs231775 SNP of CTLA-4 was associated with RA risk in Asians but not in Caucasians and Africans, and the rs5742909 SNP was not associated with RA risk in both Caucasians and Africans.

The CTLA-4 gene, located on chromosome 2q33, encodes a 223 amino acid receptor protein on T cell surface which is responsible for T cell immune regulation. As an antagonist of the costimulatory receptor CD28 which bind the same ligand B7 as CTLA-4, CTLA-4 with higher affinity transmits an inhibitory signal and subsequently plays a suppressive role in regulating T-cell activation [62], which suggest it is involved in the pathological processes of many autoimmune disorders [12–15]. It is widely believed that RA is a T cell-mediated autoimmune disease [63], of which the chronic
inflammation and damage of the joints are typical [1]. Therefore, the effect of CTLA-4 on RA pathogenesis has get growing attentions.

Previous research had found that serum levels of soluble CTLA-4 were increased in RA patients and had a positive correlation with Disease Activity Score in RA patients and even proposed that serum levels of CTLA-4 could serve as a new marker of RA disease activity [64, 65]. Besides, function experiments in vivo indicated that gene delivery of CTLA4 by intra-articular injection could alleviated experimental arthritis [66]. Furthermore, CTLA-4Ig administration on RA synovial macrophages and T helper cells downregulated the production of proinflammatory cytokines, and these evidences suggested that CTLA-4 constituted a treatment target for RA [67, 68]. In fact, blockade of CTLA-4 by CTLA-4Ig had been successfully applied to treatment for RA [69].

As we all know, the protein level, structure and function in large part are determined by gene. Apart from these function research, numerous studies on correlation between CTLA-4 and RA risk from gene level also had been conduct to investigate genetic factors [8, 9, 13–15, 17–20, 24–27, 32–61]. However, the results were inconsistent or contrary likely due to the ethnic background, geographic environment, clinical heterogeneity, limited sample size, insufficient data and so on. Thus, it was urgently necessary to perform a comprehensive up-date Meta-analysis as an effective methodology to draw an overall objective appraisal on the association between CTLA-4 polymorphism and RA susceptibility.

In the present meta-analysis, we extracted 66 studies with 21681 cases and 23457 controls to inspect the correlation between three most-often SNPs in the CTLA-4 gene and the risk of RA. There were 22 studies with 16394 cases and 17453 controls for rs3087243 SNP, 34 studies with 11452 cases and 12444 controls for rs231775 SNP, and 10 studies with 2477 cases and 2941 controls for rs5742909 SNP. For rs3087243 polymorphism, our findings demonstrated a decreased susceptibility of RA both in total and in Caucasians in any gene mode. In total, carriers with allele A reduced an approximate 13% risk of RA than ones with allele G and genotype AA reduced 20% or so than genotype GG. Moreover, a decreased susceptibility of RA was respectively also found among Asians in the allele and homozygote comparison and among Latin Americans in the heterozygote and dominant comparison. However, only one study was included in Latin Americans and so it needed to enlarge sample size to further research. For rs231775 polymorphism, significant association did exist among the whole population in all genetic models except recessive model: compared with allele A and genotype AA, allele G and genotype GG and GA respectively was associated with an increased risk of RA. The same association was observed in Asians and Latin Americans in the subgroup analysis. On the contrary, no significant association between rs231775 SNP and RA risk could be detected in Caucasians and Africans using any gene model after excluding the Elshazli R's study [24] with the apparent heterogeneity. Here, it should be noted that only one case–control study was included in Africans and Latin Americans, so the conclusions should be cautiously explained. For rs5742909 polymorphism, no significant association between this locus polymorphism and RA risk was observed among any population in any model. Although the heterogeneity existed in some genetic model, but no obvious change had happened in heterogeneity and P value for the pooled ORs when each study was removed by sensitivity analysis.

With regard to the diverse results of the same SNP on different populations, it maybe be attributed to clinical and genetic real heterogeneity of RA, interaction of genetic background and region environment, and even lack of vigorous statistical power.

It should be pointed out that previous several meta-analyses have summarized the effect of CTLA-4 polymorphism on RA risk [18–20][70]. But a few points need be taken notice. On one hand, the conclusions they achieved were discordant as the following: the conclusion of Li's (2014) study [70] on the association of rs231775 SNP of CTLA-4 with RA was different from the others; the genetic models which indicated significant association were diverse in these analyses. These differences were mainly originated from divergent diagnostic criteria, limited number of studies and sample sizes in previous meta-analyses. On the other hand, all these meta-analyses only focused one of the three well-studied loci except Li's study [23] on two. As we all know, the expression and function of the protein are determined by the whole gene.
Therefore, it is of great necessity to investigate simultaneously the effect of all the 3 SNPs on RA risk to obtain an overall evaluation. Besides, the number of included studies in previous meta-analyses was small. Some original association studies [9, 15, 24–27] have emerged in the past few years and they can be incorporated. Taking these points into considerations, we updated the meta-analysis to achieve a more valid and comprehensive estimation on the association of CTLA-4 gene and RA susceptibility.

To our knowledge, this is a latest meta-analysis with the largest number of included studies, the largest sample size and the most-wide studied SNPs, so the conclusion draw is relatively most reliable and authentic. However, some limitations of our study should be acknowledged. Firstly, the sample sizes in some specific population such as African or Latin American and in some individual studies with less than thousands of participants were not sufficient enough to detect the genetic association between CTLA-4 SNPs and RA susceptibility. Especially, the results of certain populations including only one study should be interpreted with the caution. Secondly, we only investigated the role of three locus polymorphisms. As CTLA-4 gene had various SNPs, the function of protein CTLA-4 depended on the whole gene and RA was a multigene susceptibility disease, more SNPs of CTLA-4 should be included. Thirdly, certain degree of heterogeneity still existed in rs5742909 polymorphism and some genetic models. Although the elimination of each single study did not distinctly alter the P value, the results must still be treated cautiously. Fourthly, inadequate raw data in individual studies result in the inability to calculate the number of the genotypes in certain studies and perform stratification analysis by gender or clinical variables such as RF etc.

Conclusion

Taken together, this meta-analysis first employed simultaneously the most-studied 3 SNPs of CTLA-4 to investigate comprehensively the effect of it on RA susceptibility. The results suggested that that rs3087243 polymorphisms were correlated with a reduced RA risk in both Asian and Caucasian populations, rs231775 polymorphisms was associated with an increased risk of RA in Asian populations, and that rs5742909 polymorphism had no association with RA risk. Larger-scale studies of populations with different ethnicities are encouraged to validate the role played by CTLA-4 on the pathogenesis of RA.

Abbreviations

RA: rheumatoid arthritis; CTLA-4: Cytotoxic T lymphocyte-associated protein 4; HLA: human leukocyte antigen; SNP: Single nucleotide polymorphism; PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analyses; CNKI: China National Knowledge Infrastructure; MeSH: Medical Subject Headings; NOS: Newcastle-Ottawa Scale; OR: Odds ratio; 95% CI, 95% confidence interval; HWE: Hardy–Weinberg equilibrium.

Declarations

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Not applicable.

Authors' contributions

J.X. and H. L. conceived and designed this study. C. Z., S.G, X. Y., Z. S. and S. L. performed the experiments. C. Z., S.G analyzed the data. C. Z. and H. L. draft the manuscript. X.S. and J.X. revised the paper. All authors have contributed to the final version and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of the study are included in the current study and in the Additional file 1.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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