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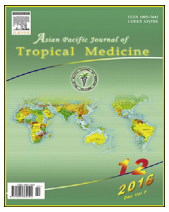

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Genetic association study of P2x7 A1513C (rs 3751143) polymorphism and susceptibility to pulmonary tuberculosis: A meta-analysis based on the findings of 11 case–control studies

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ABSTRACT

Objective: To summarize the precise association between pulmonary tuberculosis (PTB) and P2x7 A1513C gene polymorphism.

Methods: PubMed and Google Scholar web-databases were searched for the studies reporting the association of P2x7 A1513C polymorphism and PTB risk. A meta-analysis was performed for the selected case–control studies and pooled odds ratios (*ORs*) and 95% confidence intervals (95% *CIs*) were calculated for all the genetic models.

Results: Eleven studies comprising 2 678 controls and 2 113 PTB cases were included in this meta-analysis. We observed overall no significant risk in all the five genetic models. When stratified population by the ethnicity, Caucasian population failed to show any risk of PTB in all the genetics models. In Asian ethnicity, variant allele (C *vs.* A: $P = 0.001$; $OR = 1.375$, 95% $CI = 1.159–1.632$) and heterozygous genotype (AC *vs.* AA: $P = 0.001$; $OR = 1.570$, 95% $CI = 1.269–1.944$) demonstrated significant increased risk of PTB. Likewise, recessive genetic model (CC + AC *vs.* AA: $P = 0.001$; $OR = 1.540$, 95% $CI = 1.255–1.890$) also demonstrated increased risk of PTB in Asians.

Conclusions: Our meta-analysis did not suggest the association of P2x7 A1513C polymorphism with PTB risk in overall or separately in Caucasian population. However, it plays a significant risk factor for predisposing PTB in Asians. Future larger sample and expression studies are needed to validate this association.

1. Introduction

Tuberculosis (TB), a chronic disease, adds profoundly for an overall problem of infectious disease [1]. It can be categorized into two classes, namely, active disease and latent infection. The active form of the disease is characterized by production of symptoms and transmission to others. However, the latent infection with *Mycobacterium tuberculosis* (*M. tuberculosis*) occurs without any clinical symptoms. The most common type of active TB affects the lungs and is known as pulmonary TB (PTB), but when it invades other organs, it is called extrapulmonary TB (EPTB). The PTB is known to be the leading cause of mortality all over the world [1]. Infection with *M. tuberculosis* occurs in approximately 1/3 of the world's population, and in 5%–15% of these infected persons the disease progresses into clinical form during their lifespan [2]. Previous findings suggest that genetic along with environmental factors add to the development of active TB [3]. Unfortunately, TB etiology is still vague and genetic variations play a key role in regulating immune response genes that impart the risk of developing active TB [4].

The purinergic receptor P2X ligand-gated ion channel 7 (P2x7) gene which is located on the chromosome (12q24) and encodes the P2x7 receptor has been reported to be significantly associated with the risk of development of active TB [5]. Earlier

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findings have shown that ATP plays an important role in triggering anti-mycobacterial activity in human macrophages [6], performs its actions through P2x7 cell surface receptor. A series of intracellular events that include opening cation channels and pores, and initiation of nuclear factor-κB is triggered by the activation of P2x7 receptor [7]. Its activation also enhances the downstream signaling events, like stimulation of cascade of caspases, which ultimately leads to apoptosis, that promotes the fusion of phagosome and lysosome and ultimately leads to death of mycobacterial cells [8]. Previous studies have reported that apoptosis induced by ATP, and the related successive killing of bacteria, were inhibited when infected macrophages were treated with P2x7 antagonist KN62 [9]. The *P2x7* gene is extremely polymorphic, with various single nucleotide polymorphisms that affect the function of this receptor. The common among them is A1513C polymorphism found in exon 13 which converts glutamic acid to alanine at position 496, accountable for loss of receptor functions [10].

Numerous studies have reported the effect of A1513C polymorphism on PTB susceptibility in different populations but the findings lack consensus and are inconclusive [10–20]. The major conflicts in these reports can be attributed to small sample sizes, diversity in the ethnicity, and decreased statistical power. Considering the biological consequences of this genetic variant, a comprehensive pool analysis is needed to provide strength to the relationship of P2x7 1513 A>C polymorphism and PTB risk. For this purpose, we employed the meta-analysis tool which is considered a powerful tool for the analysis of collective data from different research studies in order to overcome small sample sizes and low statistical power [21]. The current study presents the updated meta-analysis of the recent data to reveal the precise association of P2x7 1513A>C polymorphism with PTB susceptibility.

2. Materials and methods

2.1. Identification and selection of pertinent studies

The PubMed (Medline) and Google Scholar web-databases were searched systematically using the following key words in combination: *P2x7* gene AND polymorphism OR mutation OR variant AND PTB to cover all the research articles published till December, 2015. The titles and abstracts of the selected studies were examined for their possible relevance to the genetic association between P2x7 and PTB. The studies matching our eligibility criteria were taken into account for this meta-analysis.

2.2. Selection criteria for inclusion and exclusion of the studies

To decrease the heterogeneity and permit the correct interpretation of the results, the studies had to pass the below mentioned criteria: (1) the study should be evaluating the P2x7 A1513C A>C polymorphism and risk of pulmonary TB, (2) the study should be only the case–control study, (3) the study should have enrolled only clinically and pathologically confirmed PTB cases and disease-free controls, (4) the study should have distribution of genotype frequency available for both the cases and the controls, and (5) the study should be published in the English language. If the same case–control study was published by more than one article, we included only the study with highest number of subjects. The main reasons for the omission of the studies from the meta-analysis were: (1) studies with overlapping of the data, (2) studies reporting cases only, and (3) review articles.

2.3. Data mining and quality evaluation

Two independent investigators assessed the methodological quality and extracted the data from all the selected research publications, individually, following a standard protocol. The accuracy of the data was determined by using a data-collection form as per our pre-set inclusion criteria. A third investigator was involved in discussion, in case of any disagreement on the retrieved data between the first two investigators, to reach a consensus. The following characters were noted from the selected studies: the first author's name with the year of publication, the country of origin, source of the study population and their numbers, study type, and genotype frequency distribution.

2.4. Statistical analysis of the data

We calculated the pooled odds ratios (*OR*s) and their respective 95% confidence intervals (*CI*s) to evaluate the relationship between the P2x7 1513A>C polymorphism and the risk of TB. The chi-square based Q-test was used to analyze the heterogeneity [22]. Lack of heterogeneity among the selected studies was revealed by Q-test significance level ($P > 0.05$). The fixed/random effects model [23,24] was employed to pool the ORs. To calculate the variability between studies (ranging between 0% and 100%, wherein, 0% suggests no observed heterogeneity and higher values specify rising degree of heterogeneity), I^2 statistics was used [25]. The Hardy–Weinberg equilibrium (HWE) in the control group was assessed using chi-square test. The funnel plot asymmetry was calculated by Egger's linear regression test – a linear regression approach. The statistically significant publication bias was indicated when *P*-value was found to be less than 0.05 as determined by *t*-test [26]. The comprehensive meta-analysis software Version 2 from Biostat, NJ, USA was used to perform the complete statistical analysis. The comprehensive meta-analysis V2 has numerous positive features in comparison to other software programs available for performing meta-analyses. The comparison of these software programs can be done using the following web-link: http://meta-analysis.com/pages/comparisons.html.

3. Results

3.1. Characteristics of the selected published studies

Initially, 28 articles were selected during literature search on PubMed (Medline), EMBASE databases, and the Google scholar. These articles were retrieved and scrutinized by their titles, abstracts and full texts for possible relevance and aptness to this meta-analysis (Figure 1). Their list of references was also screened in order to find other associated studies. Studies predicting survival or investigating the response to therapy in subjects with P2x7 polymorphism were excluded, as were those investigating P2x7 mRNA levels or the protein expression, and also review articles. After conforming to very stringent criteria for article selection, only case–control or cohort design studies mentioning the genotype frequency distribution were included.

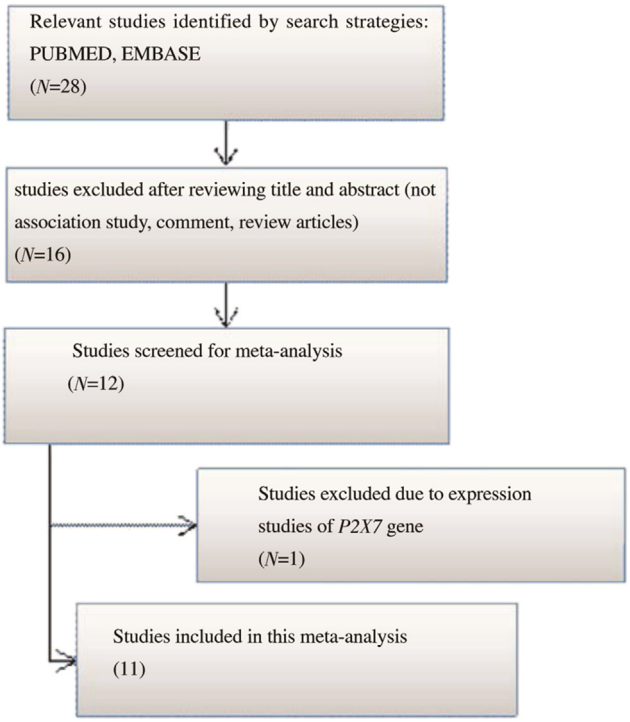

Figure 1. PRISMA flow-diagram showing the selection process (inclusion/exclusion) of the pertinent studies dealing with P2x7 A1513C (rs 3751143) polymorphism and PTB risk.

Eventually, 11 original studies as shown in Table 1 were found eligible and included for this meta-analysis. The comprehensive PRISMA flowchart of the involved selection process (inclusion/exclusion) of the studies is depicted in Figure 1. The genotype distribution, HWE *P*-values for the controls, and susceptibility to TB is given in Table 2.

3.2. Detection of publication bias

The Begg's funnel plot and Egger's test were used to estimate the publication bias among the included studies (Table 3). We observed no evidence of publication bias, by the funnel plots shape and the Egger's test results, for all comparison models (C *vs.* A, CC *vs.* AA, AC *vs.* AA, CC + AC *vs.* AA, and CC *vs.* AA + AC).

3.3. Heterogeneity test

We tested the heterogeneity by the Q-test and I^2 statistics among the selected studies. Three of the genetic models showed heterogeneity, viz., allele (C *vs.* A), heterozygous (AC *vs.* AA), and recessive (CC + AC *vs.* AA), hence random model was adopted; whereas for homozygous (CC *vs.* AA) and dominant (CC *vs.* AA + AC) models, combined OR and 95% *CI* were calculated by fixed effect model (Table 3).

Table 1
Main characteristics of the selected studies for this meta-analysis.

First authors	Year	Country of origin	Ethnicity	Genotyping method	Cases	Controls	Source of genotyping	Association*
Li *et al.* [11]	2002	Gambia	African	PCR-RFLP	325	297	Blood	No risk
Niño-Moreno *et al.* [12]	2007	Mexico	Mixed	PCR-RFLP	94	110	Blood	Risk with C-allele
Fernando *et al.* [10]	2007	Southeast Asia (Liverpool cohort)	Asian	ABI-PRISM	56	167	Blood	No risk
Fernando *et al.* [10]	2007	Australia	Caucasian	ABI-PRISM	49	102	Blood	No risk
Mokrousov *et al.* [13]	2008	Russia	Caucasian	PCR-RFLP	188	126	Blood	Border line risk
Xiao *et al.* [14]	2009	China	Asian	PCR-RFLP	41	384	Blood	No risk
Taype *et al.* [15]	2010	Peru	Caucasian	PCR-RFLP	498	513	Blood	No risk
Sambasivan *et al.* [16]	2010	India	Asian	PCR-RFLP	156	100	Blood	No risk
Sharma *et al.* [17]	2010	India	Asian	PCR-RFLP	181	177	Blood	Risk
Ben-Selma *et al.* [18]	2011	Tunisia	Caucasian	PCR-RFLP	168	150	Blood	No risk
Singla *et al.* [19]	2012	India	Asian	PCR-RFLP	286	392	Blood	Risk with C-allele
Ozdemir *et al.* [20]	2014	Turkey	Caucasian	PCR-RFLP	71	160	Blood	No risk

PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; *Association of P2x7 1513A/C & PTB.

Table 2
Distribution of P2x7 A1513C gene polymorphism studies included in this meta-analysis.

Authors and year	Control genotype				Case genotype				HWE
	AA	AC	CC	Minor allele	AA	AC	CC	Minor allele	
Li *et al.* 2002 [11]	256	37	4	0.07	261	58	6	0.10	0.05
Niño-Moreno *et al.* 2007 [12]	70	38	2	0.19	53	33	8	0.26	0.21
Fernando *et al.* 2007 [10]	105	55	7	0.20	34	17	5	0.24	0.95
Fernando *et al.* 2007 [10]	64	34	4	0.20	28	21	0	0.21	0.84
Mokrousov *et al.* 2008 [13]	96	27	3	0.13	120	59	9	0.20	0.43
Xiao *et al.* 2009 [14]	221	119	44	0.26	21	18	2	0.26	0.00
Taype *et al.* 2010 [15]	347	149	17	0.17	352	130	16	0.16	0.83
Sambasivan *et al.* 2010 [16]	71	21	8	0.18	89	55	12	0.25	0.002
Sharma *et al.* 2010 [17]	126	48	3	0.15	102	75	4	0.22	0.51
Ben-Selma *et al.* 2011 [18]	104	40	6	0.17	130	34	4	0.12	0.39
Singla *et al.* 2012 [19]	258	123	11	0.18	162	112	12	0.23	0.41
Ozdemir *et al.* 2014 [20]	76	63	21	0.32	44	18	9	0.25	0.17

HWE: Hardy–Weinberg Equilibrium.

Table 3

Statistics to test publication bias and heterogeneity: Overall analysis.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model
	Intercept	95% *CI*	*P*-value	Q-value	$P_{heterogeneity}$	I^2 (%)	
C *vs.* A	0.62	−3.13 to 4.38	0.71	27.68	0.004	60.27	Random
CC *vs.* AA	0.23	−1.99 to 2.45	0.82	12.13	0.350	9.34	Fixed
AC *vs.* AA	0.61	−3.05 to 4.28	0.71	30.90	0.001	64.41	Random
CC + AC *vs.* AA	0.67	−3.08 to 4.44	0.69	30.72	0.001	64.19	Random
CC *vs.* AA + AC	−0.03	−2.11 to 2.05	0.97	10.73	0.460	0.001	Fixed

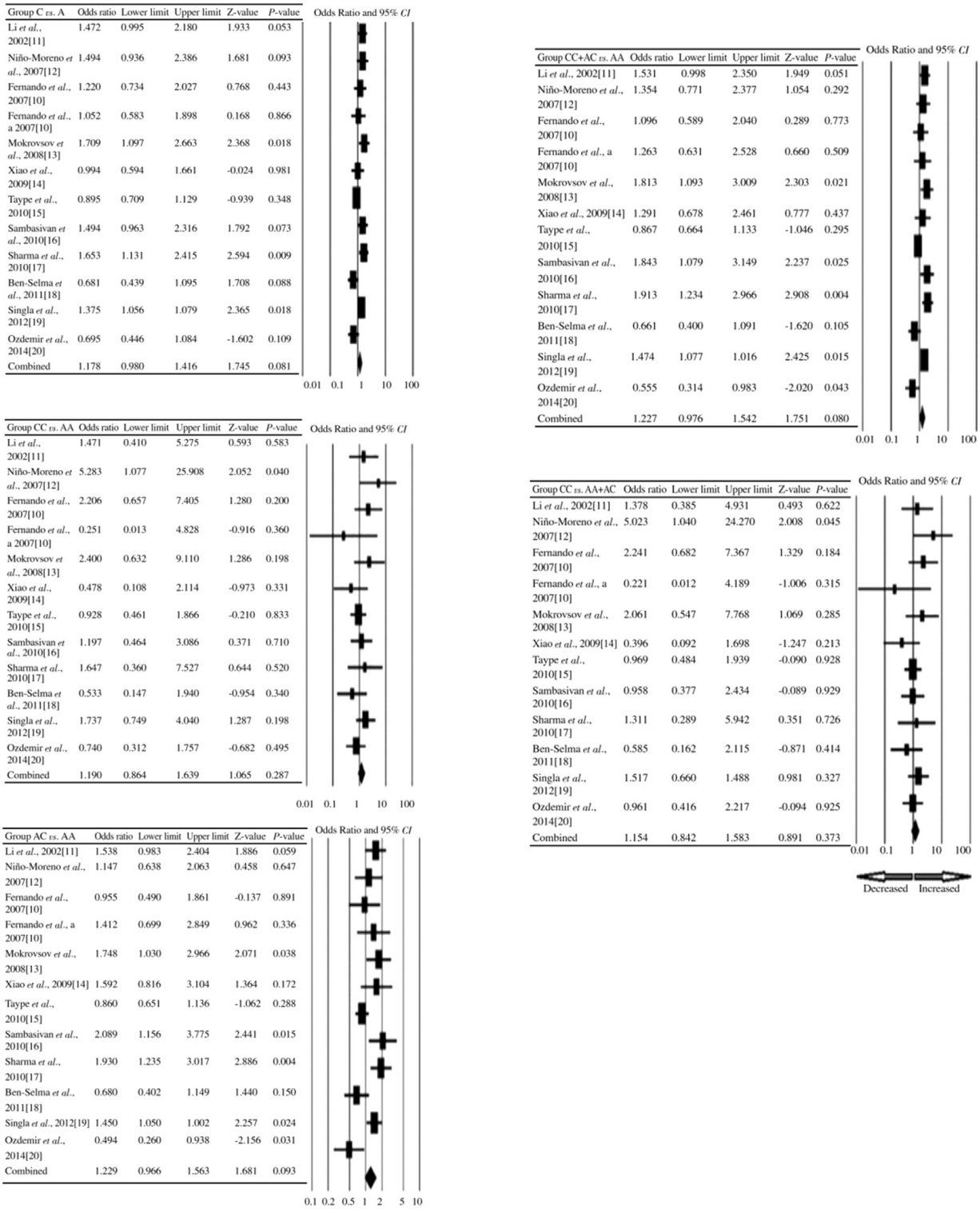

Figure 2. Forest plot for overall analysis (allele: C *vs.* A; homozygous: CC *vs.* AA; heterozygous: AC *vs.* AA; recessive: CC + AC *vs.* AA; dominant: CC *vs.* AA + AC) showing OR with 95% CI to evaluate the association of the P2x7 A1513C (rs 3751143) polymorphism and PTB risk.

3.4. Meta-analysis for P2x7 1513A>C polymorphism and its association with PTB susceptibility

Combining all the eleven studies together resulted in total of 2678 controls and 2113 confirmed PTB cases. The overall association between the P2x7 1513 A>C polymorphism and the PTB development risk was assessed by the fixed effects and random effects models which were decided on the basis of heterogeneity (Figure 2). Overall, no significant risk of PTB with P2x7 1513 A>C polymorphism was observed with variant allele (C *vs.* A: $P = 0.081$; $OR = 1.178$, 95% $CI = 0.980–1.416$), homozygous (CC *vs.* AA: $P = 0.287$; $OR = 1.190$, 95% $CI = 0.864–1.639$), heterozygous AC (AC *vs.* AA: $P = 0.093$; $OR = 1.229$, 95% $CI = 0.996–1.563$), recessive (CC + AC *vs.* AA: $P = 0.080$; $OR = 1.227$, 95% $CI = 0.976–1.542$), or in dominant (CC *vs.* AA + AC: $P = 0.373$; $OR = 1.154$, 95% $CI = 0.842–1.583$) genetic models (Figure 2).

3.5. Sensitivity analysis

In order to examine the effect of each individual study on the pooled *ORs*, we successively omitted one study at a time from the analysis. We observed that no individual study influenced the pooled *ORs* considerably, hence results of this meta-analysis were relatively stable and credible (data not shown).

3.6. Sub-group analysis

We have divided the performed studies based on ethnicity to perform sub-group analysis between the P2x7 1513 A>C polymorphism and risk of PTB in Caucasian and Asian population. The sub-group analysis included five studies from Caucasian and five studies from Asian populations.

3.7. Caucasian population

In Caucasian ethnicity, publication bias did not exist. Heterogeneity was found in three genetic models (C *vs.* A, AC *vs.* AA, CC + AC *vs.* AA), so random effect model was applied for the evaluation (Table 4). No genetic models, *i.e.*, allele (C *vs.* A: $P = 0.330$; $OR = 0.921$, 95% $CI = 0.781–1.087$), homozygous (CC *vs.* AA: $P = 0.587$; $OR = 0.879$, 95% $CI = 0.553–1.397$), heterozygous genotype (AC *vs.* AA: $P = 0.350$; $OR = 0.908$, 95% $CI = 0.742–1.111$), recessive (CC + AC *vs.* AA: $P = 0.318$; $OR = 0.907$, 95% $CI = 0.748–1.099$) and dominant (CC *vs.* AA + AC: $P = 0.850$; $OR = 0.957$, 95% $CI = 0.606–1.511$) showed any risk of PTB occurrence in Caucasian population (Figure 3).

3.8. Asian population

Heterogeneity and publication bias was not found in Asian ethnicity, so we employed the fixed effect model to assess the

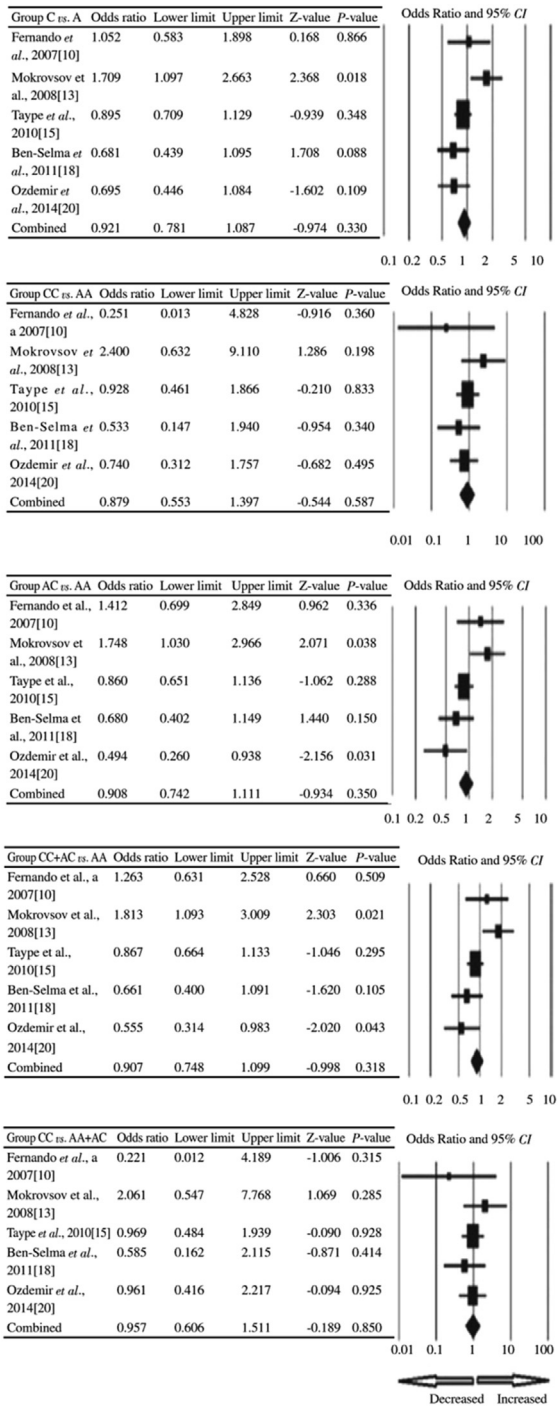

Figure 3. Forest plot for Caucasian population (allele: C *vs.* A; homozygous: CC *vs.* AA; heterozygous: AC *vs.* AA; recessive: CC + AC *vs.* AA; dominant: CC *vs.* AA + AC) showing OR with 95% CI to evaluate the association of the P2x7 A1513C (rs 3751143) polymorphism and PTB risk.

Table 4
Statistics to test publication bias and heterogeneity in the meta-analysis: Caucasian population.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model
	Intercept	95% *CI*	*P*-value	Q-value	$P_{heterogeneity}$	I^2 (%)	
C *vs.* A	0.61	−7.5 to 8.73	0.82	11.05	0.02	63.81	Random
CC *vs.* AA	−0.47	−4.4 to 3.46	0.72	3.61	0.46	0.001	Fixed
AC *vs.* AA	0.52	−7.53 to 8.59	0.84	12.19	0.01	67.19	Random
CC + AC *vs.* AA	0.61	−7.70 to 8.94	0.82	12.54	0.01	68.11	Random
CC *vs.* AA + AC	−0.72	−3.98 to 2.54	0.53	2.80	0.59	0.001	Fixed

Table 5
Statistics to test publication bias and heterogeneity in this meta-analysis: Asian population.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model
	Intercept	95% *CI*	*P*-value	Q-value	$P_{heterogeneity}$	I^2 (%)	
C *vs.* A	−1.14	−5.97 to 3.67	0.50	2.78	0.59	0.001	Fixed
CC *vs.* AA	−1.27	−6.87 to 4.33	0.52	2.94	0.56	0.001	Fixed
AC *vs.* AA	−0.12	−5.82 to 5.57	0.94	4.08	0.39	2.140	Fixed
CC + AC *vs.* AA	−0.67	−5.58 to 4.22	0.68	2.88	0.57	0.001	Fixed
CC *vs.* AA + AC	−1.28	−7.8 to 5.24	0.57	3.82	0.43	0.001	Fixed

association (Table 5). We found that variant allele (C *vs.* A: *P* = 0.001; *OR* = 1.375, 95% *CI* = 1.159–1.632) and heterozygous genotype (AC *vs.* AA: *P* = 0.001; *OR* = 1.570, 95% *CI* = 1.269–1.944) revealed significant increased risk to PTB.

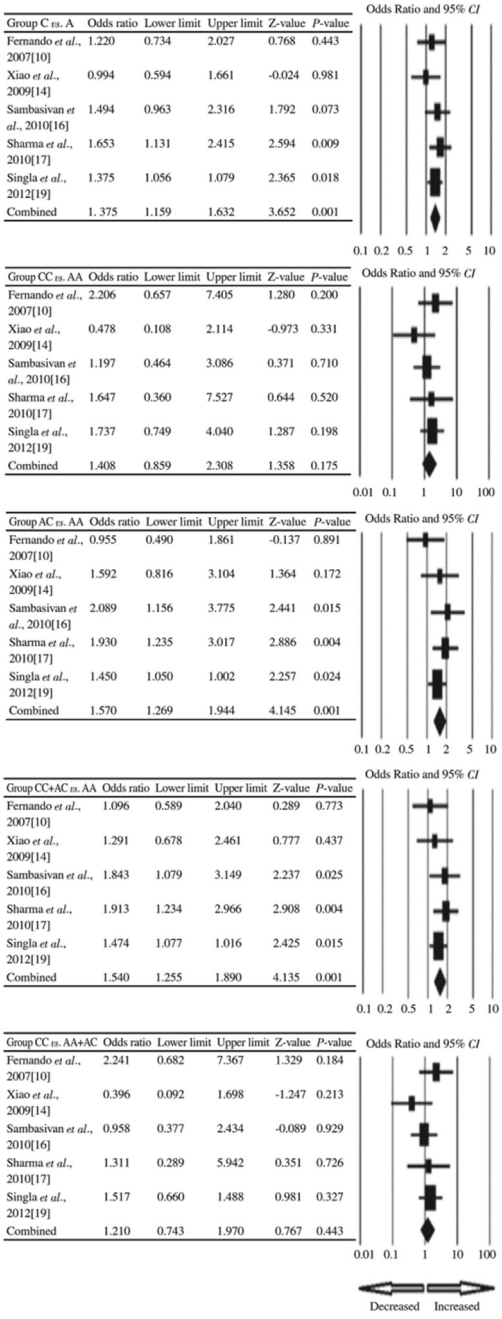

Figure 4. Forest plot for Asian population (allele: C *vs.* A; homozygous: CC *vs.* AA; heterozygous: AC *vs.* AA; recessive: CC + AC *vs.* AA; dominant: CC *vs.* AA + AC) showing OR with 95% CI to evaluate the association of the P2x7 A1513C (rs 3751143) polymorphism and PTB risk.

Likewise, recessive (CC + AC *vs.* AA: *P* = 0.001; *OR* = 1.540, 95% *CI* = 1.255–1.890) genetic model also presented increased risk of PTB. However, homozygous genotype (CC *vs.* AA: *P* = 0.175; *OR* = 1.408, 95% *CI* = 0.859–2.308) and dominant (CC *vs.* AA + AC: *P* = 0.443; *OR* = 1.210, 95% *CI* = 0.743–1.970) genetic models failed to show any risk of PTB (Figure 4).

4. Discussion

Susceptibility of PTB to genetic variations has recently led to increased awareness regarding studies investigating association of various gene polymorphisms with PTB. This has resulted in tremendous increase in exploration of numerous candidate genes for possible relationship of their modulations with risk of PTB across different populations around the world. Till date, various studies regarding P2x7 1513 A>C gene polymorphism and the threat of developing PTB have been done to assess this relationship. These studies are limited largely by the inconsistency and inconclusiveness of their results. So, to provide a comprehensive and reliable conclusion, we performed the present meta-analysis to improve the statistical power of the association, if any, between the P2x7 1513 A>C polymorphism and risk of PTB. We in this analysis combined the data from 11 studies from different populations which were selected on the basis of stringent criteria adopted during the process. Combining data from several different studies has the benefit of reducing the random errors [27].

We did not find any significant association of this polymorphism with increased or decreased risk of acquiring PTB, in overall population, in all the genetic models compared with wild type genotype. Likewise, ethnicity based analysis did not show any risk of PTB in Caucasian ethnicity. Whereas, variant allele, heterozygous genotype and recessive model were associated with significantly increased risks of PTB, in comparison to the wild type genotype, in Asian ethnicity. This finding clearly indicates that the P2x7 1513 A>C polymorphism may play a potential susceptibility to PTB development in Asian ethnicity. Our results are attuned with Wu *et al.* [28], where they reported significant increased risk in Asian ethnicity. But, due to limited database selection, Wu *et al.* [28] failed to include all the relevant published studies, as the research article available in one database is not necessarily available in another one. So, every meta-analysis has certain limitations of database selection, also some relevant studies may have been published in languages other than English. In the present meta-analysis we searched most reliable and vast databases (*i.e.* PubMed/Google Scholar) and tried to increase the number of subjects including new studies along with some of the missing studies which were not included in previous studies and concluded accordingly. Our current findings are concurrent with the previously published

study of Wang *et al.* [29] where they reported no association between P2x7 1513 A>C polymorphism and overall susceptibility to PTB based on six studies comprising 2525 subjects. Also, this meta-analysis reports increased risk of PTB in Asian population in relation with P2x7 1513 A>C polymorphism. Comparatively, our study has major improvements with respect to the earlier reports as it included more number of relevant published studies and ultimately higher number of subjects (4791), which strengthens the results and corroborated the previous findings of Wang *et al.* [29] and Wu *et al.* [28].

Based upon the statistical data finings presented above and the significant role of P2x7 in the pathogenesis of PTB, it is plausible to say that the 1513A>C polymorphism may modulate the PTB risk. The data also suggest that P2x7 could be a potential genetic factor for differences between individuals in PTB susceptibility in Asian population. The P2x7 receptor is a crucial molecule in the clearance of *M. tuberculosis* in macrophages by ATP-induced *Mycobacterial* killing. It has been shown that the P2x7 receptor 1513A>C polymorphism has an effect on the sensitivity of the P2x7 receptor to ATP and *ex vivo* cytokine release [30]. Heterozygous genotype of P2x7 1513 A>C polymorphism resulted in halving the cell function compared to the cells with germline P2x7 protein [31]. Studies have also shown that *M. tuberculosis* infected P2x7 mice have bigger *M. tuberculosis* burden in their lung tissues as compared to infected wild-type mice [32].

Tuberculosis is known to be caused by polygenic susceptibility [33]; hence, variations in a single gene cannot be considered adequate for risk of this dreaded disease. The important feature of this genetic variation is that its occurrence varies among different races and ethnicities. There are certain limitations to this meta-analysis which need to be mentioned here before reaching any conclusion. The first limitation was that we detected significant heterogeneity in three genetic models in the overall and Caucasian population analysis. Whereas, two of the studies included in this meta-analysis deviated from HWE in the control group, which may be because of potential errors in the laboratory or genotyping, stratification of population or bias in selection in choosing controls and also other confounding factors [34], we performed sensitivity analysis and found our results and conclusions to be stable. The second limitation was that only studies in English language were included. The third limitation was that the studies indexed only by PubMed, EMBASE, Google Scholar databases were included; there is the possibility that some articles published in other languages, not known to us, may have been missed. The fourth limitation was that other factors such as HIV status, severity of TB, and interactions of the gene with the environment were not considered due to lack of adequate information available in the primary selected articles.

Despite these drawbacks, there are some advantages as well associated with this meta-analysis. First, this meta-analysis involved more studies than the earlier published researches making this to be more balanced and robust. Second, the subgroup analysis was made on population and found that P2x7 1513 A>C polymorphism have the more likelihood to develop PTB in Asians, whereas, no risk of developing PTB in Caucasian population.

In summary, this meta-analysis advocates that the P2x7 1513A>C polymorphism is associated with PTB susceptibility in Asian population. Nevertheless, future larger well-designed, comprehensive studies, considering interactions of various environmental and confounding factors, are warranted to validate this association.

Conflict of interest statement

The authors state no conflict of interest.

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