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ORIGINAL ARTICLE

Genetic variants of *SLC11A1* are associated with both autoimmune and infectious diseases: systematic review and meta-analysis

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A systematic review and meta-analyses were undertaken to investigate the association of *SLC11A1* genetic variants with disease occurrence. Literature searching indentified 109 publications to include in the meta-analyses assessing the association of 11 *SLC11A1* variants with autoimmune and infectious disease. The $(GT)_n$ promoter alleles 2 and 3 (rs534448891), which alter *SLC11A1* expression, were significantly associated with tuberculosis (OR = 1.47 (1.30–1.66), OR = 0.76 (0.65–0.89), respectively) and infectious disease (OR = 1.25 (1.10–1.42), OR = 0.83 (0.74–0.93), respectively). However, although no association was observed with autoimmune disease, a modest significant association was observed with type 1 diabetes (allele 2 OR = 0.94 (0.89–0.98)). On the basis of a stronger association of $(GT)_n$ allele 2 with tuberculosis, compared with the protective effect of allele 3, we hypothesise that allele 2 is likely the disease-causing variant influencing disease susceptibility. Significant associations were observed between the 469+14G/C polymorphism (rs3731865) and autoimmune disease (OR = 1.30 (1.04–1.64)) and rheumatoid arthritis (OR = 1.60 (1.20–2.13)) and between the −237C/T polymorphism (rs7573065) and inflammatory bowel disease (OR = 0.60 (0.43–0.84)). Further, significant associations were identified between the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms (rs3731865, rs17235409 and rs17235416, respectively) and both infectious disease *per se* and tuberculosis. These findings show a clear association between variants in the *SLC11A1* locus and autoimmune and infectious disease susceptibility.

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INTRODUCTION

Solute Carrier Family 11A Member 1 (SLC11A1), formerly NRAMP1, has an immunomodulatory role in influencing macrophage activation status and the T-helper 1/T-helper 2 bias. SLC11A1 appears to have multiple functions, having a role in both the resolution of infections and erythrophagocytosis.[1–5] Localised to the endosomal/lysosomal compartment of macrophages, SLC11A1 functions as a divalent cation symporter[6,7] which, when recruited to the phagosomal membrane, transports ions out of the phagosome along the proton gradient.[8–10] SLC11A1 elicits a range of pleiotropic effects on macrophage function, including increased expression of pro-inflammatory cytokines (interleukin-1β and tumour necrosis factor-α), production of pro-inflammatory effector molecules (increased inducible nitric oxide synthase expression, resulting in increased L-arginine flux, and subsequent production of nitric oxide and oxidative burst), and modulation of an adaptive immune response (increased major histocompatibility complex class II expression and enhanced antigen presentation to T-cells).[11–15] How divalent cation transport by SLC11A1 mediates these pleiotropic effects is currently unknown (i.e. through either a direct effect or as a secondary result of SLC11A1 activity); however, these pleiotropic effects are essential in the resolution of infection and also in the initiation and perpetuation of Th1-mediated autoimmune diseases.

Owing to the immunomodulatory capabilities of SLC11A1, the encoding gene is a strong candidate for influencing autoimmune and infectious disease susceptibility. Infectious and autoimmune diseases are complex multi-factorial diseases with multiple genetic (both host and pathogen) and environmental factors having an aetiological role. An understanding of the host genetic factors involved in these complex diseases will help to develop new preventative and therapeutic strategies. Although murine models show a strong correlation between the expression of functional *Slc11a1* and both resistance to macrophage-tropic pathogens and susceptibility to autoimmune disease,[1,5,16–18] familial- and case-control association studies analysing the association of *SLC11A1* variants with disease incidence in humans have produced inconsistent results.

Of the most commonly assessed *SLC11A1* variants, the polymorphic $(GT)_n$ microsatellite repeat has been shown to alter the level of *SLC11A1* expression,[19,20] and is therefore a strong candidate for influencing disease incidence. Several alleles of different repeat length have been identified, with $(GT)_n$ allele 2 conferring lower *SLC11A1* expression compared with the more commonly occurring $(GT)_n$ allele 3. It has therefore been hypothesised that allele 3 would provide protection against infectious disease by driving high *SLC11A1* expression and a resultant Th1-mediated immune response. However, allele 3 would also be associated with an increased susceptibility to Th1-mediated autoimmune diseases.[19] Other *SLC11A1* variants, including the −237C/T promoter and 1730G/A (D543N) polymorphisms, have also been suggested to modulate expression or alter the functional capacity of SLC11A1 to transport divalent cations, respectively.[20,21] Although several meta-analyses

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assessing the association of *SLC11A1* polymorphisms with the incidence of tuberculosis ($(GT)_n$ repeat, 1730G/A and two additional variants)[22–26] and autoimmune disease ($(GT)_n$ repeat only)[27,28] have been completed, no study to date has systematically reviewed the literature and completed meta-analyses for all *SLC11A1* polymorphisms (Figure 1). The objective of this study was to systematically review the literature to identify all the case-control association studies and where possible complete the meta-analyses to determine if *SLC11A1* variants are associated with autoimmune and infectious disease occurrence.

The current meta-analysis was undertaken for a number of reasons. Firstly, there has been a twofold increase in the number of case-control association studies completed since the most current meta-analysis of the association of the $(GT)_n$ promoter polymorphism with autoimmune disease incidence was completed.[27,28] Secondly, the current meta-analysis is more inclusive than all other meta-analyses, including all infectious diseases (excluding viruses). Previous meta-analyses have only assessed pulmonary tuberculosis publications.[22–26] Finally, this meta-analysis assessed a number of polymorphisms within *SLC11A1* for which meta-analyses to determine disease association had not been previously performed because of insufficient numbers of published studies. Specifically, we present novel findings of the association of 17 *SLC11A1* variants with autoimmune and infectious diseases.

Overall, the present study constitutes the largest and most inclusive meta-analysis examining the association of *SLC11A1* polymorphisms with the incidence of infectious and autoimmune diseases conducted to date. Furthermore, based on the findings, inferences about possible functional variants responsible for the identified associations are presented.

RESULTS

A total of 131 case-control studies were identified through literature searches and cross-referencing, of which 117 publications were included in the meta-analysis as they assessed the association of *SLC11A1* variants with autoimmune or infectious disease (Figure 2, Supplementary Tables S1–S3). Furthermore 8 publications were excluded from the analysis owing to duplicate reporting of identical data. From the 36 identified publications covering the autoimmune disease, 11 *SLC11A1* polymorphisms had been investigated in a sufficient number of association studies to allow completion of a meta-analysis (a total of 160 associations; Table 1). Of the 84 publications investigating infectious disease, 10 *SLC11A1* variants had been examined in a sufficient number of case-control studies to perform meta-analyses (274 associations in total; Table 1).

Associations of the $(GT)_n$ promoter variants with the incidence of autoimmune disease

Meta-analyses assessing the association of *SLC11A1* $(GT)_n$ alleles 2 and 3 with autoimmune disease (28 data sets) yielded non-significant pooled odds ratio (OR) estimates of 0.93 (confidence interval (CI):0.83–1.05) and 1.07 (0.94–1.22; Table 2, Supplementary Tables S4 and S5). Analysis of the funnel plots from the meta-analyses did not indicate bias within the data sets. Further analysis of the association of $(GT)_n$ allele 3 with individual autoimmune diseases found a significant association with the incidence of type 1 diabetes (pooled OR estimates 1.07 (1.01–1.12); Table 2). Conversely, the association of $(GT)_n$ allele 2 with the incidence of type 1 diabetes showed a significant protective effect (OR = 0.94 (CI: 0.89–0.98); Table 2). No association was observed between either $(GT)_n$ allele 2 or 3 and the occurrence of, specifically, inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis and sarcoidosis (Table 2). When stratified according to ethnicity, a significant association was observed between both alleles 3 (OR = 1.75 (1.19–2.59)) and 2 (protective effect OR = 0.58 (0.35–0.96)) and autoimmune disease incidence in the African population; however, similar findings were not observed in either of the Asian, European or Mediterranean populations (Table 3).

The 469+14G/C (INT4) variant is significantly associated with the incidence of autoimmune disease and rheumatoid arthritis

Prior to this study, the $(GT)_n$ promoter polymorphism had been the only *SLC11A1* genetic variant to be analysed for association with autoimmune disease, as there were insufficient association studies on other *SLC11A1* variants to enable meta-analyses to be completed.[28] In addition to the $(GT)_n$ repeat polymorphism (Table 2, Supplementary Tables S6–S15), we report, for the first time, the results of meta-analyses assessing the associations of 10 additional *SLC11A1* variants with autoimmune disease. Analysis of

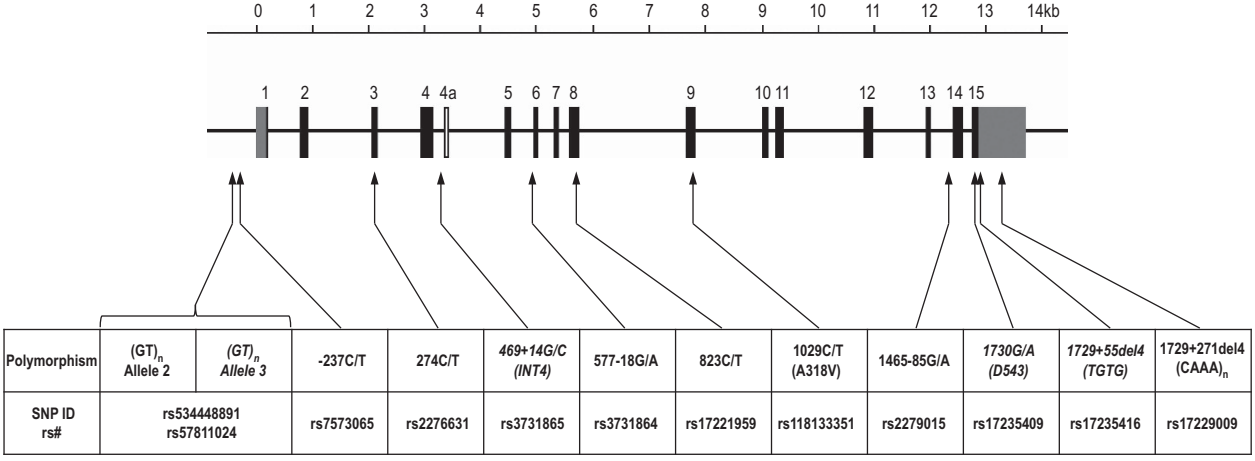


Figure 1. Location of *SLC11A1* polymorphisms analysed in the meta-analysis. Associations between the occurrence of these polymorphisms and the incidence of autoimmune and infectious disease were analysed using meta-analyses. The 15 exons of the gene are shown as black boxes with their respective numbers. The corresponding scale above indicates the length (kb) of the gene. The grey boxes indicate the 3′- and 5′-untranslated regions, and the introns and flanking regions are represented by a thin line. The arrows indicate the position of sequence variants. Below each polymorphism is the reference single-nucleotide polymorphism (rs#) identification number. Genetic variants shown in italics are those for which meta-analyses have previously been performed.

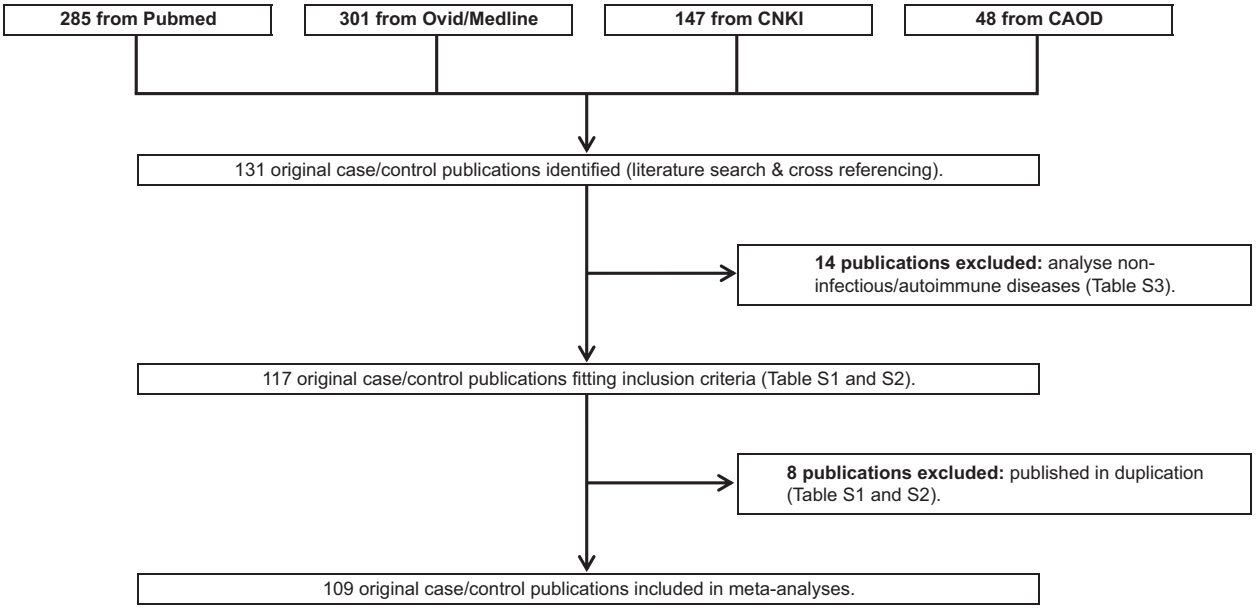


Figure 2. Results of the search strategy showing the number of case-control publications identified and excluded from the meta-analyses.

Table 1. Summary of identified publications, data sets, analysed and numbers of cases and controls

Polymorphism	*Autoimmune disease*					*Infectious disease*				
	Publications[a]	*Data sets*[b]	*Analysed*[c]	*Cases*	*Controls*	*Publications*[a]	*Data sets*[b]	*Analysed*[c]	*Cases*	*Controls*
$(GT)_n$ allele 3	29	31	28	10 602	10 797	29	30	25	5411	6118
$(GT)_n$ allele 2	29	31	28	10 664	10 919	29	30	19	3753	3622
−237C/T	7	9	9	6408	6233	7	8	6	1321	1425
274C/T	9	9	9	6546	7074	13	15	14	2847	3593
469+14G/C	15	15	15	10 806	12 540	48	52	47	7029	8170
577−18G/A	6	6	5	711	691	3	3	3	162	291
823C/T	8	8	8	922	952	4	4	3	270	355
1029C/T	7	7	4	850	775					
1465−85G/A	9	8	8	6342	6639	7	8	7	1705	1690
1730G/A	17	16	16	8010	8149	55	59	54	8174	8698
1729+55del4	18	17	16	10 321	11 790	56	58	52	8864	10 290
1729+271del4	3	3	3	480	309	6	7	7	1648	2455

[a]Total number of published studies identified from the literature search meeting the inclusion criteria of the meta-analysis. [b]Total number of data sets from the identified publications for inclusion into the meta-analysis. [c]The number of data sets analysed in the meta-analysis after the removal of data sets containing zero observations for both cases and controls and when data to determine the OR was not forthcoming from the corresponding authors.

the 469+14G/C (INT4) polymorphism identified that the less frequent C variant was significantly associated with the occurrence of autoimmune disease (OR = 1.30 (CI:1.04–1.64); Table 2, Supplementary Table S8). Surprisingly, the observed association of the C variant with disease occurrence is in opposition to the significant protective effect identified in the large sample size ($n = 8787$ cases, 10 611 controls) of the study of Yang *et al*.[29] Re-analysis in the absence of this large study did not alter the observed association (OR = 1.39 (CI:1.24–1.56)). Further analysis of the 469+14G/C polymorphism identified a significant association between the less frequent C variant and the occurrence of rheumatoid arthritis (OR = 1.60 (1.20–2.13)), but not sarcoidosis (Table 2).

No significant associations were identified between the *SLC11A1* polymorphisms, −237C/T, 274C/T, 577-18G/A, 823C/T, 1029C/T, 1465-85G/A, 1730G/A, 1729+55del4 and 1729+271del4, and the incidence of autoimmune disease (Table 2). However, although the −237C/T polymorphism was not associated with autoimmune disease as a whole, further analysis of the −237C/T polymorphism found that the less frequent T variant exerted a putative protective effect over the onset of inflammatory bowel disease (combined Crohn's disease and ulcerative colitis; OR = 0.60 (CI:0.43–0.84)).

Associations of the $(GT)_n$ promoter variants with the incidence of infectious disease

The meta-analyses of the association of $(GT)_n$ alleles 2 and 3 with the incidence of infectious disease included 19 and 25 data sets, respectively (Table 1, Supplementary Tables S16 and S17). The meta-analyses showed that $(GT)_n$ allele 2 was significantly associated with the incidence of infectious disease (OR = 1.25 (1.10–142)), whereas $(GT)_n$ allele 3 was shown to be protective against the occurrence of infectious disease (OR = 0.83 (0.74–0.93); Table 4). An analysis of the funnel plots indicated the presence of bias within the data sets (see Supplementary Tables S16 and S17). Although the trim and fill method was previously used to adjust for bias,[28] use of the trim and fill method in the current analysis was not required, because if the funnel plots did not show bias

(i.e., the 'missing' studies were filled in) they would be located in a position that would strengthen the pooled OR estimate.

Further analysis of the association of $(GT)_n$ alleles 2 and 3 with the incidence of tuberculosis alone, revealed a stronger association than those observed with the occurrence of infectious disease *per se*, with fixed- and random-effects pooled ORs of 1.47 (1.30–1.66) and 0.75 (0.69–0.82), respectively (Table 4). The results of the current meta-analysis show that the association of $(GT)_n$ allele 2 with the incidence of tuberculosis alone is more significant than the protective effect putatively exerted by $(GT)_n$ allele 3. No association was identified between $(GT)_n$ allele 3 and the incidence of leprosy (Table 4).

Stratification of the data based on ethnicity revealed that $(GT)_n$ allele 2 was significantly associated with infectious disease susceptibility in the African population, with a susceptibility trend that failed to reach significance among the Asian and European populations (Table 3). Furthermore, no association was found in the South-American population. Allele 3 was found to be significantly associated with resistance to infectious disease in the African and Asian populations; however, no association was found among the European and South American populations (Table 3). Although the lack of association of both $(GT)_n$ alleles 2 and 3 with the occurrence of infectious disease in the South-American population may be because of the small numbers of publications completed to date ($n = 2$), conflicting results were observed with the association of the $(GT)_n$ alleles with infectious disease in the European population. The results from the European population indicate that allele 2 may be associated with the incidence of infectious disease (OR = 1.24), whereas allele 3 appears to have no role in affording disease protection (OR = 1.01), suggesting that allele 2 exerts a greater influence over infectious disease susceptibility in the European population, compared with allele 3.

Table 2. Pooled OR estimates of the association of *SLC11A1* polymorphisms with the incidence of autoimmune disease

Polymorphism association	*Test of heterogeneity* χ^2 (P-*value*)	*Pooled OR estimate (CI)*
$(GT)_n$ allele 3	93.77 ($P < 0.01$)	1.07 (0.94–1.22)[a]
IBD	17.71 ($P = 0.01$)	1.05 (0.81–1.37)[a]
MS	12.80 ($P < 0.01$)	1.22 (0.80–1.85)[a]
RA	18.08 ($P < 0.01$)	1.06 (0.75–1.51)[a]
SA	30.06 ($P < 0.01$)	1.16 (0.59–2.28)[a]
T1D	1.68 ($P = 0.64$)	**1.07 (1.01–1.12)**[b]
$(GT)_n$ allele 2	73.35 ($P < 0.01$)	0.93 (0.83–1.05)[a]
IBD	4.70 ($P = 0.70$)	0.91 (0.78–1.06)
MS	14.58 ($P < 0.01$)	0.84 (0.53–1.33)[a]
RA	15.48 ($P < 0.01$)	0.91 (0.65–1.26)[a]
SA	24.43 ($P < 0.01$)	0.96 (0.52–1.80)[a]
T1D	3.91 ($P = 0.27$)	**0.94 (0.89–0.98)**[b]
−237C/T	12.43 ($P = 0.13$)	0.92 (0.83–1.02)
IBD	5.82 ($P = 0.32$)	**0.60 (0.43–0.84)**[b]
274C/T	18.41 ($P = 0.01$)	1.16 (0.96–1.40)[a]
469+14G/C	86.50 ($P < 0.01$)	**1.30 (1.04–1.64)**[a,b]
RA	1.82 ($P = 0.61$)	**1.60 (1.20–2.13)**[b]
SA	21.17 ($P < 0.01$)	1.07 (0.53–2.18)[a]
577-18G/A	2.87 ($P = 0.58$)	0.74 (0.50–1.09)
823C/T	23.71 ($P < 0.01$)	1.02 (0.67–1.56)[a]
1029C/T	1.57 ($P = 0.67$)	0.48 (0.21–1.11)
1465-85G/A	10.98 ($P = 0.14$)	0.98 (0.93–1.03)
1730G/A	46.45 ($P < 0.01$)	1.14 (0.86–1.51)[a]
RA	14.45 ($P < 0.01$)	1.29 (0.62–2.68)[a]
1729+55del4	34.75 ($P < 0.01$)	1.21 (0.96–1.54)[a]
RA	19.09 ($P < 0.01$)	1.52 (0.67–3.44)[a]
1729+271del4	1.79 ($P = 0.41$)	0.98 (0.80–1.22)

Abbreviations: CI, confidence interval; IBD, inflammatory bowel disease; MS, multiple sclerosis; OR, odds ratio; RA, rheumatoid arthritis; SA, sarcoidosis; T1D, type 1 diabetes. [a]Random-effects pooled OR estimate. [b]Statistically significant ($P < 0.05$, bold).

The 469+14G/C, 1730G/A and 1729+55del4 polymorphisms are associated with the incidence of infectious disease

Meta-analyses assessing the association of the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms with the incidence of infectious disease included 47, 54 and 52 data sets, respectively (Table 1, Supplementary Tables S20, S24 and S25). The meta-analyses revealed that the presence of the less frequent variant for each polymorphism was significantly associated with the incidence of infectious disease, with random-effects pooled OR estimates of 1.27 (1.12–1.43), 1.23 (1.08–1.40) and 1.25 (1.13–1.38) for the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms, respectively (Table 4). Furthermore, analysis of the association of the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms with the incidence of tuberculosis alone identified a significant association consistent with previous meta-analyses,[23,24,26] with OR estimates of 1.31, 1.24 and 1.31, respectively (Table 4). Significant heterogeneity, as determined by the Cochran *Q*-value, was identified within the data sets of the meta-analyses assessing both infectious disease and tuberculosis alone for all three polymorphisms (Table 4). No association between the occurrence of the 1729+55del4 polymorphism and the incidence of leprosy was identified (Table 4). No asymmetry was identified in the data from the analysis of the funnel plots for the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms.

Analysis of the association of the 469+14G/C, 1730G/A and 1729+55del4 polymorphisms with the occurrence of infectious disease among different ethnicities identified a trend in which the less frequent variant for each polymorphism was associated with the incidence of infectious disease (Table 3). In particular, a significant association was identified between each polymorphism and the incidence of infectious disease in the Asian population. The 469+14C/C and 1730G/A polymorphisms were significantly associated

Table 3. Pooled OR estimates of the association of *SLC11A1* variants and disease occurrence stratified by ethnicity

Ethnicity	*Autoimmune disease*		*Infectious disease*				
	$(GT)_n$ allele 3	*$(GT)_n$ allele 2*	*$(GT)_n$ allele 3*	*$(GT)_n$ allele 2*	*469+14G/C*	*1730G/A*	*1729+55del4*
African	**1.75 (1.19–2.59)**[a]	**0.58 (0.35–0.96)**[a]	**0.80 (0.66–0.97)**[a,b]	**1.45 (1.22–1.71)**[a]	**1.37 (1.14–1.65)**[a]	**1.57 (1.11–2.24)**[a,b]	1.11 (1.00–1.21)
Asian	0.85 (0.69–1.03)	0.86 (0.68–1.09)	**0.76 (0.64–0.92)**[a,b]	1.23 (0.98–1.53)[b]	**1.53 (1.15–2.04)**[a,b]	**1.33 (1.14–1.55)**[a,b]	**1.34 (1.16–1.57)**[a,b]
European	1.17 (0.97–1.42)[b]	0.84 (0.70–1.01)[b]	1.01 (0.69–1.48)[b]	1.24 (0.97–1.57)	1.08 (0.93–1.24)	0.95 (0.72–1.25)	1.49 (0.87–2.14)
Mediterranean	0.97 (0.74–1.30)[b]	1.14 (0.89–1.45)[b]	—	—	1.06 (0.79–1.41)	**0.38 (0.24–0.59)**[a]	0.92 (0.40–2.14)
South American	—	—	1.02 (0.74–1.41)	1.00 (0.72–1.40)	—	1.16 (0.96–1.41)	1.21 (1.00–1.47)

Abbreviation: OR, odds ratio. [a]Statistically significant ($P < 0.05$, bold). [b]Random-effects pooled OR estimate.

with the incidence of infectious disease in the African population. However, a protective effect appeared to be conferred by the less frequent 1730A variant in the Mediterranean population (Table 3).

Table 4. Pooled OR estimates of the association of *SLC11A1* polymorphisms with the incidence of infectious disease

Polymorphism association	*Test of heterogeneity* χ^2 (P-*value*)	*Pooled OR estimate (CI)*
$(GT)_n$ allele 3	61.93 ($P < 0.01$)	**0.83 (0.74–0.93)**[a,b]
Mycobacterium spp.	58.73 ($P < 0.01$)	**0.82 (0.71–0.95)**[a,b]
Tuberculosis	40.54 ($P < 0.01$)	**0.76 (0.65–0.89)**[a,b]
Leprosy	4.48 ($P = 0.11$)	1.11 (0.92–1.35)
$(GT)_n$ allele 2	30.77 ($P = 0.03$)	**1.25 (1.10–1.42)**[a]
Mycobacterium spp.	20.80 ($P = 0.07$)	**1.37 (1.23–1.53)**[a]
Tuberculosis	12.23 ($P = 0.27$)	**1.47 (1.30–1.66)**[a]
−237C/T	6.33 ($P = 0.28$)	1.03 (0.83–1.29)
Tuberculosis	1.37 ($P = 0.50$)	0.63 (0.37–1.08)
274C/T	17.33 ($P = 0.18$)	1.01 (0.92–1.11)
Tuberculosis	13.19 ($P = 0.07$)	1.12 (0.91–1.37)
469+14G/C	115.8 ($P < 0.01$)	**1.27 (1.12–1.43)**[a,b]
Mycobacterium spp.	109.16 ($P < 0.01$)	**1.30 (1.13–1.49)**[a,b]
Tuberculosis	21.17 ($P < 0.01$)	**1.31 (1.12–1.54)**[a,b]
Leprosy	4.34 ($P = 0.11$)	1.22 (0.85–1.76)
577-18G/A[c]	1.28 ($P = 0.53$)	0.96 (0.60–1.55)
823C/T[c]	7.63 ($P = 0.02$)	0.67 (0.29–1.53)[b]
1465-85G/A	3.40 ($P = 0.76$)	1.00 (0.90–1.11)
Tuberculosis	2.85 ($P = 0.58$)	1.05 (0.88–1.26)
1730G/A	128.81 ($P < 0.01$)	**1.23 (1.08–1.40)**[a,b]
Mycobacterium spp.	125.59 ($P < 0.01$)	**1.26 (1.09–1.46)**[a,b]
Tuberculosis	101.93 ($P < 0.01$)	**1.24 (1.07–1.44)**[a,b]
1729+55del4	112.86 ($P < 0.01$)	**1.25 (1.13–1.38)**[a,b]
Mycobacterium spp.	109.47 ($P < 0.01$)	**1.27 (1.14–1.41)**[a,b]
Tuberculosis	79.11 ($P < 0.01$)	**1.31 (1.18–1.46)**[a,b]
Leprosy	1.63 ($P = 0.80$)	1.06 (0.89–1.26)
1729+271del4	4.53 ($P = 0.61$)	1.00 (0.91–1.11)
Tuberculosis	2.12 ($P = 0.71$)	1.02 (0.87–1.19)

Abbreviation: OR, odds ratio. [a]Statistically significant ($P < 0.05$, bold). [b]Random-effects pooled OR estimate. [c]Publications only analyse tuberculosis.

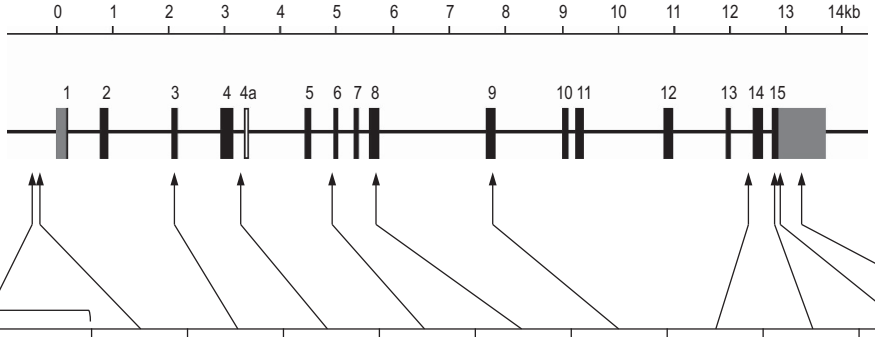
However, this analysis incorporated only two publications, suggesting that the observed association may be largely attributable to random variation.

No significant association was identified between the occurrence of the −237C/T, 274C/T, 577-18G/A, 823C/T, 1465-85G/A and 1729+271del4 polymorphisms and the incidence of infectious disease or tuberculosis alone (Table 4, Supplementary Tables S18, S19, S21–S23 and S26). The association of the −237C/T polymorphism with tuberculosis failed to reach statistical significance and this is likely attributable to the small number of publications that have been completed to date. The results suggest that the −237C/T promoter polymorphism may be associated with the occurrence of tuberculosis, however more association studies are required to confirm such an observation.

DISCUSSION

The results of the current meta-analyses have shown that genetic variants throughout *SLC11A1* are associated with the incidence of both infectious and autoimmune disease (Figure 3). Of the 17 new *SLC11A1* variants assessed, this meta-analysis has identified a significant association between the 469+14G/C polymorphism and the incidence of autoimmune disease as a whole and rheumatoid arthritis in particular, and the −273C/T polymorphism with the occurrence of inflammatory bowel disease. Similar to previous meta-analyses, the current analysis did not identify a significant association between either $(GT)_n$ allele 2 or 3 with a reduced or increased incidence of autoimmune disease, respectively.[28] However, stratification according to disease did reveal a significant association with type 1 diabetes incidence, suggesting that the $(GT)_n$ polymorphism may exert a minor effect on some autoimmune diseases.

The 469+14G/C, 1730G/A and 1729+55del4 polymorphisms were significantly associated with the incidence of infectious disease as a whole and with tuberculosis in particular, with pooled OR estimates determined in the current analyses being similar to previously reported OR estimates.[23,24] Similarly, consistent with previous reports, $(GT)_n$ allele 3 was found to be significantly protective of infectious disease and tuberculosis, whereas, for the first time, a significant association between $(GT)_n$ allele 2 and an increased susceptibility to infectious disease and tuberculosis was shown to exist.



	$(GT)_n$ Allele 2	$(GT)_n$ Allele 3	-237C/T	274C/T	469+14G/C (INT4)	577-18G/A	823C/T	1029C/T (A318V)	1465-85G/A	1730G/A (D543)	1729+55del4	1729+271del4 $(CAAA)_n$
Autoimmune	0.93 (0.83-1.05)	1.07 (0.94-1.22)	0.92 (0.83-1.02)	1.16 (0.96-1.40)	1.30 (1.04-1.64)	0.74 (0.50-1.09)	1.02 (0.67-1.56)	0.48 (0.21-1.11)	0.98 (0.93-1.03)	1.14 (0.86-1.51)	1.21 (0.96-1.54)	0.98 (0.80-1.22)
Infection	1.25 (1.10-1.42)	0.83 (0.74-0.93)	1.03 (0.83-1.29)	1.01 (0.92-1.11)	1.27 (1.12-1.43)				1.00 (0.90-1.11)	1.23 (1.08-1.40)	1.25 (1.13-1.38)	1.06 (0.91-1.11)
Tuberculosis	1.47 (1.30-1.66)	0.76 (0.65-0.89)	0.63 (0.37-1.08)	1.12 (0.91-1.37)	1.31 (1.12-1.54)	0.96 (0.60-1.55)	0.67 (0.29-1.53)		1.05 (0.88-1.26)	1.24 (1.07-1.44)	1.31 (1.18-1.46)	1.02 (0.87-1.19)

Figure 3. Summary of the results from the meta-analyses (pooled OR estimates and 95% CI interval) assessing the association of the different *SLC11A1* polymorphisms with the incidence of autoimmune disease, infectious disease and tuberculosis alone.

A meta-analysis assessing the association of $(GT)_n$ allele 2 with the occurrence of infectious disease or tuberculosis alone has not been completed prior to the current study. Previous meta-analyses,[23,24,26] and case-control association studies, have focused primarily on the association of allele 3 with infectious disease,[30–35] and associations of allele 2 with infectious disease incidence have not been investigated. However, the results of the current meta-analysis show that the association of $(GT)_n$ allele 2 with the incidence of tuberculosis alone is more significant than the protective effect putatively exerted by $(GT)_n$ allele 3.

This was highlighted in the European population, where allele 2 showed a trend for increased susceptibility to tuberculosis, however, allele 3 showed no protective effect. In addition, the $(GT)_n$ allele 2 data set was found to be homogenous ($\chi^2 = 12.23$, $P = 0.27$), however, heterogeneity was identified within the $(GT)_n$ allele 3 data set, as well as all other variants associated with tuberculosis (Table 4). It is envisaged that a sequence variant which alters the propensity of an individual to contract an infectious disease like tuberculosis would be common to all studies irrespective of other factors responsible for heterogeneity. In such a case, the ORs for the individual studies in the meta-analysis would be expected to be homogenous, as is observed with the meta-analysis examining the association of allele 2 with the incidence of tuberculosis. Therefore, the data suggests that allele 2 may exert a greater influence on the incidence of infectious disease than the previously thought $(GT)_n$ allele 3. Owing to this stronger association, we hypothesise that $(GT)_n$ allele 2, and not allele 3, is the disease-causing variant at the $(GT)_n$ microsatellite, which exerts the selective pressure at the *SLC11A1* locus to influence infectious disease susceptibility.

The question then arises as to how might $(GT)_n$ allele 2 function to alter infectious and autoimmune disease susceptibility? Reporter studies show different *SLC11A1* expression levels in the presence of different $(GT)_n$ alleles, with $(GT)_n$ allele 2 driving lower expression than $(GT)_n$ allele 3.[19,20] The $(GT)_n$ microsatellite has endogenous transcriptional enhancer activity owing to the ability of repetitive GT units to form Z-DNA.[36,37] Furthermore, alleles 2 and 3 which differ by a single 2-bp-GT repeat are reported to influence the transcription through altered transcription factor binding to the *SLC11A1* promoter. Specifically, the transcription factors HIF-1α and ATF-3/JunB have been shown to bind with in and adjacent to the $(GT)_n$ repeat, respectively.[36–38] Thus altered transcription factor binding, in the presence of the different repeat lengths may alter *SLC11A1* expression to influence macrophage phenotype and susceptibly to infectious and autoimmune disease. Indeed, murine studies show modest reductions in Slc11a1 expression result in significant phenotypic consequences,[2,4,16] suggesting a similar reduction in *SLC11A1* promoter activity with $(GT)_n$ allele 2 will also result in an altered cellular phenotype to influence disease susceptibility. Consistent with this hypothesis is the observation that allele 2 carriers have increased expression of the anti-inflammatory cytokine interleukin-10, compared with individuals who do not carry allele 2,[39] and murine macrophages which lack functional Slc11a1 show higher interleukin-10 expression after infectious challenge.[11,18,40–43] Future work should aim to explore further the role of $(GT)_n$ allele 2 in infectious disease occurrence.

The current meta-analysis identified positive associations between polymorphisms within the 5′-region of *SLC11A1*, but not within the 3′-region, and the incidence of autoimmune disease, although the polymorphisms located in the 5′- and 3′-regions of *SLC11A1* were associated with the incidence of infectious disease (Figure 4). Previous publications have identified the existence of significant linkage disequilibrium (LD) between the $(GT)_n$, −237C/T, 274C/T and 469+14G/C variants and markers 110 kb upstream of the *SLC11A1* locus, including the interleukin8Rb locus (termed 5′LD haplotype end). Furthermore, significant LD has been observed between the 823C/T, 1465-85G/A, 1730G/A and 1729+55del4 variants and markers 110 kb downstream of the *SLC11A1* locus (termed as 3′LD haplotype end). However, LD is not observed between variants located in the 5′- and 3′-LD haplotype ends of the *SLC11A1* locus (Figure 4).[44]

The *SLC11A1* polymorphisms identified to be significantly associated with disease incidence in the current analysis may be the functional cause of the association(s), or, alternatively, the associations observed may be because of the particular polymorphism being either positively or negatively selected because it is in LD with the true disease-causing variant. In the latter case, a genetic variant which alters disease incidence provides either a positive or negative selective pressure for the inheritance of all of the neutral variants within that LD block (hitchhiker effect).[45] Because of the complex LD pattern which exists at the *SLC11A1* locus,[44,46–48] the findings suggest that at least one functional polymorphism exists within the 5′-LD region of *SLC11A1*, which alters the cellular phenotype to influence autoimmune disease susceptibility, whereas at least two functional polymorphisms, one in the 5′ region and a second in the 3′ region, influence the occurrence of infectious disease (Figure 4). Thus polymorphisms in LD with the significantly associated *SLC11A1* polymorphisms should also be considered as potential functional candidates for disease susceptibility. Of the *SLC11A1* variants significantly associated with infectious disease, the $(GT)_n$ and the 1730G/A polymorphisms are putative candidates for the alteration of disease incidence observed at the 5′- and 3′-LD ends, respectively. These two polymorphisms are likely candidates as they have putative functional effects, being able to either influence the level of *SLC11A1* expressed[19,20] or alter the ability of *SLC11A1* to transport divalent cations,[21,49] respectively. These putative functional effects result in an altered phenotype, which may explain the reason for the associations with infectious disease identified in this study.

Of all polymorphisms examined, the 469+14G/C is the only variant to show an association with the incidence of both autoimmune and infectious disease and is therefore another potential disease-causing variant within the 5′-LD block of *SLC11A1*. Surprisingly, the C variant was associated with increased risk of developing both infectious and autoimmune disease. The 469+14G/C polymorphism is located in intron 4 of *SLC11A1*, near an alternatively spliced exon designated 4a, that produces a truncated transcript and non-functional protein. It has been suggested that the 469+14G/C polymorphism may alter the ratio of truncated-to-functional transcripts (which is normally relatively low at approximately 1:5).[50] However, Yang *et al.*[29] did not identify any difference in *SLC11A1* expression or the ratio of truncated-to-functional transcripts between differing genotypes of the 469 +14G/C polymorphism, suggesting that the 469+14G/C polymorphism may influence the SLC11A1 function through an yet unidentified mechanism. Further functional tests are required to identify the polymorphic variants that may result in an altered cellular phenotype to influence infectious/autoimmune disease susceptibility.

Future association studies should ideally analyse cases and controls through haplotype analyses, rather than adopting a narrow binomial approach of analysing only single polymorphisms. For example, although the current meta-analyses suggest an association between the $(GT)_n$ repeat and the incidence of infectious disease, the $(GT)_n$ repeat does not function independently to alter *SLC11A1* expression, as reporter studies show that both the $(GT)_n$ and −237C/T polymorphisms function synergistically to determine *SLC11A1* expression levels.[20] Therefore, association studies which analyse the effect of the $(GT)_n$ repeat and −237C/T polymorphisms independently will not be able to assess the complex interaction that determines the level of *SLC11A1* expressed. In addition, there are other polymorphisms within *SLC11A1* that putatively exert phenotypic effects to alter *SLC11A1* expression/function (e.g. 1730G/A). Therefore, an

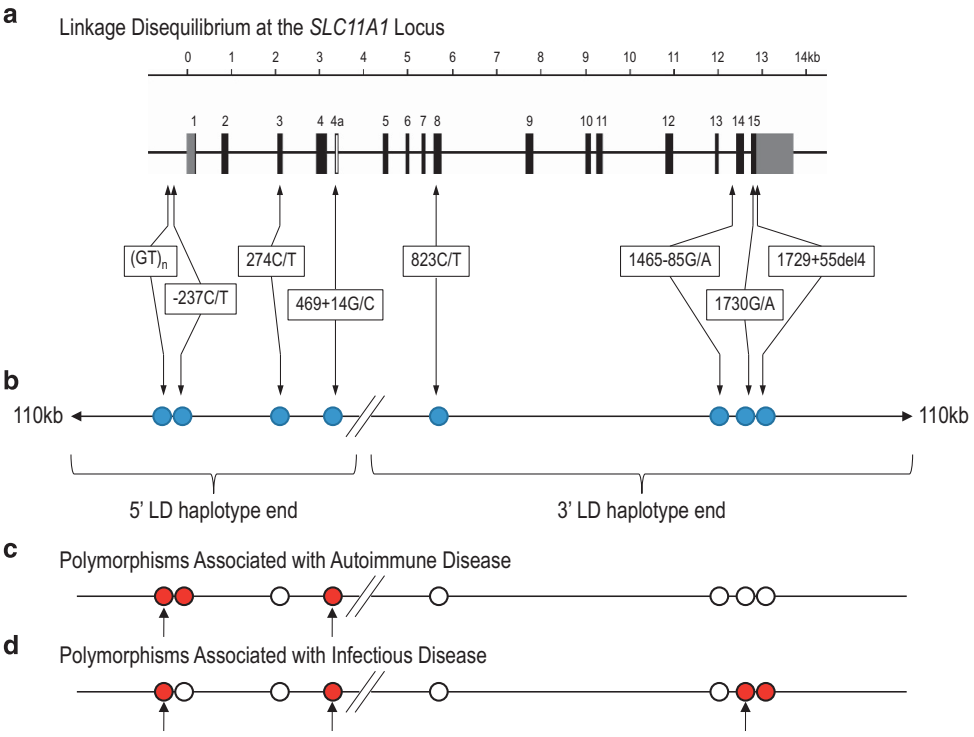


Figure 4. Linkage disequilibrium at the *SLC11A1* locus and location of polymorphisms associated with the incidence of autoimmune and infectious disease. (**a**) Genomic organisation of *SLC11A1* and location of studied sequence variants. The 15 exons of the gene are shown as black boxes with their respective numbers and the corresponding scale above indicates the length (kb) of the gene. The grey boxes indicate the 3′- and 5′-untranslated regions and the introns and flanking regions are represented by a thin line. The arrows indicate the position of sequence variants. (**b**) LD located within the *SLC11A1* locus. The blue circles indicate the location of the *SLC11A1* polymorphisms, with the thin line representing the flanking DNA regions. The two LD blocks (termed 5′-LD haplotype end and 3′-LD haplotype end) are shown, with the double-dashed line designating the weak LD observed between 5′- and 3′-*SLC11A1* regions. (**c**) Polymorphisms within the 5′-LD haplotype end but not the 3′-end are associated with the incidence of autoimmune disease (red circles indicate an association, whereas white circles indicate no association). (**d**) Polymorphisms in both the 5′- and 3′-LD haplotype blocks were found to be associated with infectious disease. The $(GT)_n$ and 469+14G/C; and 1730G/A are candidate polymorphisms in the *SLC11A1* locus influencing autoimmune and infectious disease susceptibility at the 5′- and 3′-LD haplotype ends, respectively (arrows).

individual's propensity to develop disease would be determined by a summation of the effects of each of the individual polymorphisms within the *SLC11A1* locus. Testament to this, association studies which have assessed *SLC11A1* haplotypes have identified more robust associations.[30,51–55]

In addition, although some polymorphisms have been assessed in a large number of association studies to allow the completion of a meaningful meta-analysis, there were still insufficient association studies completed for several polymorphisms which showed a trend with disease incidence, however, the pooled OR estimates did not reach significance. It is possible that the existence of more association studies may have allowed statistical significance to be attained. This includes, for example, analyses of the association of the −237C/T and 1029C/T (A318V) polymorphisms with the incidence of tuberculosis and autoimmune disease, respectively. Both of these polymorphisms may exert effects on SLC11A1 expression/function and show a significant trend with disease incidence, but in the absence of sufficient numbers of studies, the existence of significant associations cannot be determined. Furthermore, the current case/control literature has focused solely on the effect of *SLC11A1* on pro-inflammatory (M1) macrophages with disease occurrence and it is unclear the effect that *SLC11A1* variation may have on M2 macrophages and disease. For example, given the role *SLC11A1* has in erythrophagocytosis, could *SLC11A1* variants influence iron homeostasis and anaemia. Future work should aim to explore the effect of *SLC11A1* variants on the phenotype of alternatively activated M2 macrophages and associated disorders.

The aim of this work was to determine, based on previously published case-control association studies, the association of *SLC11A1* polymorphisms with the incidence of infectious and autoimmune disease. Of the 23 data sets covering 11 *SLC11A1* variants, associations were found for 9, with 4 of the 23 data sets investigated showing trends, possibly because of the low numbers of association studies available. On the basis of the findings of the current meta-analyses, the *SLC11A1* locus appears to have a role in influencing susceptibility to both infectious and autoimmune diseases. The findings of this meta-analysis are significant in helping to determine the multiple host genetic factors involved in complex diseases. Identification of these host genetic factors will help to prevent, control and treat these complex diseases.

MATERIALS AND METHODS

Literature search and inclusion criteria

Publications included in the meta-analysis were identified by searching the literature databases (PubMed, Medline/Ovid, Chinese National Knowledge Infrastructure (CNKI) and Asia/China on demand) using the search terms 'SLC11A1', 'NRAMP1', 'autoimmunity', 'tuberculosis' and 'infection', individually and in combination (from 1996 to 2012). In addition papers were sourced by cross-referencing original and review publications. Inclusion criteria for the meta-analysis were that studies assessed *SLC11A1* polymorphisms in patients diagnosed with a specific autoimmune or infectious disease and used non-familial subjects as controls. Studies analysing cancer, viral infections or pathology owing to infection were excluded. Furthermore, all publications included in the meta-analyses had to assess HIV-negative cases and controls. When duplicate association

studies were encountered, studies published in English or containing the more informative data were included in the analyses.

Data collection

Information regarding the disease studied, the population analysed and the study findings were extracted from all publications meeting the inclusion criteria. Total study numbers (individuals and alleles) and allelic frequencies (numbers and percentages) were also tabulated for all relevant data sets within a publication. When a publication contained several data sets/associations for a single polymorphism, each data set was assessed as an individual association when the populations/diseases were different between the data sets. Alternatively, data was pooled if the same population/disease was analysed. Allele frequencies were inferred from genotype frequencies when reported. In the few cases where carrier frequencies were reported, the genotype frequencies were first determined and then allele frequencies were inferred. Corresponding authors were contacted by email if the information to determine the OR was unavailable or if the published data was ambiguous. When publications assessed specific *SLC11A1* polymorphisms, but concluded that an analysis was not completed because of a low frequency of the less commonly occurring variant, the data was omitted from the analysis. The data extracted from all publications satisfying the inclusion criteria for the meta-analysis was reanalysed to ensure that the extracted data was correct.

Statistical analyses

Statistical analyses were completed using the Rmeta package in the program R.[56,57] Using the relevant data sets, the OR and 95% CI were determined for each individual association included in each of the meta-analyses. Associations which contained zero observations for both cases and controls were excluded from analyses, whereas the reciprocal of the opposite treatment size method was used to allow studies with a zero observation in either case or control groups to be included.[58]

The association of a polymorphism with disease incidence, from the individual associations, was completed by the determination of the fixed-effects pooled OR estimate (Mantel–Haenszel method). The Cochran *Q*-test was utilised to determine whether heterogeneity was present in the analysed data set. If the Cochran *Q*-test identified the presence of heterogeneity within the data set, the random-effects pooled OR estimate (DerSimonian–Laird method) was determined. Pooled OR were determined from individuals with the respective infection, independent of clinical manifestation. Funnel plots were assessed to determine the presence of publication bias.

Only polymorphisms that had been investigated in three or more individual association studies were included in the analysis. Where a large number of data sets were available for a particular polymorphism, smaller meta-analyses were completed, where possible, analysing the association of individual diseases (e.g. type 1 diabetes, tuberculosis), or geographical location, with the *SLC11A1* polymorphisms. In these cases, analyses were performed from as many as two association studies.

Although nine alleles of a polymorphic *SLC11A1* promoter $(GT)_n$ microsatellite repeat (rs534448891) have been identified to date, seven of these alleles (alleles 1 and 4–9) occur at low frequencies. Therefore, association studies have focused on the association of the most common alleles 2 and 3 with disease occurrence. Meta-analyses of both $(GT)_n$ allele 3 and allele 2 were completed to determine the association of these alleles with the incidence of autoimmune and infectious disease. For the analysis of allele 3, the frequency data for alleles 1, 2 and 4–9 were pooled and compared against the frequency of allele 3. Likewise, for the analysis of allele 2, the frequencies of alleles 1 and 3–9 were pooled and compared against the frequency of allele 2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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