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GENETICS, EVOLUTION, AND PHYLOGENY - REVIEW

Susceptibility to leishmaniasis is affected by host *SLC11A1* gene polymorphisms: a systematic review and meta-analysis

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Abstract

Leishmaniases are cutaneous, mucocutaneous, and visceral diseases affecting humans and domesticated animals mostly in the tropical and subtropical areas of the planet. Host genetics have been widely investigated for their role in developing various infectious diseases. The *SLC11A1* gene has been reported to play a role in neutrophil function and is associated with susceptibility to infectious and inflammatory diseases such as tuberculosis or rheumatoid arthritis. In the present meta-analysis, we investigate the genetic association of *SLC11A1* polymorphisms with susceptibility to leishmaniasis. Genotypes and other risk-related data were collected from 13 case-control and family-based studies (after literature search). Conventional random-effects meta-analysis was performed using STATA 13. To pool case-control and family-based data, the weighted Stouffer's method was also applied. Eight polymorphisms were investigated: rs2276631, rs3731865, rs3731864, rs17221959, rs201565523, rs2279015, rs17235409, and rs17235416. We found that rs17235409 (D543N) and rs17235416 (1729 + 55del4) are significantly associated with a risk for cutaneous leishmaniasis (CL), whereas rs17221959, rs2279015, and rs17235409 are associated with visceral leishmaniasis (VL). Our results suggest that polymorphisms in *SLC11A1* affect susceptibility to CL and VL. These findings open new pathways in understanding macrophage response to *Leishmania* infection and the genetic factors predisposing to symptomatic CL or VL that can lead to the usage of predictive biomarkers in populations at risk.

Keywords Leishmaniasis · *SLC11A1* · Meta-analysis · Genetic association · Predictive biomarkers · Genetic risk

Introduction

Leishmaniases are a group of human cutaneous (CL), mucocutaneous (ML), and visceral (VL) diseases of major public health importance in endemic areas (Kaye and Scott 2011; Murray 2002; WHO 2015). They are caused by the transmission of more than 20 different protozoan parasite *Leishmania* species to mammalian hosts by the bite of sandflies. *Leishmania* belongs to the class of Kinetoplastea and to Trypanosomatidae family of protozoans that parasitize both invertebrate (sandflies of the genus *Phlebotomus* or *Lutzomyia*) and vertebrate hosts. The most severe and potentially fatal form of the disease, if left untreated, is VL (kala-azar) (Desjeux 2001; Murray 2002) caused by parasites of the *Leishmania donovani* (*L. donovani*) complex. The outcome of *L. donovani* infection ranges from asymptomatic carriership to symptomatic disease characterized by prolonged fever, splenohepatomegaly, pancytopenia, and hypergammaglobulinemia. VL is endemic in 62 countries, and approximately 200 million individuals are at risk (Desjeux 1996). As estimated, 50,000 new cases of VL and over 20,000 deaths due to all forms of leishmaniasis occur annually while the total number of possible cases reaches 2.5 million (Bora 1999; Desjeux 1996; Kaye and Scott 2011; Murray 2002). The disease affects some of the poorest people on earth, and is associated with malnutrition, a weak immune system, population

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displacement, poor housing, lack of financial resources, or even wars (Rehman et al. 2018; WHO 2015). Importantly, in 2015, more than 90% of reported to WHO VL cases occurred in six countries: India, Sudan, Ethiopia, Kenya, Somalia, and Brazil (WHO 2015).

One major challenge facing the disease elimination initiatives carried out thus far (WHO 2011) is that only a small proportion of all *L. donovani* infections are manifested as clinical disease. This research field is challenged by the fact that the precise immune mechanisms underlying human VL are still not fully understood, and that the responses necessary for protection by vaccination are not as clear as in the mouse model (Gumy et al. 2004). Other problems of the research field are (a) the absence of validated markers for asymptomatic *L. donovani* infection, since so far, diagnostic assays for VL have been evaluated primarily on their capacity to detect clinical disease; (b) severe side effects and high cost of the existing drugs against *Leishmania* parasites and development of drug resistant parasite strains [5], and (c) the absence of a vaccine offering efficient protection to humans.

The impact of host genetics on the susceptibility to leishmaniasis (Cabello et al. 1995; Leish et al. 2013; Peacock et al. 2001) or other infectious diseases caused by pathogenic agents such as tuberculosis (Meilang et al. 2012), meningococcal disease (Brouwer et al. 2010; Martinon-Torres et al. 2016), periodontitis (da Silva et al. 2017; Dimou et al. 2010), and malaria (Ziakas et al. 2013) has been extensively evaluated and studied. The importance of host genetic variability in the interaction with parasites (viruses, bacteria, protozoans) is a much-discussed issue in the literature, and it has been suggested that the co-evolution of hosts and parasites may be responsible even for a portion of the genetic diversity found in both host and pathogen natural populations (Koella and Boete 2003; May and Anderson 1983).

Solute carrier family 11 member 1 (*SLC11A1*) is one of the few genes investigated thus far for its potential role in susceptibility to leishmaniasis disease symptoms. *SLC11A1*, formerly known as natural resistance-associated macrophage protein 1 (NRAMP1), is a member of the solute carrier family 11 encoding a multi-domain integral membrane protein (Fleming et al. 1997; Gunshin et al. 1997; Vidal et al. 1993) and is involved in iron metabolism and host resistance to certain pathogens. It is a divalent metal ion (Fe^{2+}, Zn^{2+}, and Mn^{2+}) transporter located in the membranes of early and late endosomes/phagosomes and lysosomes in macrophages. From there, it pumps the metal ions out of the microbiophorous phagosomes (Blackwell et al. 2003). It has been proposed that this mechanism deprives the pathogen enclosed in the phagosome of iron, an element which is a nutrient component of the pathogen, thus eliminating its development (Cassat and Skaar 2013). On the other hand, *Leishmania* upregulates iron transport mechanisms that compete with the host iron transporter (*SLC11A1*) in order to acquire iron within parasitophorous vacuoles of host macrophages (Blackwell et al. 2001; Flannery et al. 2013; Zaidi et al. 2017; Zwilling et al. 1999). *SLC11A1* was first positionally cloned as the gene that confers part of the resistance and susceptibility to VL by *Leishmania donovani* in mice (Blackwell et al. 2003). It has also been shown that polymorphisms of this gene are associated with leishmaniasis development or resistance in dogs (Altet et al. 2002; Sanchez-Robert et al. 2008). Moreover, it has been shown that *SLC11A1* polymorphisms are associated with many infectious diseases such as HIV, tuberculosis, leprosy, meningococcal meningitis (bacterial) as well as autoimmune diseases including rheumatoid arthritis, juvenile rheumatoid arthritis, diabetes, sarcoidosis, and Crohn's disease [reviewed in (Blackwell et al. 2001; Blackwell et al. 2003)]. From the above findings, it becomes evident that *SLC11A1* is highly likely to play a pivotal role in leishmaniasis susceptibility and that the mechanism by which it is involved in the host-parasite interaction disserves investigation.

Various polymorphisms in *SLC11A1* have been investigated, in case-control studies, regarding their role in leishmaniasis, but the results were inconsistent. In the present study, a comprehensive systematic review and meta-analysis is performed to consolidate disparate evidence of the association of host *SLC11A1* genotypes with the risk of developing leishmaniasis.

Materials and methods

Data search strategy

Pubmed and Scopus databases were searched in order to identify all relevant publications regarding genetic association studies for the implication of *SLC11A1* polymorphisms in all types of leishmaniasis up to October 2018. Terms used for the search included "NRAMP1," "SLC11A1," combined with "mutant," "variant," "polymorphism," "SNP," and "leishmaniasis." To avoid selection bias, no restrictions were imposed on the study selection procedure regarding study design, language, or other quality measures (Pan et al. 2005; Stroup et al. 2000). Retrieved abstracts were scrutinized and only the relevant ones were included in our study. In particular, the inclusion criteria were that the selected studies should provide an estimate for the relative risk such as the odds ratio and its variance, a *p* value, or the necessary data from which it could be calculated (allele or genotype frequencies of human *SLC11A1* polymorphisms). It should be noted that both case-control and family-based studies were included in the quantitative synthesis. References of the retrieved studies were scrutinized to include additional information (articles that the query missed, or other material from the gray literature).

Data extraction

Data extraction from each manuscript was performed by two researchers (GB, PK) according to eligibility criteria. Problems involving disagreement were resolved after discussion with a third reviewer (PB). The extracted data were recorded on a spreadsheet. From each article, Pubmed ID, the first author's name, year of publication, the total number of subjects (cases/controls if available), as well as population ethnicity were recorded on the spreadsheet. We also recorded genotype and allele counts for cases/controls and p values (or z-scores) from the family-based tests (Supplementary Table 1).

Statistical methods

Odds ratio (OR) was used to test the association between each SNP and the susceptibility to leishmaniasis in case-control studies, along with their 95%CIs (confidence intervals). When a cell had zero value, a continuity correction was applied by adding the value 0.5 to all cells of the contingency table. Data were analyzed using the random-effects meta-analysis method (DerSimonian and Laird 1986). Three different contrasts were investigated corresponding to co-dominant, dominant, and recessive modes of inheritance (risk vs. wt, risk/risk + risk/wt vs. wt/wt, and risk/risk vs. risk/wt + wt/wt for each polymorphism, respectively). The between-studies heterogeneity was evaluated using the chi-square-based Cochran's Q statistic and the consistency index (I^2) (Higgins et al. 2003). Control populations from case-control studies were tested for Hardy-Weinberg equilibrium (HWE) for each polymorphism. The family-based studies were not based on the well-known family trio using the transmission disequilibrium test (TDT), but rather on extended versions like the S-TDT or the P-TDT. Thus, existing methods that would allow for the integration of such studies into the meta-analysis could not be used (Bagos et al. 2011). In order to include data of these studies, as a sole alternative, we used a weighted version of the Stouffer's method which uses the p value of each study and a normal approximation (through the z-statistic) (Affandi et al. 2013; Stouffer 1949). The weighting was performed using the total sample size of each study. To estimate possible publication bias, the rank correlation method of Begg and Mazumdar (Begg and Mazumdar 1994), and additionally the fixed-effects regression method of Egger were applied (Egger et al. 1997). Influential meta-analysis was performed by removing each individual study and re-calculating the statistical significance. In order to estimate a possible time trend in the results over the years, a bias called "Proteus phenomenon" (Bagos and Nikolopoulos 2009; Ioannidis and Trikalinos 2005), we performed cumulative meta-analysis. Two methods were used: (a) the standard cumulative meta-analysis approach (Ioannidis and Trikalinos 2005; Lau et al. 1995), where we visually inspected the plot and (b) the GLS regression-based method (Bagos and Nikolopoulos 2009). In all analyses, STATA 13 (Stata 2013) was used and results with p value < 0.05 were considered statistically significant.

Linkage disequilibrium data analysis

D' and r^2 values were recovered from all studies. Mean values were calculated for populations of the same origin, if they were more than one. These D' and r^2 values were compared with D' and r^2 data retrieved from the 1000 Genomes Project, phase 3 (variant rs numbers are indexed based on dbSNP build 142) from corresponding populations using the online tool for exploring linkage disequilibrium, LDlink (NIH 2017).

Results

Studies included in the meta-analysis

The literature search performed to initially identify all studies assessing the association of *SLC11A1* polymorphisms with any type of leishmaniasis yielded 66 publications. However, only 10 published articles (Bucheton et al. 2003; Castellucci et al. 2010; Ejghal et al. 2014; Fattahi-Dolatabadi et al. 2016; Hernandez-Rivera et al. 2016; Mehrotra et al. 2011; Mohamed et al. 2004; Ortiz-Flores et al. 2015; Samaranayake et al. 2010; Sophie et al. 2017) encompassing 13 studies provided adequate data according to the inclusion criteria, and were therefore further included in the meta-analysis (Fig. 1). From the seven studies on CL, six were case-control genetic association ones (Castellucci et al. 2010; Fattahi-Dolatabadi et al. 2016; Hernandez-Rivera et al. 2016; Ortiz-Flores et al. 2015; Samaranayake et al. 2010; Sophie et al. 2017), whereas one was a family-based study (Castellucci et al. 2010). The studies assessing VL included three case-control (Ejghal et al. 2014; Mehrotra et al. 2011; Ortiz-Flores et al. 2015) and three family-based ones (Bucheton et al. 2003; Mehrotra et al. 2011; Mohamed et al. 2004). All studies were conducted in the endemic countries. From the 13 studies analyzed, we extracted various types of data (i.e., the frequency of genotypes and alleles, z-scores or p values) from cases, controls, and families referring to eight different polymorphisms (Supplementary Table 1). In total, for CL, we analyzed data from 1955 individuals and for VL from 3615 individuals. The characteristics of the studies, the ethnicity of the individuals, the studied polymorphisms, and the existence of Hardy-Weinberg equilibrium (HWE) in the controls of each study are shown in Table 1.

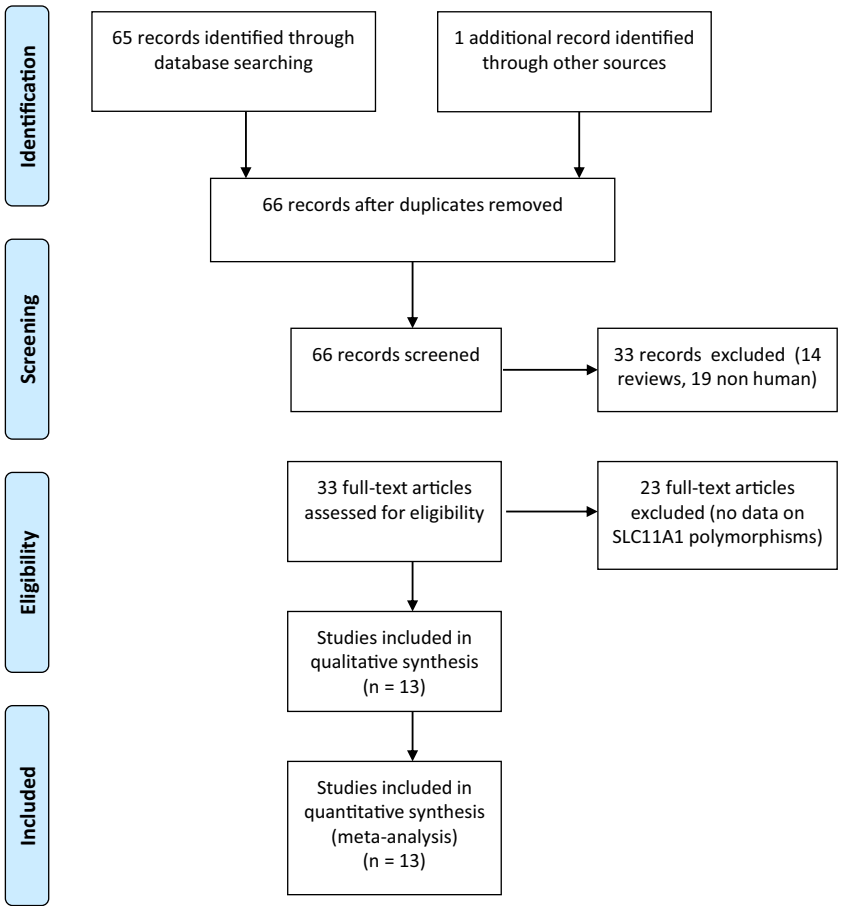

Fig. 1 Prisma flow chart for screening studies to assess association of *SLC11A1* polymorphisms with leishmaniasis

Qualitative synthesis and study design

The eight polymorphisms tested for their putative association with any type of leishmaniasis are rs2276631 (274C/T), a synonymous mutation in exon 3; rs3731865 (469 + 14G > C) in intron 4; rs3731864 (577-18G/A) in intron 5; rs17221959 (823C/T), a synonymous mutation in exon 8; rs201565523 (A318V or 1029C/T), converting a GCG to a GTG codon in exon 9; rs2279015 (1465-85G/A) in intron 13; rs17235409 (D543N, or 1730G/A), converting a GAC to an AAC codon in exon 15; and rs17235416 (1729 + 55del4, TGTG), a four base-pair insertion/deletion in the 3′UTR.

All putative associations with all forms of leishmaniasis were analyzed according to the co-dominant mode of inheritance, based on the allele contrast, using the random-effects model. Meta-analyses were also performed according to dominant and recessive modes of inheritance (with respect to the minor frequency allele); however, only the statistically significant associations are shown for these two modes (Fig. 2). The allele contrast (co-dominant mode of inheritance) analysis was also used to allow integration with the family-based studies.

SLC11A1 polymorphisms associated with leishmaniasis

Conventional meta-analysis with pooled ORs revealed that no polymorphism of the above is associated with leishmaniasis (Table 2). However, when the weighted Stouffer's method was applied to incorporate data from family-based studies for all polymorphisms, rs2279015 was found to be statistically significantly associated with all forms of leishmaniasis since the p value was 0.0007 (Table 2). The meta-analysis of only the case-control studies resulted in an OR 0.80 and the 95% CI was 0.60, 1.06. The meta-analysis showed moderate heterogeneity (I^2 = 43.2%), and no publication bias according to Begg's and Egger's methods (p value = 0.931). The results altogether suggest that the minor frequency allele A of rs2279015 is associated with lower risk of developing symptoms of leishmaniasis.

Subgroup analysis by form of leishmaniasis

In an attempt to deal with inherent differences among studies and to improve the quality and the biological and medical impact of our conclusions, we performed coherent subgroup

Table 1 Characteristics of studies included in the present meta-analysis along with studied polymorphisms

Study	PMID	Author	Year	Country	Ethnicity	Form of leishmaniasis	Individuals family-based	Individuals case-controls	Cases	Controls	Polymorphisms studied	HWE
1	28061874	Sophie et al.	2017	Pakistan	SE Asian[b]	Cutaneous		393	274	119	rs17235409	Yes
											rs201565523	Yes
											rs2276631	Yes
											rs17235416	No
											rs3731864	Yes
2	27681549	Fattahi-Dolatabadi et al.	2016	Iran	SE Asian[b]	Cutaneous		286	150	136	rs17235409	Yes
											rs201565523	Yes
3	27830154	Hernández-Rivera et al.	2016	Mexico	Admixed American	Cutaneous		184	115	69	rs17235409	Yes
											rs2276631	Yes
											rs17235416	No
											rs17221959	Yes
4	25603101	Ortiz-Flores et al.	2015	Mexico	Admixed American	Cutaneous		205	79	126	rs17235409	No
											rs2276631	Yes
											rs17221959	Yes
											rs17235416	No
											rs2279015	Yes
											rs3731864	Yes
5	25603101	Ortiz-Flores et al.	2015	Mexico	Admixed American	Visceral		104	15	89	rs17235409	Yes
											rs2276631	Yes
											rs17221959	Yes
											rs17235416	Yes
											rs2279015	Yes
											rs3731864	Yes
6	25151047	Ejghal et al.	2014	Morocco	Arab	Visceral		243	106	137	rs17235409	Yes
											rs3731865	Yes
											rs17221959	Yes
7	21599885	Mehrotra et al.	2011	India	SE Asian[b]	Visceral		1933	941	992	rs17235409	NA[a]
											rs2276631	NA[a]
											rs17235416	NA[a]
											rs3731865	NA[a]
											rs2279015	NA[a]
8	21599885	Mehrotra et al.	2011	India	SE Asian[b]	Visceral	836				rs2276631	NA[a]
											rs17235416	NA[a]
											rs3731865	NA[a]

Table 1 (continued)

Study	PMID	Author	Year	Country	Ethnicity	Form of leishmaniasis	Individuals family-based	Individuals case-controls	Cases	Controls	Polymorphisms studied	HWE
											rs2279015	NA[a]
9	20214763	Samaranayake et al.	2010	Sri Lanka	SE Asian[b]	Cutaneous		395	199	196	rs17235409	Yes
											rs2276631	Yes
											rs3731865	Yes
10	20089160	Castellucci et al.	2010	Brazil	Admixed American	Cutaneous		180	60	120	rs17235416	NA[a]
11	20089160	Castellucci et al.	2010	Brazil	Admixed American	Cutaneous	312				rs17235416	NA[a]
12	14523377	Mohamed et al.	2004	Sudan	African	Visceral	312				rs17235409	NA[a]
											rs2276631	NA[a]
											rs17235416	NA[a]
											rs3731865	NA[a]
13	12618857	Bucheton et al.	2003	Sudan	African	Visceral	187				rs2276631	NA[a]
											rs17235416	NA[a]
											rs3731865	NA[a]
Total							1647	3923				
Total							5570					

[a] Not applicable due to the family-based design of the study or lack of information

[b] South East Asian

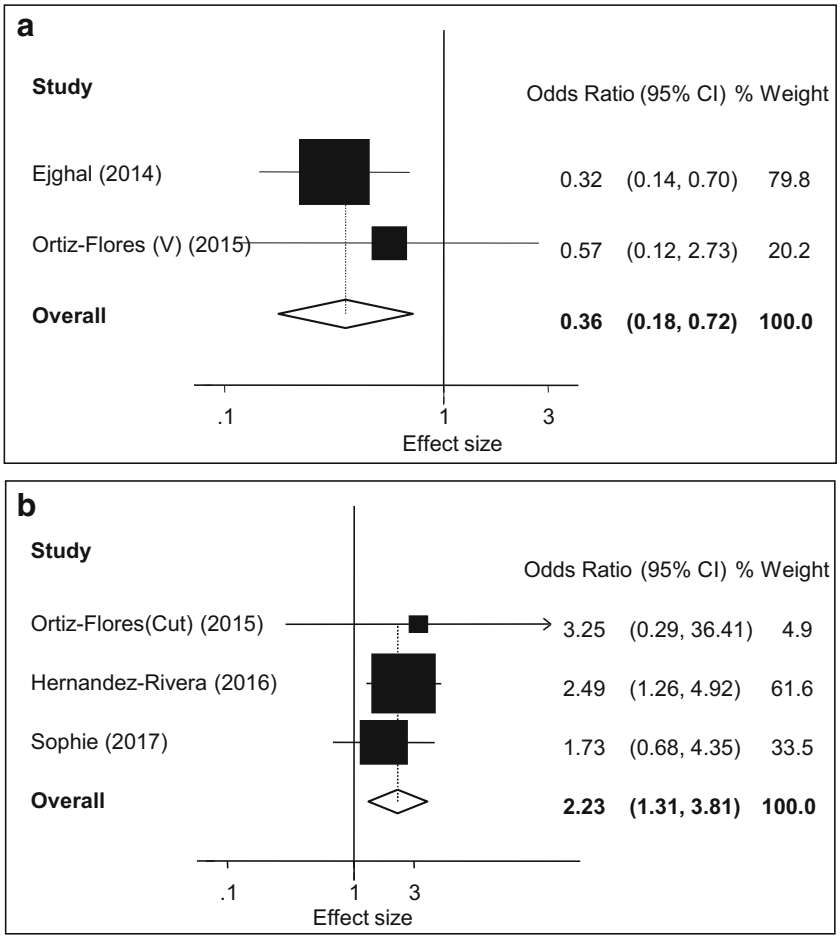

Fig. 2 **a** Forest plot of meta-analysis for the association of rs17235409 (D543N) polymorphism with VL according to the dominant mode of inheritance. **b** Forest plot of meta-analysis for the association of rs17235416 (1729 + 55del4, TGTG) polymorphism with CL according to the recessive mode of inheritance**.** As effect sizes, odds ratios (ORs) are depicted

analyses for the various forms of leishmaniasis. The analysis for each polymorphism was stratified into two subgroups: cutaneous and visceral leishmaniasis.

rs17221959 (823C/T) polymorphism

Conventional meta-analysis of the T vs. C contrast of rs17221959 polymorphism according to the co-dominant mode of inheritance yielded a significant association with VL. The OR was 0.43 and the 95% CI was 0.27, 0.71 (Table 2). With an OR < 1 (for the T vs. C contrast), this result suggests that the T allele carriers have a reduced risk of developing VL. No heterogeneity between the studies ($I^2 = 0.0\%$, p value = 0.819) or publication bias (p value = 0.317) were revealed.

rs2279015 (1465-85G/A) polymorphism

Conventional meta-analysis of the A vs. G allele contrast, for rs2279015 polymorphism, resulted in a marginally non-significant association with VL. However, a statistically significant association was revealed when the weighted Stouffer's method was applied and data from one additional family-based study were integrated (p value = 0.0003). Both the OR of the conventional meta-analysis (0.70; 95%CI 0.46, 1.05), and the direction of the effect of the Stouffer's method ($z = -3.3865$) point to a conclusion that the minor allele (A) is associated with a lower risk of disease development (i.e., the G allele increases the odds of developing the disease after infection). Between-studies heterogeneity for the conventional meta-analysis was low ($I^2 = 33.8\%$, p value = 0.219) and Begg and Egger's statistical tests indicated an absence of publication bias ($p = 0.317$).

rs17235409 (D543N) polymorphism

Analysis of the association of rs17235409 (D543N) polymorphism resulted in some remarkable findings. No statistically significant association was found from the meta-analysis of eight case-control studies (OR 1.15, 95%CI 0.82, 1.60), or with seven case-control studies, the controls of which were within Hardy-Weinberg equilibrium (OR 1.17, 95%CI 0.79, 1.75), or with the weighted Stouffer's method with which we included an additional family-based study (p value 0.0530) (Table 2).

However, stratified meta-analysis for CL including data from five case-control studies yielded statistically significant

Table 2 *SLC11A1* polymorphisms are associated with leishmaniasis. Effect sizes of all tested associations according to the co-dominant mode of inheritance (allele contrasts)

Study	Polymorphism	Form of leishmaniasis	Number of studies				Number of individuals		OR		95%CI		*p* value (Stouffer's)
			Total	Case-control	Case-control in HWE	Family-based	Cases/controls	Family-based	Case-control studies	Case-control studies in HWE	Case-control studies/	Case-control studies in HWE	
1	rs2276631 (274C/T)	All	9	6	6	3	1622/1594	1335	0.91	0.91	0.77, 1.09	0.77, 1.09	0.3207
		VL	5	2	2	3	956/1081	1335	0.72	0.72	0.35, 1.50	0.35, 1.50	0.6323
		CL	4	4	4	0	666/513	0	0.94	0.94	0.74, 1.18	0.74, 1.18	0.2698
2	rs3731865 (469 + 14G/C)	All	6	3	3	3	1227/1289	1335	1.06	1.06	0.90, 1.24	0.90, 1.24	0.2862
		VL	5	2	2	3	1030/1091	1335	1.11	1.11	0.84, 1.46	0.84, 1.46	0.2576
		CL	1	1	1	0	197/198	0	1.01	1.01	0.55, 1.85	0.55, 1.85	0.9851
3	rs3731864 (577-18G/A)	All	3	3	3	0	368/334	0	2.55	2.55	0.31, 20.86	0.31, 20.86	ND[d]
		VL	1	1	1	0	15/89	0	5.85	5.85	0.11, 300.5	0.11, 300.5	ND[d]
		CL	2	2	2	0	353/245	0	1.84	1.84	0.15, 21.99	0.15, 21.99	ND[d]
4	*rs17221959* (*823C/T*)	All	4	4	4	0	315/421	0	1.17	1.17	0.31, 4.42	0.31, 4.42	ND[d]
		VL	*2*	*2*	*2*	*0*	*121/226*	*0*	*0.43*	*0.43*	*0.27, 0.71*	*0.27, 0.71*	*ND*[d]
		CL	2	2	2	0	194/195	0	3.42	3.42	0.15, 76.5	0.15, 76.5	ND[d]
5	rs201565523 (A318V)	CL	2	2	2	0	424/255	0	0.44	0.44	0.17, 1.16	0.17, 1.16	ND[d]
6	*rs2279015* (*1465-85G/A*)	*All*	*4*	*3*	*3*	*1*	*1028/1057*	*836*	*0.80*	*0.80*	*0.60, 1.06*	*0.60, 1.06*	*0.0007*
		VL	*3*	*2*	*2*	*1*	*949/931*	*836*	*0.70*	*0.70*	*0.46, 1.05*	*0.46, 1.05*	*0.0003*
		CL	1	1	1	0	79/126	0	1.03	1.03	0.69, 1.53	0.69, 1.53	0.7873
7	*rs17235409* (*D543N*)	All	9	8	7	1	1878/1691	312	1.15	1.17	0.82, 1.60	0.79, 1.75	0.0530
		VL[a]	4	3	3	1	1061/1045	312	0.65	0.65	0.26, 1.62	0.26, 1.62	0.8123
		CL	*5*	*5*	*4*	*0*	*817/646*	0	*1.42*	*1.59*	*1.03, 1.95*	*1.16, 2.18*	*0.0044*
8	*rs17235416* (*1729 + 55del4, TGTG*)	All	10	6	2	4	1342/1383	1647	1.30	1.05	0.81, 2.08	0.80, 1.37	0.8143
		VL	5	2	2	3	814/949	1335	1.05	1.05	0.80, 1.37	0.80, 1.37	0.7727
		CL[b]	5	4	NA[c]	1	528/434	250	1.51	NA[c]	0.68, 3.33	NA[c]	0.9879

Significant associations or significantly associated polymorphisms are in italics

[a] Evidence for this association according to dominant mode of inheritance is shown in Fig. 2a

[b] Evidence for this association according to the recessive mode of inheritance is shown in Fig. 2b

[c] Not applicable because 1 study gave data for only *z*- and *p* values. Three studies deviated from HWE

[d] Not determined because only case-control studies were included

association (OR 1.42 and 95%CI 1.03 to 1.95) for the A vs G contrast (co-dominant mode of inheritance). The significance of the association was further verified by the weighted Stouffer's method (p value = 0.0044) (Table 2). Further tests showed no heterogeneity (p value = 0.25, I^2 = 25.4%) and no publication bias according to Begg's and Egger's tests (p value = 0.163). The meta-analysis did not demonstrate a time trend ("Proteus phenomenon") and influential analysis did not uncover any specific study to greatly influence the overall association (data not shown). It is worth noting that although the control genotypes in one study for the D543N analysis (Ortiz-Flores et al. 2015) for CL were not within the HWE, the significance of the association outcome remained when this study was omitted from the meta-analysis (Table 2). Moreover, when the AA+AG vs. GG contrast was investigated, denoting the dominant mode of inheritance, the rs17235409 (D543N) polymorphism was found to be significantly associated with a reduced risk for VL since the OR was 0.36 and the 95%CI was 0.18, 0.72 (Fig. 2a). No heterogeneity (I^2 = 0.0%, p value = 0.518) or publication bias (p = 0.317) were observed.

rs17235416 (1729 + 55del4) polymorphism

Meta-analysis (allele contrast) of 10 (six case-control and four family-based) studies revealed no association of rs17235416 polymorphism with any form of leishmaniasis. Stratification by form of leishmaniasis, similarly, did not reveal any association either with VL or with CL. However, when the recessive mode of inheritance was investigated with conventional meta-analysis evaluating data from the three case-control studies that held genotype data, the rs17235416 (1729 + 55del4) polymorphism was found to be associated with CL with OR 2.23 and 95%CI 1.31, 3.81 (Fig. 2b). No overall between-studies heterogeneity (I^2 = 0.0%, p value = 0.78) or publication bias were detected (p value = 0.901). There was no time trend as revealed by the GLS regression-based test (p value = 0.505) and no study significantly influenced the overall association result. It should be mentioned that the controls of three studies included in this conventional meta-analysis concerning the rs17235416 polymorphism association with CL deviate from the HWE.

The rs2276631, rs3731865, rs3731864, and rs201565523 polymorphisms

The four remaining polymorphisms, i.e., rs2276631, rs3731865, rs3731864, and rs201565523 were not found to be associated with any form of symptomatic leishmaniasis under any type of contrast or mode of inheritance (co-dominant, dominant, recessive) (Table 2 and data not shown).

Stratification by ethnicity

Since the populations of our study are not of the same origin, the individuals may differ systematically in both genetic ancestry and phenotype. Thus, subgroup analysis by ethnicity was performed. The subgroups analyzed were Admixed American, African, Arab, and Southeast Asian. Only the rs17235409 (D543N) polymorphism was shown to be associated with leishmaniasis in Southeast Asians with OR 1.44 and 95% CI 1.01, 2.04 (Supplementary Table 2).

Linkage disequilibrium

Because linkage disequilibrium (LD) has been reported to exist for at least some of the *SLC11A1* polymorphisms in Asian and African populations (Castellucci et al. 2010; Ejghal et al. 2014; Mohamed et al. 2004; Ortiz-Flores et al. 2015; Soborg et al. 2007; Sophie et al. 2017; Yip et al. 2003), we set out to compare pair-wise LD data between SNPs in the separate populations of our study, with LD data retrieved from comparable populations from the 1000 Genomes Project (Phase 3), using the LDlink (NIH 2017), an interactive web tool for exploring linkage disequilibrium. Table 3 shows the way population comparison was performed. As shown in Fig. 3, LD was high (r^2 = 0.87 to 0.92) between the pair rs2276631 and rs3731865 in Brazilian and Mexican (Admixed American) and Pakistani (Southeast Asian) populations. A moderate degree of LD was suggested for the pairs rs2276631-rs2279015 and rs3731865-rs2279015 in the American Admixed populations (Fig. 3a, b). A moderate LD for the pair rs3731865-rs17235409 seems to stand only for the Southern American Admixed populations and not for the Mexicans (Fig. 3a, b). It is noteworthy that our data revealed another strong LD between rs17235409 and rs17235416 with r^2 = 0.78 to 0.8 for Mexican and Pakistani populations, an LD that was not supported by LDlink (Fig. 3b, c). It is interesting to mention that in our analysis, both polymorphisms were associated with CL.

Discussion

Thus far, application of meta-analytical approaches has highlighted the fact that many gene variants are involved in the pathogenesis of infectious diseases. Tuberculosis is associated with polymorphisms in vitamin-*D receptor* (*VDR*), *intereleucin-10* (*IL-10*), *interferon gamma* (INFγ), and *SLC11A1* (Archer et al. 2015; Gao et al. 2010; Meilang et al. 2012; Mosaad et al. 2010). Polymorphisms in *TLR4* have been implicated in a predisposition to malaria, brucellosis, CL, neurocysticercosis, and typhoid fever (Ziakas et al. 2013). A relatively recent meta-analysis showed genetic association of *SLC11A1* polymorphisms with the incidence of autoimmune

Table 3 Information on LD data from the included studies and presentation of comparable populations for which 1000 Genomes Project holds LD data

Author	Form of leishmaniasis	Country of study	D' data	r^2 data	Comparable with 1000 Genomes Project populations		
Castelluci et al.	CL	Brazil	Yes	Yes	PUR	Puerto Ricans from Puerto Rico	AMR
					CLM	Colombians from Medellin, Colombia	AMR
					PEL	Peruvians from Lima, Peru	AMR
Ortiz-Flores et al.	CL	Mexico	Yes	Yes	MXL	Mexican Ancestry from Los Angeles USA	AMR
Ortiz-Flores et al.	VL	Mexico	Yes	Yes	MXL	Mexican Ancestry from Los Angeles USA	AMR
Sophie et al.	CL	Pakistan	Yes	Yes	GIH	Gujarati Indian from Houston, Texas	SAS
					PJL	Punjabi from Lahore, Pakistan	SAS
					BEB	Bengali from Bangladesh	SAS
					STU	Sri Lankan Tamil from the UK	SAS
					ITU	Indian Telugu from the UK	SAS
Mohamed et al.	VL	Sudan	Yes	No	YRI	Yoruba in Ibadan, Nigeria	AFR
					LWK	Luhya in Webuye, Kenya	AFR
					GWD	Gambian in Western Divisions in the Gambia	AFR
					MSL	Mende in Sierra Leone	AFR
					ESN	Esan in Nigeria	AFR
					ASW	Americans of African Ancestry in SW USA	AFR
					ACB	African Caribbeans in Barbados	AFR
Ejghal et al.	VL	Morocco	Yes	No	–	–	–

AMR, Ad Mixed American; *EAS*, East Asian; *EUR*, European; *SAS*, South Asian

diseases such as rheumatoid arthritis, type 1 diabetes, and inflammatory bowel disease (Archer et al. 2015).

This meta-analysis investigates the relationship of the host genetic background and susceptibility to leishmaniasis. The present meta-analysis, including case-control and family-based studies, comprises a comprehensive attempt to quantify the risk of carriers of *SLC11A1* polymorphisms for developing either VL or CL. By including all relevant data existing in the literature, we investigated the effect of eight polymorphisms: rs17235409, rs201565523, rs2276631, rs17235416, rs3731865, rs17221959, rs2279015, and rs3731864. We documented that rs2279015 is associated with all forms of leishmaniasis. In addition, we showed that rs17235409 (D543N) and rs17235416 (1729 + 55del4) are significantly associated with CL according to the co-dominant and recessive modes of inheritance, respectively. Another interesting finding is the significant association of the three neighboring polymorphisms rs17221959 (823C/T), rs2279015 (1465-85G/A), and rs17235409 (D543N) with a lower risk of VL. These findings are in line with another study reporting that *SLC11A1* carrying the D543N polymorphism confers protection against *Mycobacterium tuberculosis* reactivation (Affandi et al. 2013). Our findings that *SLC11A1* [a protein that possesses immunomodulatory properties (Blackwell et al. 2003)] is associated with leishmaniasis risk in humans are further supported by findings of GWAS studies performed on canines. These studies had identified genes responsible for leishmaniasis development involved in the activity and signaling of macrophages and T helper cells (Batista et al. 2016; Utsunomiya et al. 2015).

Apart from the standard methods of meta-analysis that we used, one of the most important advantages of the present study is the integration of both case-control and family-based studies using the weighted Stouffer's method as a meta-analytical tool. This way, we achieved an increase in statistical power by increasing the number of studies and patients that were analyzed.

Nevertheless, we acknowledge the limitations that need to be considered when interpreting our results. For example, even though we did not restrict our analysis to English-written papers in order to avoid local literature bias (Pan et al. 2005), publication bias due to gray literature cannot be completely ruled out. Furthermore, since the studies on which we based our analysis did not contain any information on the severity of the leishmaniasis symptomatology or other existing background diseases, we could not stratify for such factors. In addition, due to the inconsistency of allele presentation and different allelic comparisons for the (GT)n polymorphism (rs534448891), a polymorphism significantly associated with tuberculosis (Archer et al. 2015; Meilang et al.

Fig. 3 Linkage disequilibrium between *SLC11A1* single nucleotide polymorphisms. r^2 values are depicted for the populations of the included studies and are compared (after slash, /) with r^2 values from comparable populations from the 1000 Genomes Project according to LDlink for **a** Brazilian, **b** Mexican, and **c** Pakistani populations. (NA, not available information)

a

Castelluci *etal.,* Brazil / PUR, CLM, PEL (1000GP)

	rs2276631	rs3731865	rs3731864	rs17221959	rs201565523	rs2279015	rs17235409	rs17235416
rs2276631								
rs3731865	**0.8/0.92**							
rs3731864								
rs17221959	0.1/0.019	0.05/0.019						
rs201565523								
rs2279015	0.15/0.44	0.1/0.451		0/ 0.039				
rs17235409	NA/0.064	NA/0.451		NA/0.012		NA/0.083		
rs17235416	0.01/NA	0.01/NA		0/NA		0.06/NA		

b

Ortiz-Flores *etal.,* Mexico / MXL (1000GP)

	rs2276631	rs3731865	rs3731864	rs17221959	rs201565523	rs2279015	rs17235409	rs17235416
rs2276631								
rs3731865	**NA/0.89**							
rs3731864	0/NA	NA						
rs17221959	0.02/0.034	NA/0.036	0/NA					
rs201565523								
rs2279015	0.51/0.495	NA/0.518	0/NA	0.01/0.017				
rs17235409	0.05/0.1	NA/0.094	0.01/NA	0/0.042		0.09/0.193		
rs17235416	0.06/NA		0/NA	0		0.09/NA	**0.8/NA**	

c

Sophie *etal.,* Pakistan / YRI, LWK, GWD, MSL, ESN, ASW, ACB (1000GP)

	rs2276631	rs3731865	rs3731864	rs17221959	rs201565523	rs2279015	rs17235409	rs17235416
rs2276631								
rs3731865	**NA/0.872**							
rs3731864	NA/0	NA/0						
rs17221959	NA/0.012	NA/0.012	NA/0					
rs201565523								
rs2279015	NA/0.179	NA/0.194	NA/0.002	NA/0.127				
rs17235409	0/0.007	NA/0.007	NA/0.013	NA/0.175		NA/0.099		
rs17235416	0/NA						**0.78/NA**	

2012), it was not possible herein to quantitatively synthesize the data and perform a meta-analysis for the association of this polymorphism with leishmaniasis. Another limitation is imposed by HWE tests. HWE tests are routinely performed to assess the quality of genetic studies and GWASs, to screen for genotyping errors and test the validity of the genetic association assumptions. Deviations from HWE may lead to inflation of type I error rate and result in false positive associations (Li and Li 2008; Moonesinghe et al. 2010; Ziegler et al. 2008). The most common method is to test HWE in the control sample of case-control studies and then exclude studies that deviate from HWE or adjust for estimates properly (Clarke et al. 2011; Salanti et al. 2005; Sato et al. 2006; Schaid and Jacobsen 1999; Trikalinos et al. 2006). However, for both approaches, complete genotype data are needed, a case that could not be applied to all our studies, especially those performed under family-based designs (for which HWE testing is not common). More importantly, when we restricted our analysis to studies in HWE, the main findings remained unchanged.

The degree of LD between the investigated SNPs is also of importance. There are studies documenting the LD between rs17235409 and rs17235416 in Asian and African populations (Soborg et al. 2007; Yip et al. 2003). In the current meta-analysis, we found that these SNPs are both associated with CL and are in LD ($r^2 \approx 0.790$) in Mexican and Pakistani populations. We cannot reason this association to either or both polymorphisms; however, we cannot exclude that one is the causative polymorphism and the other is simply in LD with the first. Despite the efforts made, there is still no evidence that could mechanistically explain the role of the D543N amino acid substitution to *SLC11A1* expression or activity. On the contrary, according to our analysis, the three polymorphisms rs17221959, rs2279015, and rs17235409 that are also found to be associated with VL are not in LD in any tested population. Nevertheless, one should also keep in mind that several of the populations studied here are admixed and may possess a hidden degree of population stratification. In fact, this may be the reason why the authors of the primary studies chose family-based tests for the analysis, which are robust to population stratification. Given the high or moderate LD found in many pair-wise comparisons, the potential effect could be traced if data permitted to perform a multipoint meta-analysis (Bagos and Liakopoulos 2010) or to use haplotype-based methods (Bagos 2011). These two approaches could account for the possible correlations between gene polymorphisms with two different strategies. However, individual patient-level data that would allow for the use of such methods were not available and perhaps in future studies, these considerations should be taken into account.

Susceptibility to leishmaniasis can be considered a complex trait with a multifactorial etiology which necessitates a clearer insight into the genetic factors associated with it. Although a GWA study in Brazilian and Indian populations uncovered only HLA-DRB1-HLA-DQA1 locus to be associated with Leishmaniasis susceptibility (Leish et al. 2013), many reasons exist to support *SLC11A1* involvement in this susceptibility. First, an interplay between many genes already suggested by various methods (GWAS, simple Genetic Association studies, or studies in other animals) to be associated with leishmaniasis (Altet et al. 2002; Batista et al. 2016; Sanchez-Robert et al. 2008; Utsunomiya et al. 2015) may occur. Second, findings from mice studies implicate 17 *Leishmania* major response (Lmr) gene loci that regulate leishmaniasis symptomatology include immunological parameters and macrophage function (Havelkova et al. 2006). On the basis of the above-mentioned as well as our findings, *SLC11A1* gene locus emerges as an important regulator of susceptibility to leishmaniasis. Our findings put forward the notion that host macrophage *SLC11A1* protein may have multiple roles in the macrophage response to *Leishmania* infection, a hypothesis that can be experimentally investigated. Although the present findings have to be confirmed and expanded with more case-control studies, they pave the way for other genetic association studies that will take into consideration the afore mentioned issues (background disease, severity of leishmaniasis, *Leishmania* species), as well as to molecular and functional studies. Since therapeutic strategies and vaccination to combat leishmaniasis are still under development, our study provides valuable insight into the *SLC11A1* function in response to *Leishmania* parasite infection, and proposes *SLC11A1* as a putative genetic biomarker for prognosis of susceptibility to leishmaniasis.

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