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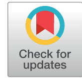
Association of *IL4R*-rs1805016 and *IL6R*-rs8192284 polymorphisms with clinical dengue in children from Colombian populations



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Background: Etiologic studies provide evidence that IL-4R and IL-6R receptors may play important roles in the regulatory mechanisms of the development of clinical dengue, especially in children which is a segment of the population with high severe dengue risk. Moreover, the allele frequencies and genetic associations may be influenced by the populational genetic background. Therefore, we performed a case-control study to evaluate possible associations between SNPs in *IL4R* and *IL6R* genes and clinical dengue in children from two Colombian populations.
Methods: We genotyped the rs1805016 (*IL4R*) and rs8192284 (*IL6R*) by PCR-RFLP method, in 298 symptomatic children and 648 asymptomatic controls. Three individual genetic ancestral proportions (APs) (European, Amerindian, African) were inferred by genotyping 29 AIMs (Ancestry informative markers). The variables gender, APs, and the population of origin were used like confusion variables.
Results: We found *IL4R*-rs1805016 GG genotype and G-allele carriers and *IL6R*-rs8192284 AA genotype associated with clinical dengue in the pooled and Huila samples. Nevertheless, we found no association of these polymorphisms in the sample of Antioquia.
Conclusions: For the first time, we report SNPs in *IL4R* and *IL6R* genes associated with clinical dengue, which contributes to understanding the genetic susceptibility to dengue disease. Moreover, these results may be influenced by genetic background and must be evaluated through functional analysis.
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Introduction

Dengue is the most rapidly spreading arboviral infectious disease in the world. There has been estimated 390 million dengue-infected people around the world, of which about 25 percent manifest clinical symptoms [1]. In Colombia, the largest historical dengue outbreak was in 2010 with 157,202 cases [2]. The four distinct serotypes of DENV, known as DENV 1–4, can cause different outcomes, from the asymptomatic subclinical infection to symptomatic illness including dengue without warning signs (DWOS), dengue with warning signs (DWWS) and severe dengue (SD) [3]. DENV disease outcome is determined by multiple interactions between viral, vector, and human genetic and immunological factors [4]. Despite the most primary DENV infections are asymptomatic [5], few studies have been focused on the factors that confer protection against clinical DENV infections. For instance, down-regulation of host defense response (innate, adaptive and matrix metalloprotease) genes has been observed in asymptomatic individuals [6]. Notwithstanding the development of a strong and specific immunologic response removes circulating DENV, a possible response in asymptomatic infections, the immune system can also exacerbate DENV pathogenesis [4]. Therefore, the impairment of responses that lead to a self-limited viral infection, may drive to clinical dengue.

Abbreviations: Afr-AP, African ancestral proportion; AIMs, Ancestry informative markers; APs, individual ancestral proportions; DENV, dengue virus; Eur-AP, European ancestral proportion; mIL-6R, IL-6R membrane anchored form membrane; sIL-6R, IL-6R soluble form.

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Recent evidence support that gene polymorphisms lead to the activation of different immuno-pathological mechanisms, which are the main determinants of the different outcomes for dengue disease [4]. Among these mechanisms, *in vitro* and *in vivo* studies suggest that DENV infection induces a predominant T helper type 1 (Th1) immune response early in the course of infection that is replaced by a T helper type 2 (Th2) response after a period of approximately three days [7], when usually strong hemorrhagic symptoms appear. Therefore, the shift from a Th1 response in mild illness to a Th2 response resulting in severe Dengue Hemorrhagic Fever (DHF) [8]. Nonetheless, it has been observed higher levels of IFN-γ and other pro-inflammatory cytokines in DHF adults and children in comparison with Dengue Fever (DF) patients. These results was observed in samples of CD4 + T-cells and blood taken in the period from 2 days of infection up to a few days after the defervescence day, which suggests the permanence of Th1 immune response in these patients [9,10]. Accordingly, it has been reported the occurrence of a Th2 immune response around the time of defervescence in a group of patients with DF, showing that Th2 is a protective response in children [7].

Among others, IL-4 and IL-6 cytokines are involved in the Th2 response. IL-4 is an anti-inflammatory cytokine which promotes the Th2 response, stimulates B-cell proliferation, and suppresses Th1 responses [11]. IL-6 is associated with dengue disease in different ways, IL-6 is a potent fever inducer, potentiates the coagulation cascade, and downregulates the production of TNFα and TNF receptors [12]. The IL-4/IL-4R interaction affects the inflammation through activation of the JAK-STAT pathway [13] which leads to the anti-inflammatory Th2 differentiation and CD4T cells expansion [14]. In particular, up-regulated *IL4R* gene was observed in asymptomatic individuals when compared to dengue symptomatic patients in a Malayan population [6], that suggests an association between IL-4R overexpression and protective responses to the development of dengue. Conversely, although the IL-6R role in the immunopathogenesis of dengue has not been studied specifically, the regulation of IL-6 responses lies in the soluble interleukin 6 receptor (sIL-6R), which forms a ligand–receptor complex with IL-6. This interaction stimulates several cellular responses including proliferation, differentiation, and modulation of inflammatory processes [15].

As we have seen, levels of cytokines indicate the activation of Th1 or Th2 responses. Nevertheless, these levels may be influenced by the genetic background. For instance, it has been reported that Afro-Colombians have higher TNFα levels than Mestizos, IL-6 levels were significantly higher in Mestizos than in Afro-Colombians, and similar IFNγ levels were detected in both ethnic groups in a Colombian population [16]. Moreover, it has been suggested that individuals with high amounts of African ancestry, may be less susceptible to serious forms of dengue [17]. Previous etiologic studies provide evidence that polymorphisms in the *IL4R* and *IL6R* genes may play important roles in the progression of dengue, and they may be influenced by the genetic background of different populations. Thus, the aim of this study was to examine the association between the polymorphisms rs1805016 in *IL4R* gene and rs8192284 in *IL6R* gene and clinical dengue in children from two Colombian populations with different ancestry background compositions.

Materials and methods

Study subjects

We used a case-control design including samples from two Colombian administrative departments (*i.e*., states): 176 dengue cases (DEN) and 311 healthy controls (HC) were from Huila; besides 122 DEN and 337 HC were from Antioquia. These departments were located southwest (Huila) and northwest (Antioquia) of the Colombian Andean region. DEN included pediatric patients admitted at the Hospital Universitario Hernando Moncaleano Perdomo (HUHMP) in Neiva (Huila), and Hospital General de Medellín, Pablo Tobón Uribe, Hospital Marco Fidel Suarez, Hospital San Vicente and Sura EPS, all in Medellín city (Antioquia). Patients of dengue, younger than 15 years of age and residents from Antioquia were recruited as cases during two dengue outbreak periods, 2006–2009 and 2010–2012, while from Huila only in the last period. Cases were defined and classified by Dengue specialist from the Colombian Institute of Tropical Medicine of Medellín, and the HUHMP of Neiva, according to the last criteria of the WHO (2009) [3]. All cases were either IgM or IgG positives. In Huila, 95.3% were IgM positive, and 72.2% were IgG positive. While in Antioquia, 97.5% were IgM positive and 25.2% were IgG positive. Moreover, all DEN were diagnosed by a clinical examination, with DENV virus detected in some of them. Immunological tests were performed in the outbreak period. Serological confirmation was performed using IgM and IgG-specific ELISA (Kit Panbio® Dengue Duo IgM and IgG Capture ELISA — PanBio Diagnostics). Most of children DEN in the sample of Antioquia were classified in DWWS (57.4%), followed by SD (7.4%) and one case with DWOS. Meanwhile, in the sample of Huila 58% of children DEN were classified as DWWS, followed by SD (35.2%) and two patients with DWOS. The 34.4% and 5.0% of DEN in the samples of Antioquia and Huila, respectively, could not be classified by lack of clinical information, and were confirmed with laboratory tests.

We included children and adults as healthy controls (HC), they were residents in Huila and Antioquia during previous dengue epidemics. HC have relatives in the region and were not related to each other or to the infant cases. The status of HC was defined by not have previous registration of consultation on suspicion of dengue or hospitalization for this illness at the time of sample collection, and they do not report any symptoms of bleeding or severe symptoms dengue during their lifetime. The HC were not evaluated for serology; therefore, this group can be formed by DENV infected asymptomatic and not DENV infected individuals. In the context of endemic regions of dengue, the composition of HC group allowed us to perform the comparisons between who developed symptoms of the dengue illness (DEN) and who not progressed to mild or severe dengue outcomes (HC).

DNA extraction and genotyping

Among 5–10 ml of whole blood sample was taken from each participant in sterile vacutainer tubes containing EDTA. Samples were taken in the respective outbreak periods and they were stored during one or two weeks until DNA extraction, performed by either the salting out or phenol–chloroform methods. Briefly, the salting out method consists of washing of the blood twice with 4× RBC (red blood cells) lysing solution (10 mM Tris–HCl; 5 mM $MgCl_2$; 10 mM NaCl) in a 15 ml tube. After 2 or 3 steps of centrifugation for 10 min at 3500 rpm, a cleaner pellet of white cells was obtained. The white pellet was resuspended in 4 ml of WBC (white blood cells) lysing solution (20 mM Tris–HCl; 20 mM EDTA; 20 mM NaCl) with 7.5 mM sodium perchlorate, 1% SDS, and 9 mM NaCl. DNA was extracted from the supernatant with 6 ml of cold absolute isopropanol, followed by 70% ethanol precipitation. The phenol–chloroform method was performed as follows. After washing with RBC lysing solution, incubation of a white pellet was made in 4 ml of WBC lysing solution with 0,05% proteinase K and 1% SDS at 54 °C overnight on a water bath. After incubation, DNA was isolated by two sequential steps of extractions with 1× phenol and 1× chloroform:isoamyl-alcohol 24:1, respectively. DNA was precipitated with 1 × 3 M sodium acetate and 3× cold absolute ethanol, followed by 70% ethanol precipitation. The DNA samples extracted

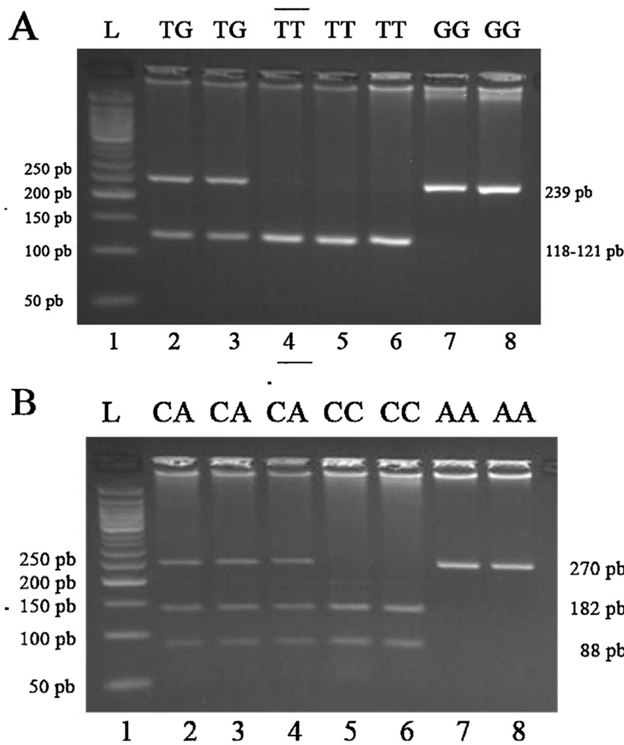

Fig. 1. PCR-RFLP assay for analyzing the rs1805016 (*IL4R*) and rs8192284 (*IL6R*). (A) *IL4R* rs1805016. Lane 1: DNA ladder (50 bp, New England); Lanes 2 and 3: TG genotypes; Lanes 4, 5 and 6: TT genotypes; Lanes 7 and 8: GG genotypes. (B) *IL6R* rs8192284. Lane 1: DNA ladder (50 bp, New England); Lanes 2, 3 and 4: CA genotypes; Lanes 5 and 6: CC genotypes; Lanes 7 and 8: AA genotypes.

by either of the two methods were resuspended in 500 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA [pH 8.0]) and were stored at −80 °C until genotyping. DNA quality and quantity were assessed using a Nanodrop 2000c (Thermo Scientific).

For the genetic ancestry determination, samples were genotyped with 29 ancestry-informative marker set (AIMs) by capillary electrophoresis and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with conditions described in a study of Colombian populations [17]. For the association study, we genotype *IL4R*-rs1805016 (S752A) and *IL6R*-rs8192284 (D358A) by PCR-RFLP using Taq DNA polymerase (Fermentas, USA).

Specifically, S752A genotyping was conducted with primers forward 5′-TCTACTCAGCCCTTACCTGC-3′ and reverse 5′-GGATTTACTCTTCTCTGAGATGC-3′. Primers for D358A genotyping were forward 5′-AGCTTGTCAAATGGCCTGTTG-3′ and reverse 5′-CAGAACAATGGCAATGCAGAG-3′. PCRs were run at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, melting temperature (S752A 57,5 °C; D358A 59,5 °C) for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were then digested with the restriction endonucleases AvaII (GĜWCC) for S752A at 37 °C overnight, and for D358A was used the HindIII (AÂGCTT) Fast Digest at 37 °C for 30 min. Primers and restriction enzymes were defined through the programs Primer3 Plus and NEBcutter version 2.0, respectively. Digestions were subjected to 3% agarose gel electrophoresis to identify the *IL4R* and *IL6R* polymorphisms on the basis of the respective size. The polymorphism genotypes of rs1805016 (*IL4R*) and rs8192284 (*IL6R*) were characterized as TT/TG/GG and CC/CA/AA, respectively (Fig. 1). Genotypes were read independently by two researchers, who were blinded to the patients' clinical status, and ambiguous results were obtained again.

Statistical analysis

Individual ancestral proportions (APs) and the average for each sample were estimated using the genotyping of 29 AIMs and allele frequencies of ancestral populations: CEU (Utah Residents with North and Western European Ancestry), CHB (Han Chinese, Beijing, China), and YRI (Yoruba in Ibadan, Nigeria) as European, Amerindian and African ancestries, respectively (1000 Genomes Project (http://www.internationalgenome.org/), and using the program ADMIXMAP v. 3.7 for Windows.

The SNPs were tested for Hardy–Weinberg Equilibrium (HWE) (chi-squared tests). The analysis of association with clinical dengue was performed using unconditional multiple logistic regression. This analysis were performed using the Genetics package in R [18]. Confounding continuous variables like age and European, Amerindian, and African APs (Eur-AP, Ame-AP, Afr-AP) ancestries were compared between DEN and HC groups by the Mann–Whitney test, and possible differences in gender were assessed with the Fisher's exact test. A posteriori power calculation was performed using DEN and HC groups and the Genetics Design package of R. All statistical analyses were performed in the R software v.3.4.1 [19].

Results and discussion

Studied population samples

We used 298 symptomatic children (DEN) and 648 healthy controls (HC). The description of populations samples was summarized in Table 1. Age was different between DEN and HC in the samples of Antioquia and Huila ($p < 0.0001$ for both). The median age of DEN was 9 (IQR = 5), while in HC was 25 (IQR = 37) in Antioquia; similarly, the median age of DEN was 6 (IQR = 5), while the median age of HC was 19 (IQR = 6.75) in Huila. Age was not used as a variable of confusion because 75.7% of controls were adults to ensure previous exposure to DENV, allowing us to compare the presentation of the disease assuming universal exposition to DENV in our sample,

Table 1
Description of study Colombian populations.

Variable	Antioquia			Huila		
	HC	DEN	p-Value	HC	DEN	p-Value
Age[c]	25 (37)	9 (5)	<0.0001[a]	19 (6.75)	6 (5)	<0.0001[a]
Gender (M/F)	(103/232)	(59/60)	0.0003[b]	(168/132)	(78/95)	0.0278[b]
Eur-AP[c]	0.68 (0.12)	0.62 (0.46)	0.0001[a]	0.58 (0.1)	0.62 (0.06)	<0.0001[a]
Ame-AP[c]	0.16 (0.08)	0.15 (0.09)	0.0096[a]	0.32 (0.15)	0.32 (0.05)	0.7188[a]
Afr-AP[c]	0.13 (0.1)	0.19 (0.45)	<0.0001[a]	0.07 (0.12)	0.07 (0.02)	0.3321[a]

HC: healthy controls. DEN: dengue cases. Eur-AP: European ancestral proportion, Ame-AP: Amerindian ancestral proportion, Afr-AP: African ancestral proportion. Gender (male/female) presented in absolute values.

[a] Mann Whitney test.
[b] Fisher's exact test. Statistical significance was set at p-Value <0.05.
[c] Median (interquartile range: difference between Q3 and Q1).

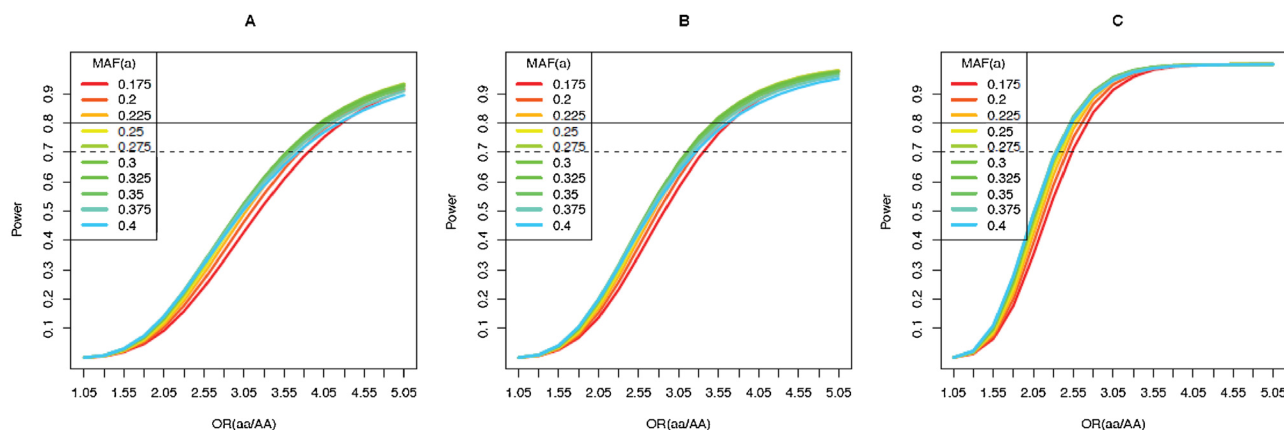

Fig. 2. Power statistical estimations for association tests in samples: (A) Antioquia. (B) Huila. (C) Pooled sample. These estimations were performed considering: (a) assumption of additive genetic model; (b) Minor Allele Frequency (MAF) between 0.175–0.40; (c) weighted average prevalence of dengue in departments (Antioquia and Huila) during years of sampling; (d) population sizes of departments; (e) D' = 0.95 between the markers and SNP associated to disease; and (f) cases/controls proportions in each sample.

especially in the context of endemic areas of dengue like Huila and Antioquia. Concerning gender, the proportion of male/female was significantly different between the DEN and HC groups in Antioquia and Huila (p-value = 0.0003, p-value = 0.0278, respectively).

There were significative differences of three ancestral proportions between DEN and HC in Antioquia (Eur-AP: p-value <0.0001; Ame-AP: p-value = 0.0096; and Afr-AP: p-value = <0.0001). In Huila, only the Eur-AP was different between DEN and HC (p-value <0.0001). Due to differences in gender and ancestral proportions, these variables were used as confounders in the association analysis. We found that the Eur-AP was similarly high in the sample of Antioquia and Huila, the second largest component was Afr-AP in the sample of Antioquia, while in the sample of Huila, the second one was Ame-AP (Table 1). These ancestral compositions were similar to the ones estimated in other two association studies carried out in Antioquia and Huila [17,20].

SNPs associated with symptomatic dengue

Statistical power estimations allowed the acceptance of the no-association hypothesis (>80%), with ORs ≥4.3 or ORs ≤0.23 in the sample of Antioquia (Fig. 2A), ORs ≥3.55 or ORs ≤0.28 in the sample of Huila (Fig. 2B), and ORs ≥2.5 or OR ≤0.4 in the pooled sample (Fig. 2C). All associations were corrected by gender and APs. The *IL4R*-rs1805016 and *IL6R*-rs8192284 were in Hardy–Weinberg Equilibrium (HWE) in the HC group (Table 3) and we found these polymorphisms associated with clinical dengue in the pooled and Huila samples, but there was no association in Antioquia (Table A1). These differences may be of the variation in the prevalence of dengue and in the genetic background between Huila and Antioquia populations. The last one is a result of the marked regional differences in pre-Columbian native population density and the extent of past European and African immigration [21].

*Interleukin 4 receptor (*IL4R*)*

We observed the *IL4R*-rs1805016 GG genotype and G-allele carriers associated with clinical dengue in the pooled sample (aOR = 2.86, p-Value = 0.0042 and aOR = 1.62, p-Value = 0.023, respectively) (Table 2) and in the sample of Huila (aOR = 5.58, p-Value = 0.0001 and aOR = 2.33, p-Value = 0.0008, respectively) (Table 3). The rs1805016 (T/G) implies the change from T-allele to G (S752A) that results in the impairment of the signaling transduction [22]. Therefore, our result is coherent with

Table 2
SNPs associated with clinical dengue in the pooled sample (Antioquia and Huila).

Gene-SNP	Frequency (%)		DEN *vs* HC	
Genotypes/alleles	HC	DEN	aOR (95% CI)[a]	p-Value
IL4R-rs1805016				
TT	323 (65.12)	117 (51.09)	Reference	
TG	148 (29.84)	83 (36.24)	1.41 (0.98–2.02)	0.2037
GG	25 (5.04)	29 (12.66)	2.86 (1.54–5.31)	**0.0042**
TG-GG	173 (34.88)	112 (48.91)	1.62 (1.15–2.27)	**0.023**
T	794 (80.04)	317 (69.21)	Reference	
G	198 (19.96)	141 (30.79)	1.64 (1.12–2.39)	**0.0696**
IL6R-rs8192284				
CC	166 (30.24)	62 (21.99)	Reference	
CA	261 (47.54)	120 (42.55)	1.47 (1–2.16)	0.18
AA	122 (22.22)	100 (35.46)	2.54 (1.66–3.9)	**<0.0001**
CA + AA	383 (69.76)	220 (78.01)	1.8 (1.25–2.57)	**0.0073**
C	593 (54.01)	244 (43.26)	Reference	
A	505 (45.99)	320 (56.74)	1.65 (1.21–2.25)	**0.0139**

HC: healthy controls. DEN: dengue cases.
[a] aOR values adjusted by gender, ancestry percentages and population of origin. p-Values in bold indicate significant association (p-Value <0.05).

Table 3
SNPs associated with clinical dengue in the sample of Huila.

Gene-SNP	Frequency (%)		DEN *vs* HC	
Genotypes/alleles	HC	DEN	aOR (95% CI)[a]	p-Value
IL4R-rs1805016				
TT	115 (59.9)	57 (42.86)	Reference	
TG	62 (32.29)	49 (36.84)	1.74 (1.01–3)	**0.0441**
GG	15 (7.81)	27 (20.3)	5.58 (2.41–12.91)	**0.0001**
TG-GG	77 (40.1)	76 (57.14)	2.33 (1.42–3.84)	**0.0008**
T	292 (76.04)	163 (61.28)	Reference	
G	92 (23.96)	103 (38.72)	2.42 (1.41–4.17)	**0.0014**
HWE (χ2; p)	2.48;0.16			
IL6R-rs8192284				
CC	97 (41.1)	46 (27.54)	Reference	
CA	105 (44.49)	69 (41.32)	1.16 (0.7–1.92)	0.5601
AA	34 (14.41)	52 (31.14)	2.63 (1.45–4.77)	**0.0015**
CA + AA	139 (58.9)	121 (72.46)	1.54 (0.97–2.45)	0.0658
C	299 (63.35)	161 (48.2)	Reference	
A	173 (36.65)	173 (51.8)	1.66 (1.07–2.56)	**0.0227**
HWE (χ2; p)	0.41;0.57			

HC: healthy controls. DEN: dengue cases.
[a] aOR values adjusted by gender and ancestry percentages. p-values in bold indicate significant association (p-value <0.05).

the impairment of IL-4/IL-4R signal transduction and with the alteration of the anti-inflammatory Th2 polarization and T-cell expansion [14]. Indeed, the up-regulated *IL4R* gene was observed in asymptomatic individuals when they were compared to dengue symptomatic patients in a Malayan population [6]. These results suggest an association between *IL4R* gene overexpression and protective responses to the development of dengue. This observation strengthens the risk role of rs1805016G allele since it alters the function of IL4-R.

*Interleukin 6 receptor (*IL6R*)*

We observed the *IL6R*-rs8192284 AA genotype, A-allele carriers and A-allele associated with clinical dengue in the pooled sample (aOR = 2.54, p-Value <0.0001; aOR = 1.8, p-Value = 0.0073; and aOR = 1.65, p-Value = 0.0139, respectively) (Table 2); and similarly, the AA genotype and A allele in the sample of Huila (aOR = 2.63, p-Value = 0.0015 and aOR = 1.66, p-Value = 0.0227) (Table 3). The IL-6R exists in two different forms, a membrane-anchored (mIL-6R) and a soluble form (sIL-6R). The latter one is generated either by proteolytic cleavage of the former or by alternative splicing. The sIL-6R alone binds to IL-6 and gp130 homodimer, this protein complex activates the JAK/STAT3 pathway and the anti-inflammatory Th2 response protein induction. At physiological levels, sIL-6R in response to IL-6 renders endothelial cells induce cytokines and chemokines, which in turn leads to leukocyte recruitment [23]. One of the SNPs related to the sIL-6R production is the *IL6R*-rs8192284 (A/C, D358A), which is located in the intron of the proteolytic site of mIL-6R. Indeed, the D358A AC and CC genotypes were already associated with the increase of the sIL-6R levels in African-Americans, European-Americans, Japanese and in some Caucasian populations [24]. Therefore, our result of clinical dengue risk D358A A-allele may be related with low sIL-6R production, and in consequence, with the low activation of the anti-inflammatory Th2 response and the decrease in the leukocyte recruitment, which may impair the antiviral response against DENV.

Conclusions

The associations that we observed of polymorphisms in *IL4R* and *IL6R* genes with clinical dengue suggest that the alteration in the cytokines receptors may be equally important than cytokines for the activation of Th1/Th2 and other immune responses, and in consequence for the immunopathogenesis of dengue. Moreover, we show that differences in genetic background may lead to differences in susceptibility to clinical dengue, associated with the diverse epidemiological features and related to the wide range of geographic landscapes occurring in Colombia.

Noteworthy, SNPs in *IL4R* and *IL6R* genes were strongly associated with clinical dengue in this study for the first time. Nevertheless, these polymorphisms must be assessed through the functional and association analyses in other Colombian populations to establish their importance as markers for the clinical dengue in this country. This information may be useful for other researchers interested in the genetic susceptibility of clinical dengue.

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Competing interests

None declared.

Ethical approval

The ethical approval was obtained from the Research Unit and Bioethics Committee of the University of Antioquia, Colombia (act number 09-12-225). The study was classified as of minimal risk (Art.11, Resolution 008430-1993, Colombian Ministry of Health). All the subjects or their legal guardians were informed of the study and gave their written informed consent to participate in the study.

Authors' contributions

GB and BNR participated in the conception of the study and together with DMS and CFN participated in its design and coordination. YMU and OC participated in the molecular assays and together with BNR, DMS and CFN participated in acquisition of data. YMU carried out the study, the statistical analysis, the interpretation of data, and drafting the manuscript. GB and BNR reviewed the manuscript critically for important intellectual content and given final approval of the version to be published.

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Appendix A

Table A1
Complete results of association analysis with polymorphisms in *IL4R* and *IL6R* genes with clinical dengue in Colombian populations.

Gene SNP	Huila				Antioquia				Pooled sample (Huila + Antioquia)			
Genotypes/alleles	Frequency (%)		aOR (95% CI)[a]	p-Value	Frequency (%)		aOR (95% CI)[a]	p-Value	Frequency (%)		aOR (95% CI)[a]	p-Value
	HC	DEN			HC	DEN			HC	DEN		
IL4R-rs1805016												
TT	115 (59.9)	57 (42.86)	Reference		208 (68.42)	60 (62.5)	Reference		323 (65.12)	117 (51.09)	Reference	
TG	62 (32.29)	49 (36.84)	1.74 (1.01–3)	**0.0441**	86 (28.29)	34 (35.42)	0.9 (0.51–1.61)	0.7238	148 (29.84)	83 (36.24)	1.41 (0.98–2.02)	0.2037
GG	15 (7.81)	27 (20.3)	5.58 (2.41–12.91)	**0.0001**	10 (3.29)	2 (2.08)	0.57 (0.12–2.83)	0.4931	25 (5.04)	29 (12.66)	2.86 (1.54–5.31)	**0.0042**
TG+GG	77 (40.1)	76 (57.14)	2.33 (1.42–3.84)	**0.0008**	96 (31.58)	36 (37.5)	0.86 (0.49–1.51)	0.6076	173 (34.88)	112 (48.91)	1.62 (1.15–2.27)	**0.023**
T	292 (76.04)	163 (61.28)	Reference		502 (82.57)	154 (80.21)	Reference		794 (80.04)	317 (69.21)	Reference	
G	92 (23.96)	103 (38.72)	2.42 (1.41–4.17)	**0.0014**	106 (17.43)	38 (19.79)	0.85 (0.43–1.68)	0.6427	198 (19.96)	141 (30.79)	1.64 (1.12–2.39)	**0.0696**
IL6R-rs8192284												
CC	97 (41.1)	46 (27.54)	Reference		88 (28.12)	48 (41.74)	Reference		166 (30.24)	62 (21.99)	Reference	
CA	105 (44.49)	69 (41.32)	1.16 (0.7–1.92)	0.5601	69 (22.04)	16 (13.91)	0.69 (0.33–1.44)	0.3233	261 (47.54)	120 (42.55)	1.47 (1–2.16)	0.18
AA	34 (14.41)	52 (31.14)	2.63 (1.45–4.77)	**0.0015**	156 (49.84)	51 (44.35)	1.02 (0.59–1.78)	0.9429	122 (22.22)	100 (35.46)	2.54 (1.66–3.9)	**<0.0001**
CA + AA	139 (58.9)	121 (72.46)	1.54 (0.97–2.45)	0.0658	225 (71.88)	67 (58.26)	0.92 (0.54–1.56)	0.7575	383 (69.76)	220 (78.01)	1.8 (1.25–2.57)	**0.0073**
C	299 (63.35)	161 (48.2)	Reference		332 (53.04)	147 (63.91)	Reference		593 (54.01)	244 (43.26)	Reference	
A	173 (36.65)	173 (51.8)	1.66 (1.07–2.56)	**0.0227**	294 (46.96)	83 (36.09)	0.86 (0.52–1.4)	0.5372	505 (45.99)	320 (56.74)	1.65 (1.21–2.25)	**0.0139**

HC: healthy controls. DEN: dengue cases.
[a] aOR values adjusted by gender and ancestry percentages. Aditionally by population of origin only for associations in the pooled sample. p-Values in bold indicate significant association (p-Value <0.05).

References

[1] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496(7446):504–7.

[2] Castrillón JC, Castaño JC, Urcuqui S. Dengue en Colombia: diez años de evolución. Rev Chilena Infectol 2015;32(2):142–9.

[3] World Health Organization. Dengue: guidelines for diagnosis, treatment, prevention, and control. New edition. Geneva: WHO Press; 2009, 147 pp.

[4] Coffey LL, Mertens E, Brehin AC, Fernandez-Garcia MD, Amara A, Despres P, et al. Human genetic determinants of dengue virus susceptibility. Microbes Infect 2009;11(2):143–56.

[5] Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. Immunol Rev 2008;255:300–13.

[6] Yeo ASL, Azhar NA, Yeow W, Talbot CC, Khan MA, Shankar EM, et al. Lack of clinical manifestations in asymptomatic dengue infection is attributed to broad down-regulation and selective up-regulation of host defence response genes. PLoS One 2014;9(4):e92240.

[7] Mabalirajan U, Kadhiravan Tamilarasu, Sharma SK, Banga A, Ghosh B. Th2 immune response in patients with dengue during defervescence: preliminary evidence. Am J Trop Med Hyg 2005;72(6):783–5.

[8] Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol 2000;28:183–8.

[9] Kadhiravan T, Saxena A, Singh A, Broor S, Sharma SK, Mitra DK. Association of intracellular TH1-TH2 balance in CD4+ T-cells and MIP-1 α in CD8+ T-cells with disease severity in adults with dengue. Immune Netw 2010;10(5):164–72.

[10] Green S, Vaughn DW, Kalayanarooj S, Nimmannitya S, Suntayakorn S, Nisalak A, et al. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J Infect Dis 1999;179:755–62.

[11] Dettogni RS, Tristao-Sa R, dos Santos M, da Silva FF, Louro ID. Single nucleotide polymorphisms in immune system genes and their association with clinical symptoms persistence in dengue-infected persons. Hum Immunol 2015;76(10):717–23.

[12] Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev 2009;22(4):564–81.

[13] Scheurer ME, Amirian E, Cao Y, Gilbert MR, Aldape KD, Kornguth DG, et al. Polymorphisms in the interleukin-4 receptor gene are associated with better survival in patients with glioblastoma. Clin Cancer Res 2008;14(20):6640–6.

[14] Zhu J, Guo L, Watson CJ, Hu-li J, Paul WE. Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. J Immunol 2001;166:7276–81.

[15] Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM. The soluble interleukin 6 receptor mechanisms of production and implications in disease. FASEB J 2001;15(1):43–58.

[16] Restrepo BN, Ramirez RE, Arboleda M, Alvarez G, Ospina M, Diaz FJ. Serum levels of cytokines in two ethnic groups with dengue virus infection. Am J Trop Med Hyg 2008;79(5):673–7.

[17] Chacón-Duque JC, Adhikari K, Avendaño E, Campo O, Ramirez R, Rojas W, et al. African genetic ancestry is associated with a protective effect on dengue severity in Colombian populations. Infect Genet Evol 2014;27:89–95.

[18] Warnes G, Leisch F. Genetics: population genetics; 2013. https://CRAN.R-project.org/package=genetics. [Accessed 28 February 2018].

[19] R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2016.

[20] Moreno DJ, Ruiz S, Rios A, Lopera F, Ostos H, Via M, et al. Association of GWAS top genes with late-onset Alzheimer's disease in Colombian population. Am J Alzheimers Dis Other Demen 2017;32(1):27–35.

[21] Rojas W, Parra MV, Campo O, Caro MA, Lopera JG, Arias W, et al. Genetic make up and structure of Colombian populations by means of uniparental and biparental DNA markers. Am J Phys Anthropol 2010;143(1):13–20.

[22] Forster T, Chapman K, Loughlin J. Common variants within the interleukin 4 receptor α gene (IL4R) are associated with susceptibility to osteoarthritis. Hum Genet 2004;114:391–5.

[23] Romano M, Sironi M, Toniatti C, Polentarutti N, Fruscella P, Ghezzi P, et al. Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. Immunity 1997;6(March):315–25.

[24] Reich D, Patterson N, Ramesh V, De Jager PL, Mcdonald GJ, Tandon A, et al. Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. Am J Hum Genet 2007;80(April):716–26.