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FUNGAL AND PARASITIC INFECTIONS

Association of *TRAF1/C5* Locus Polymorphisms with Epilepsy and Clinical Traits in Mexican Patients with Neurocysticercosis

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ABSTRACT Neurocysticercosis is caused by the establishment of *Taenia solium* cysts in the central nervous system. Murine cysticercosis by *Taenia crassiceps* is a useful model of cysticercosis in which the complement component 5 (C5) has been linked to infection resistance/permissiveness. This work aimed to study the possible relevance for human neurocysticercosis of single nucleotide polymorphisms (SNPs) in the C5-*TRAF1* region (rs17611 *C/T*, rs992670 *G/A*, rs25681 *G/A*, rs10818488 *A/G*, and rs3761847 *G/A*) in a Mexican population and associated with clinical and radiological traits related to neurocysticercosis severity (cell count in the cerebrospinal fluid [CSF cellularity], parasite location and parasite load in the brain, parasite degenerating stage, and epilepsy). The *AG* genotype of the rs3761847 SNP showed a tendency to associate with multiple brain parasites, while the *CT* and *GG* genotypes of the rs17611 and rs3761847 SNPs, respectively, showed a tendency to associate with low CSF cellularity. The rs3761847 SNP was associated with epilepsy under a dominant model, whereas rs10818488 was associated with CSF cellularity and parasite load under dominant and recessive models, respectively. For haplotypes, *C5*- and the *TRAF1*-associated SNPs were, respectively, in strong linkage disequilibrium with each other; thus, these haplotypes were studied independently. For *C5* SNPs, carrying the *CAA* haplotype increases the risk of showing high CSF cellularity 3-fold and the risk of having extraparenchymal parasites 4-fold, two conditions that are related to severe disease. For *TRAF1* SNPs, the *GA* and *AG* haplotypes were associated with CSF cellularity, and the *AG* haplotype was associated with epilepsy. Overall, these findings support the clear participation of *C5* and *TRAF1* in the risk of developing severe neurocysticercosis in the Mexican population.

KEYWORDS neurocysticercosis, genetic polymorphism, C5 complement, TRAF1, association, SNP, TRAF1/C5

Human neurocysticercosis (NCC) is caused by the establishment of the larval stage of *Taenia solium* in the central nervous system (CNS); it is a prevalent infectious disease in nondeveloped countries of Asia, Africa, and Latin America (1). NCC is a clinically and radiologically pleomorphic disease. Indeed, some infected subjects can be completely asymptomatic, while others exhibit a severe, acute, life-threatening clinical picture (2, 3). This variability has been linked to radiological heterogeneity, i.e., differences in parasite load and parasite location in the CNS, and to the various degenerating stages of parasites (4). Seizures are the most frequent sign of NCC, and it is a significant contributor to late-onset epilepsy in tropical regions worldwide according to a recent meta-analysis study (5). Among the factors involved in the clinical and radiological heterogeneity of NCC, those related to the host immunoinflammatory and endocrino-

Citation Villegas M, Sciutto E, Rosetti M, Fleury A, Fragoso G. 2019. Association of *TRAF1/C5* locus polymorphisms with epilepsy and clinical traits in Mexican patients with neurocysticercosis. Infect Immun 87:e00347-19. https://doi.org/10.1128/IAI.00347-19.

Editor De'Broski R. Herbert, University of Pennsylvania

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Received 2 May 2019
Returned for modification 11 June 2019
Accepted 20 September 2019
Accepted manuscript posted online 30 September 2019
Published 18 November 2019

logical response have been found to play a role in the pathogenesis of the disease (6–8), and previous studies have demonstrated the relevance of the host genetic background in modulating these factors (9–12).

Particularly, the participation of the complement component 5 (C5) in the early protective inflammatory response was observed in murine cysticercosis by *Taenia crassiceps* (10). The complement system is an innate immune component with a prominent role in modulating the inflammatory response against several sterile and nonsterile pathological conditions (13). The complement system is comprised of more than 30 proteins, from which the fragments C3a, C4a, and C5a act as anaphylatoxins and chemotactic factors, triggering inflammation. *C5* gene polymorphisms have been found associated with chronic inflammatory diseases such as bronchial asthma, rheumatoid arthritis, liver fibrosis, periodontitis, and stroke (14–17). The role of complement in the outcome of neuroinflammation in sterile and nonsterile conditions has been widely studied and recently reviewed (18). Indeed, the complement system, especially the C5a fragment, plays an important role in some neuropathologies closely related to inflammation. C5 mice and C5 receptor (C5aR) knockout mice or mice therapeutically treated with specific antibodies against either C5 or C5aR showed a notable improvement in neurological disorders like traumatic brain injury, spinal cord injury, and Alzheimer's disease (19–21). Until now, no study has evaluated the levels of C5 or C5a in NCC. Proximal to *C5* is the *TRAF1* gene, which codes for the tumor necrosis factor receptor (TNFR)-associated factor 1 (TRAF1); this protein associates with and mediates the signal transduction of various receptors of the TNFR superfamily. TRAF1 plays a key role in the prosurvival downstream signaling of TNFR superfamily members such as TNFR2, LMP1, 4-1BB, and CD40; in addition, an independent role of the tumor necrosis factor (TNF) receptor was proposed as a negative regulator of the Toll-like receptor (TLR) and Nod-like receptor signaling pathways (22). TRAF1 is an essential molecule of the TNF signaling cascade, promoting the expression of inflammatory cytokines such as TNF-α through the NF-κB pathway (22), and it was recently suggested to have a regulatory influence on the expression of C5 (23). Single nucleotide polymorphisms (SNPs) of the *TRAF1* region have been associated with inflammation in rheumatoid arthritis (24). Considering this, three *C5* and two *TRAF1* SNPs, the most widely studied SNPs in these regions, were studied here to evaluate the contribution of their alleles, genotypes, and haplotypes to the clinical and radiological heterogeneity of NCC in a Mexican population, in which their significance for NCC has not been yet defined.

RESULTS

General traits of NCC patients. All patients included met the most recently validated NCC diagnosis criteria (25). The demographic and clinical-radiological characteristics of the patients enrolled are shown in Table 1. Since our study aimed to evaluate genetic factors associated with parasite location and degenerating stage, patients with mixed parasite locations and/or parasite degenerating stages were excluded from the study.

Genotypic and allelic frequencies of the five SNPs studied in all NCC patients were compared to the information reported for the Mexican migrant population in Los Angeles (MXL) in the HapMap project (see Fig. S1 in the supplemental material). As shown, a heterozygous genotype was the most frequent finding in both populations except for rs3761847, for which *AA* was the most frequent genotype in NCC patients, with a similar frequency to the heterozygous (44.49 versus 41.52). With respect to allele frequency, our results agree with the HapMap study except for the rs25681 SNP, for which we found a higher frequency of the allele *A*, while the HapMap study reported a higher frequency of the allele *G*.

Allele and genotype frequencies of *C5* and *TRAF1* SNPs and association with susceptibility to develop a severe disease. Tables S1 to S5 show the genotype and allele frequencies of the five SNPs studied for each disease-related variable (parasite location, parasite load, cerebrospinal fluid [CSF] cellularity, parasite degenerating stage, and epilepsy), along with the results of the Hardy-Weinberg equilibrium (HWE) test. A

TABLE 1 Dichotomized clinical and radiological traits of NCC patients included in this study

Trait	Category[a]	*n*
Sex	Male	110
	Female	111
Parasite degenerating stage	Vesicular (viable)	95
	Colloidal or calcified (damaged)	82
	Mixed or ND†	44
Parasite load in the brain	Single	67
	Multiple	154
Parasite location in the brain	Parenchymal	90
	Extraparenchymal	123
Cell count in CSF (CSF cellularity)	High, >15/ml	85
	Low, <15/ml	113
	ND	23
Epilepsy	Yes	101
	No	84
	Unknown	36

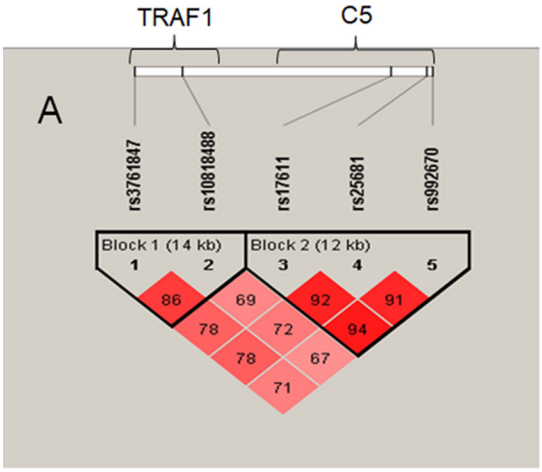
[a]ND, not determined; †, not included in SNP analysis.

deviation of the HWE test was observed in the *C5* rs17611 SNP with respect to parasite load, parasite location in the brain, CSF cellularity, and epilepsy, while a deviation in the HWE was observed in the *C5* rs992670 and *TRAF1* rs3761847 SNPs with respect to parasite load only.

The effect of each SNP on the disease was evaluated by regression analysis on individual genotypic variants (Tables S6 to S10) and under two genetic models of inheritance (dominant and recessive) (Table S11). Odds ratio (OR) data and *P* values of the frequency of association of the SNPs are shown. The phenotypes associated with increased inflammation are extraparenchymal location, multiple brain parasites in the degenerating state, and a CSF cellularity of >15 cells/ml. As observed, none of the individual SNPs was found to be associated with the risk of having parenchymal or extraparenchymal NCC, with the risk of having vesicular or damaged parasites, or with the risk of suffering epilepsy ($P > 0.05$; Tables S6, S9, and S10, respectively). Although the AG genotype of the *TRAF1* rs3761847 SNP showed a slight tendency to associate with multiple brain parasites (Table S7), in this variable only age was statistically significant ($P < 0.05$). The *CT* and *GG* genotypes of the *C5* (rs17611) and *TRAF1* rs3761847 SNPs, respectively, showed a tendency toward association with low CSF cellularity (Table S8), with a statistically significant effect for sex as a covariate of these SNPs ($P < 0.05$), but not for age, since a higher inflammatory CSF was found in women ($P < 0.05$). According to the analysis of the effect of these SNPs under two inheritance models, the *TRAF1* rs10818488 (*A/G*) SNPs have a strong tendency toward association at the limit of significance ($P = 0.0511$) with the parasite location in the brains of NCC patients in a recessive model. In a dominant model, this SNP was significantly associated with CSF cellularity ($P < 0.05$) and showed a tendency to associate with epilepsy ($P = 0.096$). Meanwhile, the *TRAF1* rs3761847 (*A/G*) SNP was found to be significantly associated with epilepsy ($P = 0.018$) in a dominant model and showed a tendency toward association with CSF cellularity ($P = 0.085$). No association of the three *C5* SNPs studied was found with any clinical-radiological trait of NCC patients (Table S11).

***C5* and *TRAF1* haplotypes and their association with disease severity.** As shown in Fig. 1, two haplotype groups were constructed, one for *C5* SNPs and the other for *TRAF1* SNPs. No linkage disequilibrium (LD) was found between *TRAF1* and *C5* haplotypes. The highest disequilibrium was found among *C5* SNPs ($D' = 0.94$).

The most common haplotypes in NCC patients for *C5* were *TAA* (51.41%) and *CGG* (35.3%); *CAA*, *CGA*, *CAG*, and *TGA* were also present at a very low frequency (3.7, 3.1, 1.2, and 1.15%, respectively). For *TRAF1*, *AG* and *GA* were the most frequent haplotypes



B

Linkage disequilibrium in the study population

		Cellularity		Number of parasites		Parasite location		Parasite stage		Epilepsy	
		D′	r^2	D′	r^2	D′	r^2	D′	r^2	D′	r^2
rs17611	rs25681	0.9	71	0.95	81	0.92	73	0.93	77	0.96	78
rs25681	rs992670	0.91	72	0.89	73	0.91	72	0.94	77	0.94	78
rs17611	rs992670	0.92	65	0.95	75	0.94	68	0.95	72	0.95	68
rs10818488	rs3761847	0.88	73	0.81	65	0.86	73	0.89	74	0.85	70

C

C5 Haplotypes	Frequency (%)
TAA	51.41
CGG	35.30
CAA	3.68
CGA	3.11
CAG	1.18
TGA	1.15
TAG	0.69
TGG	0.44

D

TRAF1 Haplotypes	Frequency (%)
AG	60.527
GA	29.222
AA	3.604
GG	2.734

FIG 1 Representative pairwise linkage disequilibrium (LD) in the *C5/TRAF1* region for CSF cellularity. Three single *C5* SNPs and two *TRAF1* SNPs were analyzed in this study. An LD test was performed using HAPLOVIEW v4.2. The relative location of each SNP along chromosome 9 is shown. (A) Diamonds in haplotype blocks represent the pairwise linkage disequilibrium between all SNPs analyzed. As shown, two blocks were constructed: one for *C5* SNPs and the other for *TRAF1* SNPs. (B) *D′* and r^2 values for each comparison (cellularity, parasite degenerating state, number and location of brain parasites, and epilepsy). (C) Frequency of the constructed haplotypes for *C5* in NCC patients. (D) Frequency of the constructed haplotypes for *TRAF1* in NCC patients.

(60.53 and 29.22%, respectively), while the least frequent haplotypes were *AA* and *GG*, at 3.6 and 2.7% frequencies, respectively (Fig. 1). Associations of SNP haplotypes with disease severity in NCC patients were obtained using SNP analyzer software (26). Among *C5* haplotypes, *CAA* was significantly associated with parasite location in the brain, CSF cellularity, and epilepsy in both additive and dominant inheritance models (Fig. 2, 3, and 4, respectively). The *TGA* haplotype was significantly associated with CSF cellularity, but only in an additive inheritance model (Fig. 3). With respect to *TRAF1* haplotypes, *GA* was found to be significantly associated with CSF cellularity in both additive and dominant inheritance models (Fig. 5), whereas the haplotype *AG* was significantly associated with CSF cellularity in additive and recessive inheritance models and with epilepsy in additive and dominant inheritance models (Fig. 5 and 6, respectively). None of the *C5* haplotypes was significantly associated with parasite degenerating stage or parasite load in the brain, whereas no *TRAF1* haplotypes were significantly associated with parasite load, parasite degenerating stage, or parasite location in the brain (data not shown).

DISCUSSION

Neurocysticercosis, due to the establishment of *T. solium* cysticerci in the CNS, is a clinically and radiologically heterogeneous disease. Symptoms vary from headaches to severe intracranial hypertension; at a radiological level, the number, location, and degenerating stage of brain parasites are highly variable among patients (4). Heterogeneity in the intensity of the inflammatory reaction is also frequent, and a clear

A

C5 haplotypes	Frequency of C5 haplotypes in NC patients with parasites located in	
	Parenchyma, N (%) Total number: 182	Extra-Parenchyma, N(%) Total Number:245
TAA	95 (52.2)	124 (50.6)
CGG	71 (39.0)	88 (35.9)
CGA	6 (3.3)	8 (3.3)
CAG	3 (1.6)	2 (0.08)
CAA	3 (1.6)	14 (5.7)
TGA	2 (1.1)	3 (1.22)
TGG	1 (0.05)	4 (1.6)
TAG	1 (0.05)	2 (0.08)

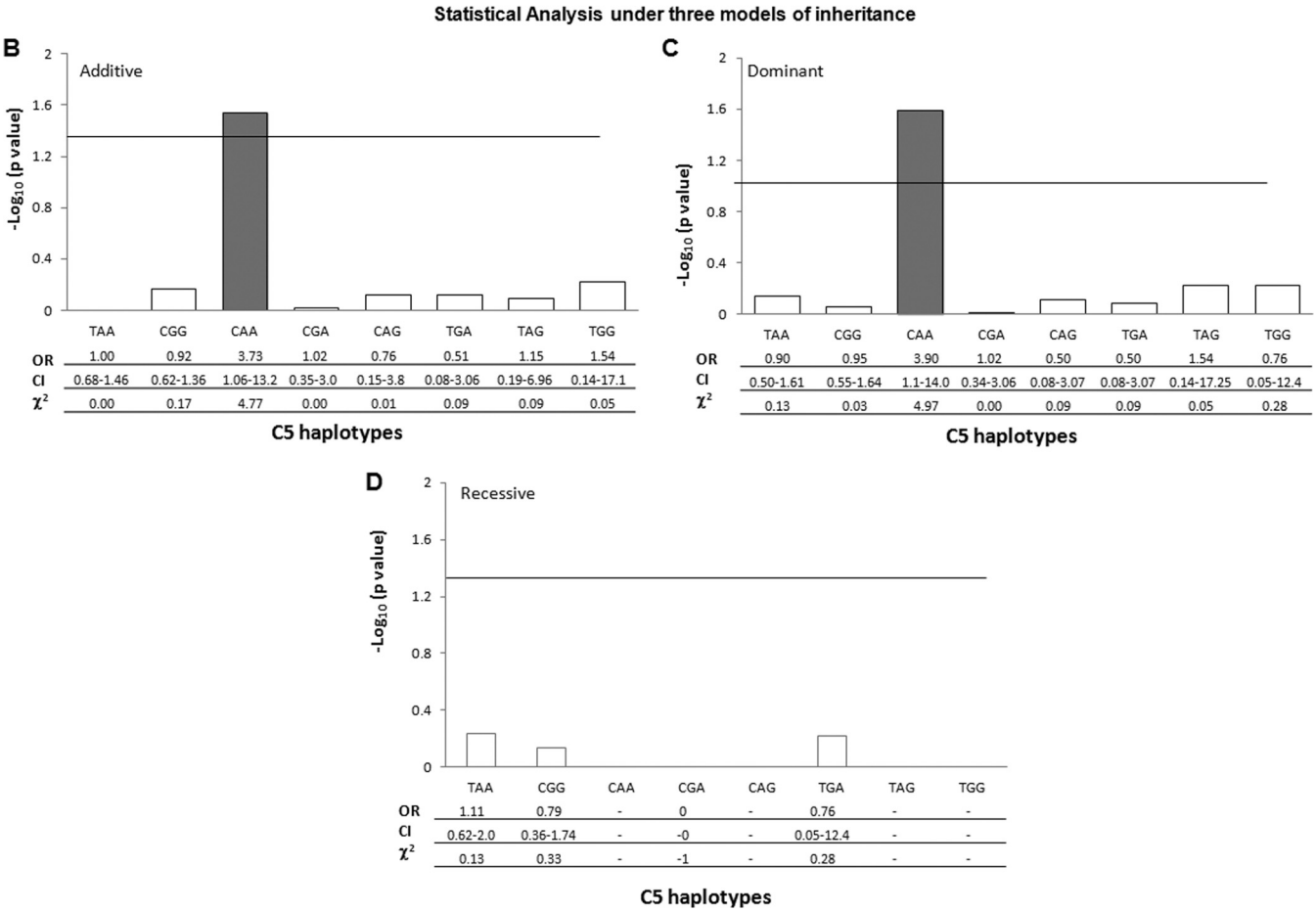


FIG 2 Association of *C5* constructed haplotypes with parasite location in NCC patients (parenchymal versus extraparenchymal). (A) The absolute numbers and percentage values of each haplotype for patients with parenchymal and extraparenchymal brain parasites are shown. Graphs were generated using the SNP analyzer software. The graphs show the statistical association of the generated C5 haplotypes with parasite location for three inheritance models (B, additive; C, dominant; and D, recessive). *P* values are graphed as the $-\log_{10} P$ and are represented in the *y* axis. The horizontal line in each model represents the significance level ($P = 0.05$). χ^2, OR, and confidence interval values are shown under each haplotype.

relationship between increased inflammation and the destruction of parenchymal parasites has been observed (4). In addition, an association between degenerated parasites and the occurrence of symptomatic seizures has been clearly described (27, 28).

The host genetic background has been reported to participate in the clinical and radiological heterogeneity of NCC. Polymorphisms in the gene coding for the Toll-like receptor 4 (*TLR4*) were found associated with calcified parasites and seizure occurrence;

A

C5 haplotypes	Frequency of C5 haplotypes in NC patients with high and low CSF cellularity	
	Low, N (%) Total number: 271	High, N(%) Total Number:172
TAA	145 (53.5)	92 (53.5)
CGG	107 (39.48)	56 (32.6)
CAA	6 (2.21)	11 (6.4)
CGA	8 (2.95)	6 (3.5)
CAG	5 (1.84)	0
TGA	0	5 (2.9)
TAG	0	2 (1.1)

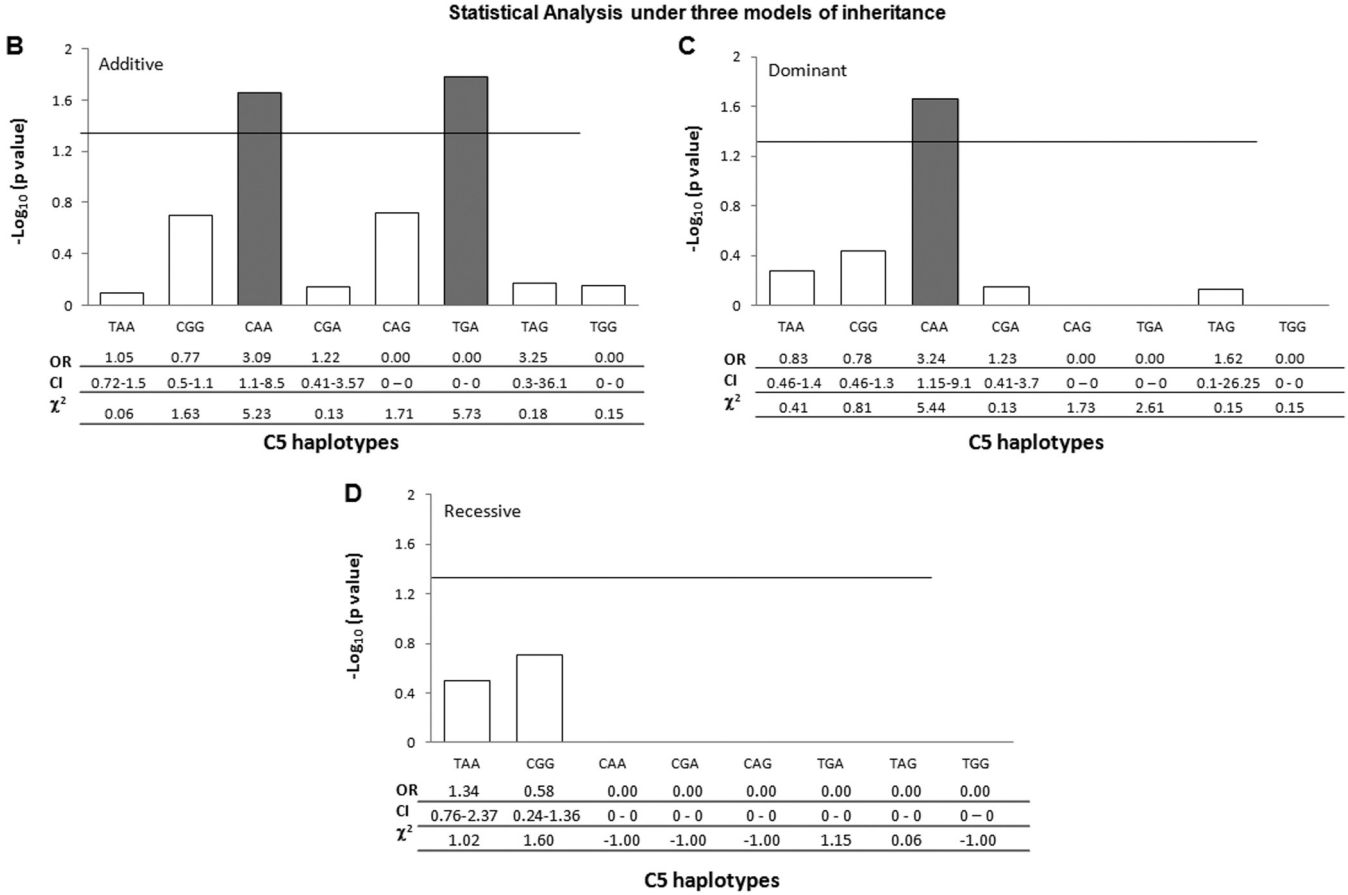


FIG 3 Association of *C5* constructed haplotypes with CSF cellularity. (A) The absolute numbers and percentage values of each C5 generated haplotype for patients with high and low CSF cellularity values are shown. Graphs were generated using the SNP analyzer software. The graphs show the statistical association of the generated C5 haplotypes with parasite location for three inheritance models (B, additive; C, dominant; and D, recessive). *P* values are graphed as the $-\log_{10} P$ and are represented in the *y* axis. The horizontal line in each model represents the significance level ($P = 0.05$). χ^2, OR, and confidence interval values are shown under each haplotype.

polymorphisms in the gene coding for matrix metalloproteinase-9 (*MMP-9*) were associated with calcified parasites (9), and polymorphisms in the gene coding for the intercellular adhesion molecule 1 (*ICAM-1*) were linked with brain edema (11).

The genes *TRAF1* and *C5* are associated with the immunoinflammatory response, and the selected SNPs have been found to be associated with the inflammatory reaction in several diseases (14, 16, 17, 29, 30). In addition, in a previous study on the murine model of cysticercosis by *T. crassiceps*, the *C5* gene was associated with susceptibility to the infection (10). Since inflammation is one of the main clinical

A

C5 haplotypes	Frequency of C5 haplotypes in epileptic and non-epileptic NC patients	
	Non-epileptic, N (%) Total number: 202	Epileptic, N(%) Total Number: 164
TAA	105(51.9)	84(52.4)
CGG	66(32.5)	64(39.1)
CAA	13(6.4)	2(3.1)
CGA	8(4.03)	5(1.16)
CAG	2(1.1)	1(0.66)
TGA	1(0.53)	0
TGG	1(0.52)	1(0.61)
TAG	0	2(0.62)

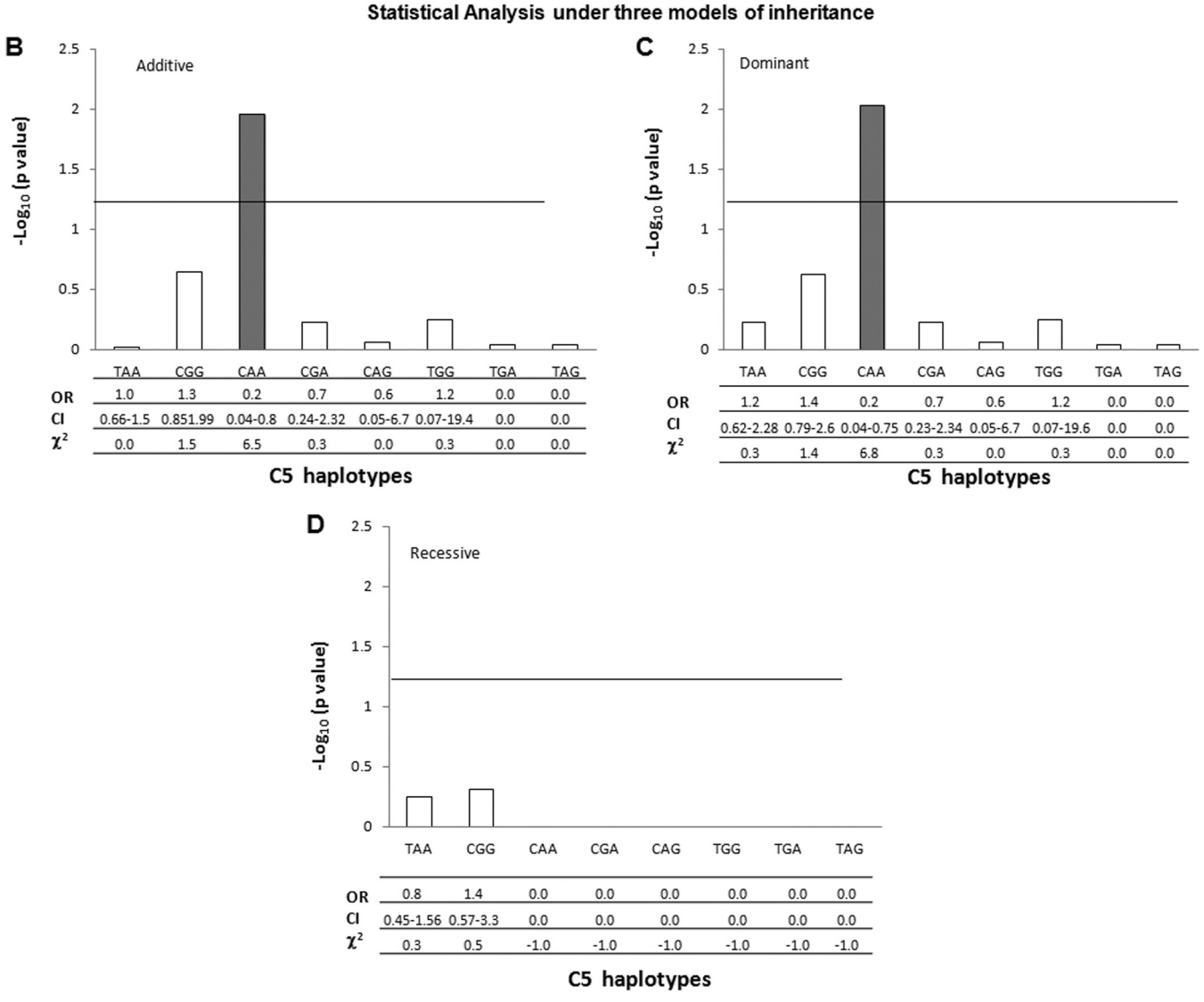


FIG 4 Association of *C5* constructed haplotypes with epilepsy presentation. (A) The absolute numbers and percentage values of each C5 generated haplotype for epileptic and nonepileptic patients are shown. Graphs were generated using the SNP analyzer software. The graphs show the statistical association of the generated C5 haplotypes with parasite location for three inheritance models (B, additive; C, dominant; and D, recessive). *P* values are graphed as the $-\log_{10}$ *P* and are represented in the *y* axis. The horizontal line in each model represents the significance level ($P = 0.05$). χ^2, OR, and confidence interval values are shown under each haplotype.

A

TRAF1 haplotypes	Frequency of TRAF1 /C5 haplotypes in NC patients with high and low number of cells in the CSF	
	Low, N (%) Total number: 274	High, N(%) Total Number: 169
GA	162 (59.12)	117 (69.2)
AG	94 (34.3)	41 (24.3)
AA	8 (2.9)	3 (1.8)
GG	10 (3.6)	8 (4.7)

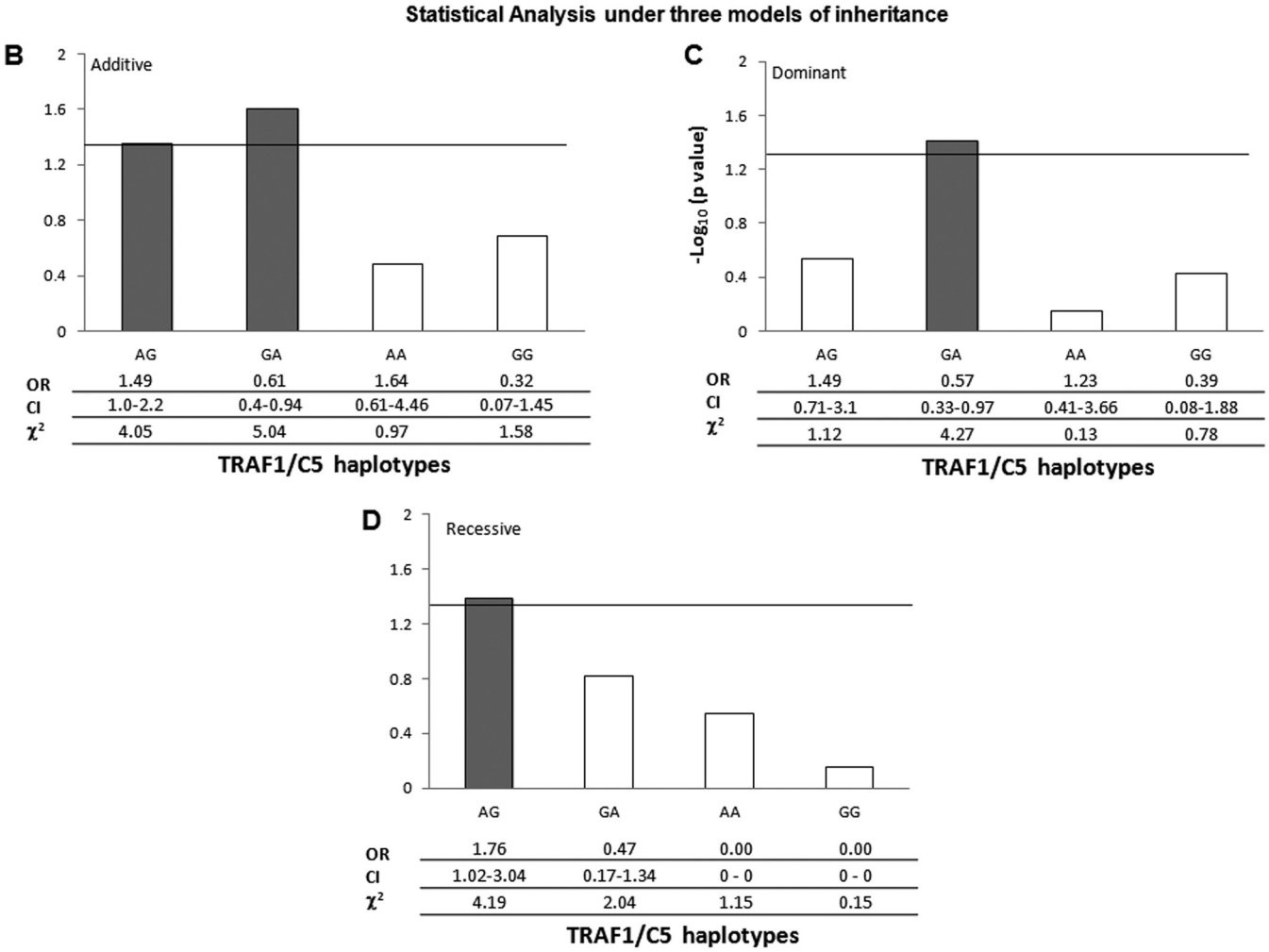


FIG 5 Association of *TRAF1/C5* constructed haplotypes with CSF cellularity. (A) The absolute numbers and percentage values of each C5 generated haplotype for patients with high and low CSF cellularity values are shown. Graphs were generated using the SNP analyzer software. The graphs show the statistical association of the generated *TRAF1* haplotypes with parasite location for three inheritance models (B, additive; C, dominant; and D, recessive). *P* values are graphed the as $-\log_{10} P$ and are represented in the *y* axis. The horizontal line in each model represents the significance level ($P = 0.05$). χ^2, OR, and confidence interval values are shown under each haplotype.

manifestations of symptomatic NCC, the relationship between five SNPs in the *TRAF1* region and NCC severity in Mexican patients was studied here.

None of the three *C5* SNPs under study was found to be significantly associated with the clinical and radiological traits of NCC patients. A tendency toward association was found between the *CT* genotype of the rs17611 SNP and a low CSF cellularity level. No significant association of the *C5* SNPs was found in either dominant or recessive inheritance models. A joint contribution of these three *C5* SNPs has been found only in one study on the human inflammatory disease caused by dental plaque in Chinese

A

TRAF1/C5 haplotypes	Frequency of TRAF1 /C5 haplotypes in epileptic and non-epileptic NC patients	
	Non-epileptic, N (%) Total number: 190	Epileptic, N (%) Total Number: 164
GA	127 (66.8)	92 (56.1)
AG	51 (26.8)	59 (36.0)
AA	8 (4.2)	7 (4.2)
GG	4 (2.1)	6 (3.6)

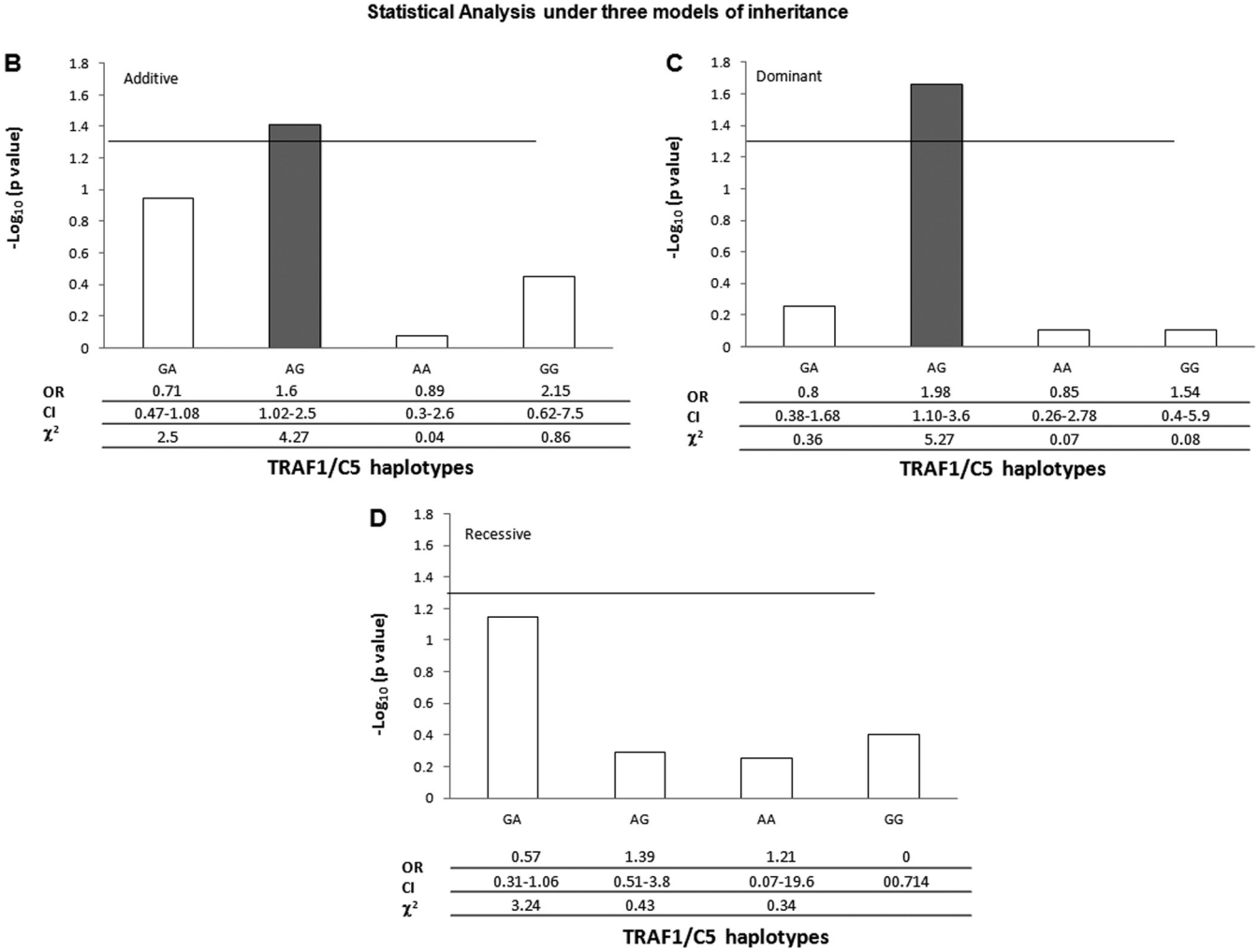


FIG 6 Association of *TRAF1/C5* constructed haplotypes with epilepsy presentation. (A) The absolute numbers and percentage values of each C5 generated haplotype for epileptic and nonepileptic patients are shown. Graphs were generated using the SNP analyzer software. The graphs show the statistical association of the generated *TRAF1* haplotypes with parasite location for three inheritance models (B, additive; C, dominant; and D, recessive). *P* values are graphed as the $-\log_{10} P$ and are represented in the *y* axis. The horizontal line in each model represents the significance level ($P = 0.05$). χ^2, OR, and confidence interval values are shown under each haplotype.

patients from Hong Kong (29). From these three *C5* SNPs, the most widely studied one in inflammatory diseases is rs17611, which is related to C5 serum levels in these patients (31). Considering the relevance of C5 in murine cysticercosis (10), it could be hypothesized that these SNPs are involved in the success of infection rather than in the clinical and radiological status of NCC patients. The involvement of C5 in the innate immunity against *Taenia taeniaeformis* supports this possibility (32). In addition, several cestodes, including *Echinococcus multilocularis* and *T. crassiceps*, are known to be damaged by the complement system (33, 34). Considering this, further family studies

(parents and NCC children) will be required to evaluate the role of these *C5* SNPs in the risk of acquiring the infection. However, the relevance of these *C5* SNPs for disease severity cannot be ruled out, and the lack of association observed here may be due to the small sample size.

TRAF1 SNPs made a greater contribution to the clinical and radiological traits of NCC patients than *C5* SNPs. Indeed, the *AG* genotype of the rs3761847 SNP showed a tendency toward association with multiple brain parasites and CSF cellularity. This SNP showed a tendency toward association with CSF cellularity and a significant association with epilepsy under a dominant model of inheritance (Table S11). None of the clinical and radiological NCC traits that have been reported to underlie epilepsy development, such as degenerating parasites and a parenchymal parasite location, were found significantly associated with this SNP. These findings indicate that epilepsy in NCC patients is a complex phenomenon that may be related to several factors beyond parasite location and degenerating state. The *TRAF1* rs10818488 (*A/G*) SNP has a strong tendency toward association, at the limit of significance ($P = 0.051$), with parasite location in the brain of NCC patients in a recessive model of inheritance and with CSF cellularity ($P = 0.022$) in a dominant model of inheritance, although with low OR values, accounting for the low relevance of this SNP in the evolution of the disease, as shown in Table S11.

TRAF1 is a member of the TNF receptor family that participates in several signaling pathways, especially in NF-κB signaling, which promotes the expression of inflammatory cytokines such as TNF-α (22) and regulates leukocyte recruitment to inflamed tissues (35); this could explain the relationship of the rs10818488 SNP with CSF cellularity. In addition, TRAF1 forms a heterodimeric complex with TRAF2, required for the activation of the TNF-α, MAPK8/JNK, and NF-κB signaling pathways, thus promoting an inflammatory response, but also required to inhibit proapoptotic proteins, thus mediating antiapoptotic signals of TNF receptors (36, 37). In swine NCC, TNF-α has been detected in the fluid surrounding degenerating cysts, a condition associated with symptomatic NCC that causes additional damage to host tissues and hence may contribute to the pathology in NCC (38). Also, higher TNF-α levels were found in symptomatic NCC patients than in healthy controls and asymptomatic NCC individuals (39). Finally, a TNF-α blockade suppresses pericystic inflammation after antihelminthic treatment in swine NCC (40). All of these findings highlight the relevance of TNF-α in promoting inflammation. The two *TRAF1* SNPs here studied have not been reported to increase the levels of TNF-α, and only correlations with the production of autoantibodies have been observed. However, since *TRAF1* modulates the expression of inflammatory cytokines, it is possible that it can contribute to the expression of TNF-α. Recently, *TRAF1* was proved to be an adapter that negatively regulates TLRs (41). Previous studies failed to show an association of the *TNF* (–238*G/A*) SNP (42) or the *TLR4* (Asp299Gly) SNP (9) with parasite infection and its clinical evolution. These findings point out the relevance of studying the joint contribution of *TRAF1* and *TLR4* SNPs in the same population sample.

In previous studies of rheumatoid arthritis, high LD values were found between the *TRAF1* and *C5* genes, a result that precluded the determination of which of these two genes is responsible for the exacerbated inflammatory response (43). However, an LD of <78 between both genes was found in our study, a value like that reported by Kurreeman et al. for these genes (16). Thus, it is possible to analyze these two genes separately. From the five SNPs studied here two haplotype groups were constructed, one for *C5* and one for *TRAF1* SNPs. Among *C5* SNPs, the two with the highest frequency in our Mexican population were *TAA* (51.41%) and *CGG* (35.30%), followed by the *CAA* haplotype, with a frequency of only 3.68%. The *CAA* haplotype was significantly associated with parasite location, CSF cellularity, and epilepsy; its expression was higher in patients with extraparenchymal parasites, patients who showed high CSF cellularity levels, and patients without epilepsy, although the latter with a very low OR (0.2 in both dominant and additive models). Thus, although individual *C5* SNPs were not related to disease severity, an overall contribution of the *C5* SNPs was found when haplotypes

were considered. Nevertheless, these results should be replicated in other NCC populations.

Our data show a more severe inflammatory disease in the small population that carries the *CAA* haplotype; indeed, carrying this haplotype increased by three times the risk of showing high CSF cellularity levels (OR = 3.09 and 3.24 for the additive and dominant models, respectively). This haplotype also increased by almost four times the risk of having parasites located in extraparenchymal regions of the brain (OR = 3.73 and 3.90 for the additive and dominant models, respectively). Both conditions are clearly linked with severe disease. With respect to *TRAF1* haplotypes, *AG* was the most common haplotype in the NCC patients included (60.52%), followed by the *GA* haplotype (29.22%). Although *GA* was significantly associated with high CSF cellularity, the risk is very low (OR = 0.61 and 0.57 for the additive and dominant models); a similar situation was observed when the analysis was performed on patients with high CSF cellularity levels and suffering from epilepsy, in whom a tendency toward association of the *AG* haplotype was found in the dominant model, although with a very low risk ($P = 0.071$, OR = 0.37). Meanwhile, the *AG* haplotype increased by 1.5 to 2.0 times the risk of having a low CSF cellularity level but suffering from epilepsy. However, the number of patients included for these analyses is very low, and a larger patient sample is required to evaluate the participation of these haplotypes in the development of epilepsy along with an inflammatory status or extraparenchymal parasites along with an inflammatory status. However, other factors beyond the expression of inflammatory genes could be influencing the clinical profile of patients, such as a genetic predisposition to develop epilepsy or physiological/anatomical conditions that favor epilepsy.

The possible contribution of these genetic variants in the *C5* and *TRAF1* regions to the severity of NCC, considering the functions of the C5 and TRAF1 proteins, is depicted in Fig. 7. As proposed in the figure, the complement cascade can be activated by the parasite, rendering C5a and C5b (as previously described for other cestodes). As an anaphylatoxin, C5a promotes mastocyte degranulation and the release of histamine, which activates the endothelial cells of the blood-brain barrier (BBB), favoring the recruitment of peripheral blood mononuclear cells into the CNS, a process also promoted by the chemotactic activity of C5a. On the other hand, C5b, together with C6, C7, C8, and C9, forms the membrane attack complex, which may affect both the oncosphere and larvae before and after entering the CNS. On the other hand, TNF-α may trigger the TNFR2 signaling pathway, involving the participation of TRAF1 with the expression of inflammatory cytokines like TNF-α, which also increase BBB permeability. Further studies will be conducted to evaluate the levels of C5, C5a, and TNF-α in these NCC patients to support this hypothetical picture.

Finally, the joint contribution of the already reported SNPs that have been found to be related to infection and disease severity (such as *TLR4*, *MMP-9*, and *ICAM1*), along with those reported in this study, could set a genetic marker to predict how an individual would respond to infection by *T. solium* and the evolution of the disease to establish personalized clinical treatments.

MATERIALS AND METHODS

Population under study and characterization of NCC cases. All of the patients included in this study met the most recently validated NCC diagnosis criteria (25). All subjects were Mexicans who attended the NCC outpatient clinic at the Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico, from January 2009 to May 2016 (700 patients in total). Written informed consent was obtained from all participants, and the protocol was approved by the INNN Ethics and Research Committee. Patients came from rural communities, as well as from urban and suburban areas of Mexico. For this study, 221 patients (of 700 total) were recruited, and a DNA sample was obtained. Of these 221 patients, 111 were females, and 110 were males, with ages ranging from 17 to 74 years (mean, 37.8 $\pm$ 13.84 years old). Complete clinical-radiological records could not be recovered for all patients. The confirmatory diagnosis of NCC was based on computed tomography (CT) and magnetic resonance imaging (MRI); all images were interpreted and analyzed by a medical specialist.

The radiological presentation of NCC was evaluated at the time of diagnosis, before treatment. Based on CT and/or MRI findings, parasite degenerating stages were defined as vesicular (parasites with a transparent membrane, clear vesicular fluid, and scarce surrounding inflammatory reaction) or damaged

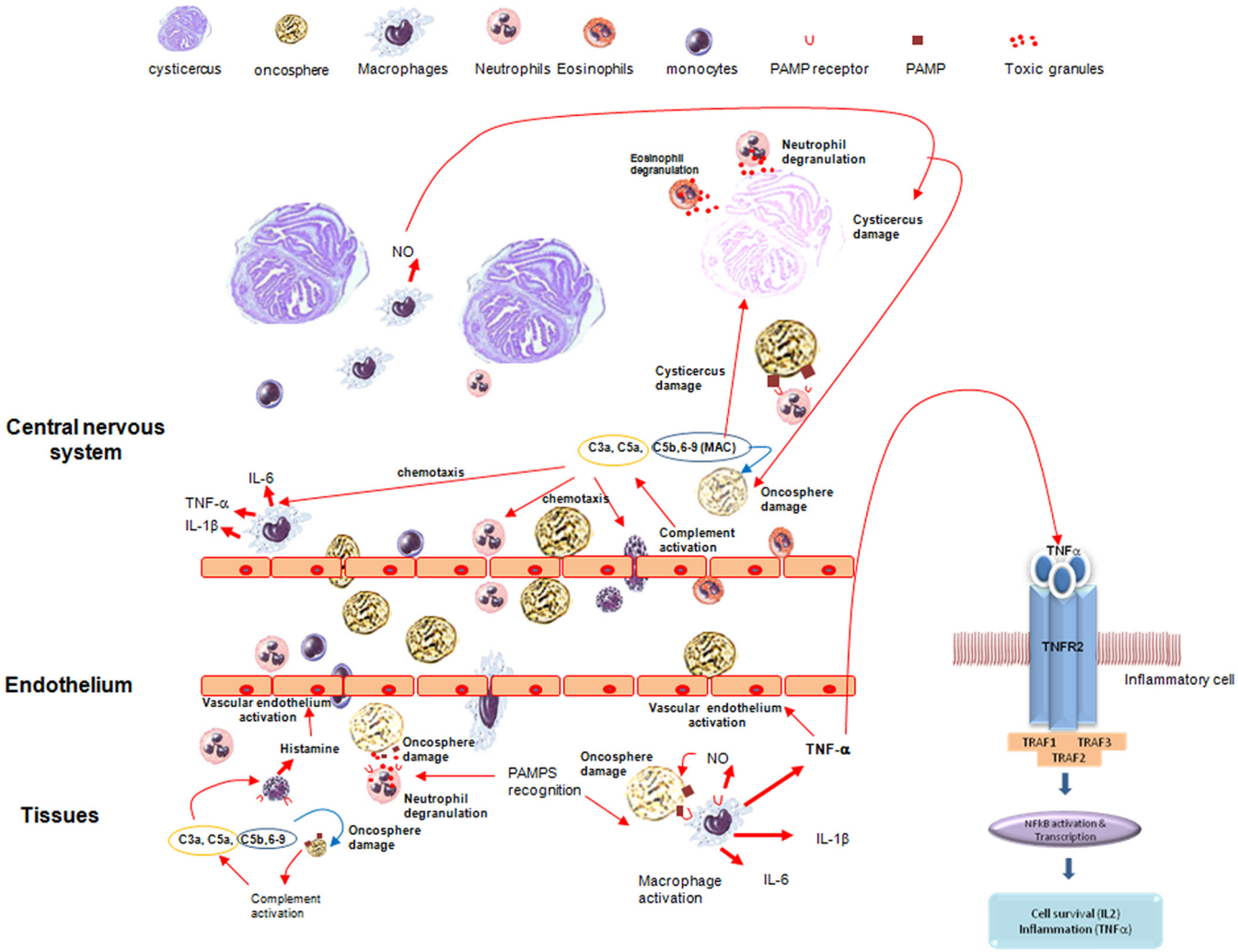


FIG 7 Schematic picture depicting the effect of the immune factors on cysticercal brain infection. Both C5a and TNF-α participate in the recruitment of peripheral blood cells (CSF cellularity increases) that may damage brain cysticerci. The exacerbated inflammation increases the severity of the disease and promotes the destruction of the parasite. Parenchymal parasites are the main cause of late-onset epilepsy in countries of endemicity, and seizure crises are clearly linked to the presence of an inflammatory reaction around the parasites. TRAF1 participates in the TNF-α signaling pathway via the TNFR2 receptor, which in turn participates in the production of TNF-α and the survival of activated cells. TNF-α, together with C5a and C3a, produced during complement activation, promotes the recruitment of peripheral macrophages, neutrophils, and eosinophils, which induce parasite damage through cell degranulation and the release of toxic molecules. On the other hand, cysticerci and oncospheres can also be damaged by the membrane attack complex of the complement.

parasites, including colloidal (degenerating parasites with turbid fluid, surrounded by an inflammatory reaction) and calcified parasites (dead parasites that appear as a mineralized granuloma).

The following information was collected from clinical and radiological studies performed for each NCC patient (provided by the medical doctor of the neurocysticercosis clinic) and dichotomized for SNP analysis: number of lesions (single versus multiple cysts), cysticercal degenerating stages (vesicular versus damaged), and CNS location (parenchymal versus extraparenchymal). To evaluate the intensity of the CNS inflammatory reaction, CSF samples were taken by lumbar puncture to determine cell counts (CSF cellularity); CSF samples were regarded as inflammatory when more than 15 cells/ml were counted. Data on epilepsy were retrieved from clinical records.

Blood samples and DNA extraction. Portions (10 ml) of EDTA-treated peripheral blood were collected; plasma and cells were separated, dispensed into aliquots, and stored at −70°C. DNA was extracted from blood leukocytes using a DNeasy blood and tissue kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. DNA was stored at −20°C until used.

SNP selection. Three *C5* SNPs (rs17611, rs25681, and rs992670), one SNP of the *TRAF1* intergenic region (rs10818488), and one of the 5′ untranslated regulatory region (5′ UTR) of *TRAF1* (rs3761847) were selected (Table 2), considering that these loci were previously identified in genetic linkage analyses as involved with permissiveness to parasite growth in *T. crassiceps* murine cysticercosis (10) and participating in many inflammatory diseases. It should be noted that, according to the HapMap project and the variations found in a Mexican population residing in Los Angeles, the changes considered for rs17611,

TABLE 2 Information of the selected SNPs in the *C5-TRAF1* region

Gene	SNP no.	Chromosome position (Chr9)[a]	Location	Base change	MAF[b] (%)
C5	rs17611	123769200	Missense	C/T	T/49
	rs25681	123780005	Intron	A/G	A/49
	rs992670	123781770	Intron	A/G	G/43
TRAF1	rs10818488	123705087	5′ UTR	A/G	A/41
	rs3761847	123690239	Regulatory region	A/G	G/39

[a]That is, the SNP position according to the NCBI database (https://www.ncbi.nlm.nih.gov/snp/) and the 1000 Genomes Project (http://phase3browser.1000genomes.org/index.html).
[b]Frequency of the minor allele for the Mexican population residing in Los Angeles (MXL) (http://phase3browser.1000genomes.org/index.html).

rs25681, rs992670, rs3761847, and rs10818488 were *C/T*, *A/G*, *A/G*, *A/G*, and *A/G*, respectively (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). In addition, the minor allelic frequency of all these SNPs was up to 40% in the Mexican migrant population of Los Angeles; this allelic frequency was used as a reference since no previous studies on these SNPs have been conducted on the mestizo Mexican population.

Genotyping *C5* and *TRAF1* SNPs. *TRAF1* (rs10818488 and rs3761847) and *C5* (rs17611, rs992670, and rs25681) SNPs and their variations were detected by quantitative PCR using TaqMan predesigned probes (Applied Biosystems, Foster City, CA), i.e., C_11720402_10, C_2359571_10, C_2783709_10, C_2783655_10, and C_2783640_10 for rs17611, rs992670, rs25681, rs10818488, and rs3761847, respectively. Briefly, 20 ng of DNA containing 20× TaqMan genotyping master mix (Applied Biosystems), including two fluorescent probes, was used, along with allele-specific primers and AmpliTaq Gold DNA polymerase. The amplification conditions were as follows: preincubation at 60°C for 30 s, incubation at 95°C for 10 min, followed by 40 two-step cycles, one for denaturalization at 95°C for 15 s and another for hybridization-elongation at 60°C for 1 min. Data on DNA quality or SNP amplification could not be obtained for all samples.

Statistical analysis. This study was designed to evaluate the relevance of three *C5* and two *TRAF1* SNPs to the severity of infection by *T. solium* in NCC patients with different clinical and radiological presentations. All patients who attended the INNN in the period from 2011 to 2015 on whom radiological and clinical studies were performed, who were diagnosed with NCC, and who met the inclusion criteria (most radiological and clinical information available and access to biological material to obtain appropriate samples [>20 ng of DNA/sample with >90% integrity]) were included. A total of 221 NCC patients of 700 met these inclusion criteria. The allele and genotype frequencies were determined, and an HWE analysis of all studied SNP and haplotype frequencies was performed for dominant and recessive models using SNP Analyzer 2.0 software (25). Haplotype construction and linkage disequilibrium (LD; $D' > 0.8$) analysis were performed using HAPLOVIEW 4.2 (http://www.broad.mit.edu/mpg/haploview/). An association between the severity of each of the studied SNPs and the relationship between the joint contribution of the SNPs and the main clinical features of the disease was also evaluated using multiple logistic regression adjusted for age and sex for the categorical response variables (number of parasites, degenerating stage, and parasite location) and a general linear model for the continuous response variables (CSF cellularity). These analyses were performed using SPSS software (IBM Statistics, v23.0.0). Statistical significance was set at $P < 0.05$. The association of the *C5* and *TRAF1* haplotypes with disease severity was tested by Bayesian statistics for the dominant, recessive, and additive models using SNP Analyzer 2.0.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/IAI.00347-19.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

We thank the subjects who participated in this study. We especially thank Juan Francisco Rodríguez for editing the manuscript and Marisela Hernández and Andrea Toledo for technical assistance. We also thank the Unidad de Investigación at Facultad de Medicina Veterinaria y Zootecnia, UNAM, for their support in the use of real-time PCR equipment.

We gratefully acknowledge the financial assistance of CONACYT to M.V. from Programa de Doctorado en Ciencias de la Producción y de la Salud Animal, UNAM. This study was supported by CONACyT (FOSISS-272519), México.

Author contributions were as follows: conception and design, M.V., E.S., and G.F.; data acquisition, interpretation, and analysis, M.V., M.R., and G.F.; drafting of the manuscript, M.V., E.S., A.F., and G.F.; and critical revision of the manuscript, E.S., A.F., and G.F. M.V. performed the experiments.

REFERENCES

1. Sciutto E, Fragoso G, Fleury A, Laclette JP, Sotelo J, Aluja A, Vargas L, Larralde C. 2000. *Taenia solium* disease in humans and pigs: an ancient parasitosis disease rooted in developing countries and emerging as a major health problem of global dimensions. Microbes Infect 2:1875–1890. https://doi.org/10.1016/S1286-4579(00)01336-8.
2. Fleury A, Cardenas G, Adalid-Peralta L, Fragoso G, Sciutto E. 2016. Immunopathology in *Taenia solium* neurocysticercosis. Parasite Immunol 38:147–157. https://doi.org/10.1111/pim.12299.
3. Raibagkar P, Berkowitz AL. 2018. The many faces of neurocysticercosis. J Neurol Sci 390:75–76. https://doi.org/10.1016/j.jns.2018.04.018.
4. Fleury A, Escobar A, Fragoso G, Sciutto E, Larralde C. 2010. Clinical heterogeneity of human neurocysticercosis results from complex interactions among parasite, host, and environmental factors. Trans R Soc Trop Med Hyg 104:243–250. https://doi.org/10.1016/j.trstmh.2010.01.005.
5. Debacq G, Moyano LM, Garcia HH, Boumediene F, Marin B, Ngoungou EB, Preux PM. 2017. Systematic review and meta-analysis estimating association of cysticercosis and neurocysticercosis with epilepsy. PLoS Negl Trop Dis 11:e0005153. https://doi.org/10.1371/journal.pntd.0005153.
6. Fleury A, Dessein A, Preux PM, Dumas M, Tapia G, Larralde C, Sciutto E. 2004. Symptomatic human neurocysticercosis: age, sex, and exposure factors relating with disease heterogeneity. J Neurol 251:830–837. https://doi.org/10.1007/s00415-004-0437-9.
7. Fleury A, Morales J, Bobes RJ, Dumas M, Yánez O, Piña J, Carrillo-Mezo R, Martínez JJ, Fragoso G, Dessein A, Larralde C, Sciutto E. 2006. An epidemiological study of familial neurocysticercosis in an endemic Mexican community. Trans R Soc Trop Med Hyg 100:551–558. https://doi.org/10.1016/j.trstmh.2005.08.008.
8. Chavarria A, Fleury A, Garcia E, Marquez C, Fragoso G, Sciutto E. 2005. Relationship between the clinical heterogeneity of neurocysticercosis and the immune-inflammatory profiles. Clin Immunol 16:271–278. https://doi.org/10.1016/j.clim.2005.04.008.
9. Lachuriya G, Garg RK, Jain A, Malhotra HS, Singh AK, Jain B, Kumar N, Verma R, Sharma PK. 2016. Toll-like receptor-4 polymorphisms and serum matrix metalloproteinase-9 in newly diagnosed patients with calcified neurocysticercosis and seizures. Medicine (Baltimore) 95:e3288. https://doi.org/10.1097/MD.0000000000003288.
10. Ramirez-Aquino R, Radovanovic I, Fortin A, Sciutto-Conde E, Fragoso-González G, Gros P, Aguilar-Delfin I. 2011. Identification of loci controlling restriction of parasite growth in experimental *Taenia crassiceps* cysticercosis. PLoS Negl Trop Dis 5:e1435. https://doi.org/10.1371/journal.pntd.0001435.
11. Singh A, Singh AK, Singh SK, Paliwal VK, Gupta RK, Prasad KN. 2014. Association of ICAM-1 K469E polymorphism with neurocysticercosis. J Neuroimmunol 276:166–171. https://doi.org/10.1016/j.jneuroim.2014.07.018.
12. Verma A, Prasad KN, Gupta RK, Singh AK, Nyati KK, Rizwan A, Pandey CM, Paliwal VK. 2010. Toll-like receptor 4 polymorphism and its association with symptomatic neurocysticercosis. J Infect Dis 202:1219–1225. https://doi.org/10.1086/656395.
13. Ricklin D, Lambris JD. 2013. Complement in immune and inflammatory disorders: pathophysiological mechanisms. J Immunol 190:3831–3838. https://doi.org/10.4049/jimmunol.1203487.
14. Karp CL, Grupe A, Schadt E, Ewart SL, Keane-Moore M, Cuomo PJ, Köhl J, Wahl L, Kuperman D, Germer S, Aud D, Peltz G, Wills-Karp M. 2000. Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. Nat Immunol 1:221–226. https://doi.org/10.1038/79759.
15. Hillebrandt S, Wasmuth HE, Weiskirchen R, Hellerbrand C, Keppeler H, Werth A, Schirin-Sokhan R, Wilkens G, Geier A, Lorenzen J, Köhl J, Gressner AM, Matern S, Lammert F. 2005. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. Nat Genet 37:835–843. https://doi.org/10.1038/ng1599.
16. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, van der Helm-van Mil AH, Allaart CF, Verduyn W, Houwing-Duistermaat J, Alfredsson L, Begovich AB, Klareskog L, Huizinga TW, Toes RE. 2007. A candidate gene approach identifies the TRAF1 region as a risk factor for rheumatoid arthritis. PLoS Med 4:e278. https://doi.org/10.1371/journal.pmed.0040278.
17. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, Liew A, Khalili H, Chandrasekaran A, Davies LR, Li W, Tan AK, Bonnard C, Ong RT, Thalamuthu A, Pettersson S, Liu C, Tian C, Chen WV, Carulli JP, Beckman EM, Altshuler D, Alfredsson L, Criswell LA, Amos CI, Seldin MF, Kastner DL, Klareskog L, Gregersen PK. 2007. TRAF1 as a risk locus for rheumatoid arthritis: a genomewide study. N Engl J Med 357:1199. https://doi.org/10.1056/NEJMoa073491.
18. Carpanini SM, Torvell M, Morgan BP. 2019. Therapeutic inhibition of the complement system in diseases of the central nervous system. Front Immunol 10:362. https://doi.org/10.3389/fimmu.2019.00362.
19. Brennnan FH, Gordon R, Lao HW, Biggins PJ, Taylor SM, Franklin RJ, Woodruff TM, Ruitenberg MJ. 2015. The complement receptor C5aR controls acute inflammation and astrogliosis following spinal cord injury. J Neurosci 35:6517–6531. https://doi.org/10.1523/JNEUROSCI.5218-14.2015.
20. Landlinger C, Oberleitner L, Gruber P, Noiges B, Yatsyk K, Santic R, Mandler M, Staffler G. 2015. Active immunization against complement factor C5a: a new therapeutic approach for Alzheimer's disease. J Neuroinflammation 12:150. https://doi.org/10.1186/s12974-015-0369-6.
21. Sewell DL, Nacewicz B, Liu F, Macvilay S, Erdei A, Lambris JD, Sandor M, Fabry Z. 2004. Complement C3 and C5 play critical roles in traumatic brain cryoinjury: blocking effects on neutrophil extravasation by C5a receptor antagonist. J Neuroimmunol 155:55–63. https://doi.org/10.1016/j.jneuroim.2004.06.003.
22. Edilova MI, Abdul-Sater AA, Watts TH. 2018. TRAF1 signaling in human health and disease. Front Immunol 9:2969. https://doi.org/10.3389/fimmu.2018.02969.
23. Messemaker TC, Frank-Bertoncelj M, Marques RB, Adriaans A, Bakker AM, Daha N, Gay S, Huizinga TW, Toes RE, Mikkers HM, Kurreeman F. 2016. A novel long noncoding RNA in the rheumatoid arthritis risk locus TRAF1-C5 influences C5 mRNA levels. Genes Immun 17:85–92. https://doi.org/10.1038/gene.2015.54.
24. Kurkó J, Besenyei T, Laki J, Glant TT, Mikecz K, Szekanecz Z. 2013. Genetics of rheumatoid arthritis: a comprehensive review. Clin Rev Allergy Immunol 45:170–179. https://doi.org/10.1007/s12016-012-8346-7.
25. Carpio A, Fleury A, Romo ML, Abraham R, Fandiño J, Durán JC, Cárdenas G, Moncayo J, Leite Rodrigues C, San-Juan D, Serrano-Dueñas M, Takayanagui O, Sander JW. 2016. New diagnostic criteria for neurocysticercosis: reliability and validity. Ann Neurol 80:434–442. https://doi.org/10.1002/ana.24732.
26. Yoo J, Lee Y, Kim Y, Rha SY, Kim Y. 2008. SNPAnalyzer 2.0: a web-based integrated workbench for linkage disequilibrium analysis and association analysis. BMC Bioinformatics 9:290. https://doi.org/10.1186/1471-2105-9-290.
27. Carpio A, Romo ML. 2014. The relationship between neurocysticercosis and epilepsy: an endless debate. Arq Neuropsiquiatr 72:383–390. https://doi.org/10.1590/0004-282x20140024.
28. Marcin-Sierra M, Arroyo M, Cadena-Torres M, Ramírez-Cruz N, García-Hernández F, Taboada D, Galicia-Martínez Á, Govezensky T, Sciutto E, Toledo A, Fleury A. 2017. Extraparenchymal neurocysticercosis: demographic, clinicoradiological, and inflammatory features. PLoS Negl Trop Dis 11:e0005646. https://doi.org/10.1371/journal.pntd.0005646.
29. Chai L, Song YQ, Zee KY, Leung WK. 2010. Single nucleotide polymorphisms of complement component 5 and periodontitis. J Periodontal Res 45:301–308. https://doi.org/10.1111/j.1600-0765.2009.01234.x.
30. Guo L, Zheng L, Guo X, Chang Y, Zhou X, Sun Y. 2016. Single-nucleotide polymorphism rs17611 of complement component 5 shows association with ischemic stroke in northeast Chinese population. Genet Test Mol Biomarkers 20:766–770. https://doi.org/10.1089/gtmb.2016.0125.
31. Gressner O, Meier U, Hillebrandt S, Wasmuth HE, Köhl J, Sauerbruch T, Lammert F. 2007. Gc-globulin concentrations and C5 haplotype-tagging polymorphisms contribute to variations in serum activity of complement factor C5. Clin Biochem 40:771–775. https://doi.org/10.1016/j.clinbiochem.2007.02.001.
32. Davis SW, Hammerberg B. 1990. *Taenia taeniaeformis*: evasion of complement-mediated lysis by early larval stages following activation of the alternative pathway. Int J Parasitol 20:587–593. https://doi.org/10.1016/0020-7519(90)90115-4.
33. Kassis AI, Tanner CE. 1977. *Echinococcus multilocularis*: complement's role *in vivo* in hydatid disease. Exp Parasitol 43:390–395. https://doi.org/10.1016/0014-4894(77)90045-5.
34. Núñez G, Villalobos N, Herrera CP, Navarrete-Perea J, Méndez A, Martinez-Maya JJ, Bobes RJ, Fragoso G, Sciutto E, Aguilar L, Del Arenal IP. 2018. Anti-GK1 antibodies damage *Taenia crassiceps* cysticerci through complement activation. Parasitol Res 117:2543–2553. https://doi.org/10.1007/s00436-018-5943-2.
35. Oyoshi MK, Barthel R, Tsitsikov EN. 2007. TRAF1 regulates recruitment of

lymphocytes and, to a lesser extent, neutrophils, myeloid dendritic cells and monocytes to the lung airways following lipopolysaccharide inhalation. Immunology 120:303–314. https://doi.org/10.1111/j.1365-2567.2006.02499.x.
36. Lu Y, Li C, Zhang P, Shao Y, Su X, Li Y, Li T. 2013. Two adaptor molecules of MyD88 and TRAF6 in *Apostichopus japonicus* Toll signaling cascade: molecular cloning and expression analysis. Dev Comp Immunol 41:498–504. https://doi.org/10.1016/j.dci.2013.07.009.
37. Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS, Jr. 1998. NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281:1680–1683. https://doi.org/10.1126/science.281.5383.1680.
38. Singh AK, Prasad KN, Prasad A, Tripathi M, Gupta RK, Husain N. 2013. Immune responses to viable and degenerative metacestode of *Taenia solium* in naturally infected swine. Int J Parasitol 43:1101–1107. https://doi.org/10.1016/j.ijpara.2013.07.009.
39. Verma A, Prasad KN, Cheekatla SS, Nyati KK, Paliwal VK, Gupta RK. 2011. Immune responses in symptomatic and asymptomatic neurocysticercosis. Med Microbiol Immunol 200:255–261. https://doi.org/10.1007/s00430-011-0198-x.
40. Mahanty S, Orrego MA, Cangalaya C, Adrianzen MP, Arroyo G, Calcina J, Gonzalez AE, García HH, Guerra-Giraldez C, Nash TE. 2017. TNF-α blockade suppresses pericystic inflammation following anthelmintic treatment in porcine neurocysticercosis. PLoS Negl Trop Dis 11:e0006059. https://doi.org/10.1371/journal.pntd.0006059.
41. Abdul-Sater AA, Edilova MI, Clouthier DL, Mbanwi A, Kremmer E, Watts TH. 2017. The signaling adaptor TRAF1 negatively regulates Toll-like receptor signaling and this underlies its role in rheumatic disease. Nat Immunol 18:26–35. https://doi.org/10.1038/ni.3618.
42. Fleury A, Alaez C, Dessein A, Rosetti M, Saenz B, Hernández M, Bobes RJ, Ramírez-Aquino R, Sciutto E, Gorodezky C, Fragoso G. 2018. No association of IL2, IL4, IL6, TNF, and IFNG gene polymorphisms was found with *Taenia solium* human infection or neurocysticercosis severity in a family-based study. Hum Immunol 79:578–582. https://doi.org/10.1016/j.humimm.2018.04.011.
43. Canhão H, Rodrigues AM, Santos MJ, Carmona-Fernandes D, Bettencourt BF, Cui J, Rocha FL, Canas Silva J, Polido-Pereira J, Pereira Silva JA, Costa JA, Araujo D, Silva C, Santos H, Duarte C, Cáliz R, Filipescu I, Pimentel-Santos F, Branco J, Sainz J, Plenge RM, Solomon DH, Bruges-Armas J, Da Silva JA, Fonseca JE, Karlson EW. 2015. TRAF1 but not PTPRC variants are potential predictors of rheumatoid arthritis response to anti-tumor necrosis factor therapy. Biomed Res Int 2015:490295. https://doi.org/10.1155/2015/490295.