The role of *ST2* and *ST2* genetic variants in schistosomiasis

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Background: Chronic schistosomiasis and its severe complication, periportal fibrosis, are characterized by a predominant T_H2 response. To date, specific single nucleotide polymorphisms in *ST2* have been some of the most consistently associated genetic variants for asthma.

Objective: We investigated the role of ST2 (a receptor for the T_H2 cytokine IL-33) in chronic and late-stage schistosomiasis caused by *Schistosoma japonicum* and the potential effect of *ST2* genetic variants on stage of disease and ST2 expression.

Methods: We recruited 947 adult participants (339 with end-stage schistosomiasis and liver cirrhosis, 307 with chronic infections without liver fibrosis, and 301 health controls) from a *S japonicum*–endemic area (Hubei, China). Six *ST2* single nucleotide polymorphisms were genotyped. Serum soluble ST2 (sST2) was measured by ELISA, and ST2 expression in normal liver tissues, Hepatitis B virus–induced fibrotic liver tissues, and *S japonicum*–induced fibrotic liver tissues was measured by immunohistochemistry.

Results: We found sST2 levels were significantly higher in the end-stage group (36.04 [95% CI, 33.85-38.37]) compared with chronic cases and controls (22.7 [95% CI, 22.0-23.4], $P < 1E-10$). In addition, *S japonicum*–induced fibrotic liver tissues showed increased ST2 staining compared with normal liver tissues ($P = .0001$). Markers rs12712135, rs1420101, and rs6543119 were strongly associated with sST2 levels ($P = 2E-10$, 5E-05, and 6E-05, respectively), and these results were replicated in an independent cohort from Brazil living in a *S mansoni* endemic region.

Conclusions: We demonstrate for the first time that end-stage schistosomiasis is associated with elevated sST2 levels and show that *ST2* genetic variants are associated with sST2 levels in patients with schistosomiasis. (J Allergy Clin Immunol 2017;140:1416-22.)

Key words: *sST2, ST2,* Schistosoma japonicum*, liver cirrhosis immunohistochemistry*

More than 200 million individuals are infected with *Schistosoma* (*S japonicum*, *S mansoni,* and *S haematobium*) worldwide.[1] *S japonicum* is the only human blood fluke that occurs in China; most recent estimates suggest that hundreds of thousands of people experience chronic or severe clinical symptoms, including liver fibrosis, liver cirrhosis, liver portal hypertension, splenomegaly, and ascites.[2,3] Thus, chronic infection and subsequent disease are responsible for a tremendous socioeconomic burden attributable to schistosomiasis in China. Long-lived adult worms inhabiting the hepatic portal vasculatures and eggs laid by the females in tissues are the cause of pathology, the severity of which is related to worm burden and intensity of the host response.[4-6] However, it has been observed in endemic areas that some individuals develop a more severe form of schistosomiasis while some develop no symptoms, despite these individuals engaging in the same activities that create contact points with the *S japonicum* habitat. This situation encourages us to identify genetic risk factors to better understand the disease.

Schistosomiasis is characterized as a polarized T_H2 immune response,[6] which is further evidenced by parasite-specific IgE responses to infection.[7] *Schistosoma* eggs trapped in tissues

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Supported by the National Institutes of Health (grant R01HL112088), the Mary Beryl Patch Turnbull Scholar Program, the Major and Special Program of National Science and Technology in Twelfth Five-year Plan of China (grant 2012ZX10002016-004), the National Natural Science Foundation of China (grant 81471612), State Key Project on Infection Disease of China (grants 2012ZX10002016-004 and 2012ZX10002010-001-004), the Natural Science Foundation of Hubei Province, China (grant 2011BCB035), and the Hepatic Surgery Clinical Study Center of Hubei Province, China (grants 2014BKB089 and 2013BCB026).

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication June 29, 2016; revised October 31, 2016; accepted for publication December 5, 2016.

Available online February 9, 2017.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.12.969

Abbreviations used
HBV: Hepatitis B virus
HWE: Hardy-Weinberg equilibrium
IHC: Immunohistochemistry
SNP: Single nucleotide polymorphism
sST2: Soluble ST2

stimulate the local infiltration of T_H2 cells that secrete IL-4, IL-5, and IL-13 in the acute stage followed by the infiltration of T_H1 cells, which is responsible for the granulomatous reaction and chronicity of lesions. ST2 has recently been identified as a T_H2 cell marker, is a member of the IL-1 receptor family, and is the receptor for the T_H2 cytokine IL-33.[8] Previous studies suggest that the interaction of IL-33 and its receptor ST2 may impact the ensuing protective immune reactions toward helminthic infection by supporting the development of a type 2 immune response.[9,10] Indeed, the absence of ST2 has been associated with impaired T_H2 immune responses following immunization with *S mansoni* eggs and, moreover, the abrogation of granuloma formation.[9] Additionally, an $ST2^{-/-}$ knockout mice model showed high susceptibility to *Toxoplasma gondi* infection.[10] ST2 is constitutively expressed or induced on several immune cell types, such as T_H2 lymphocytes, invariant natural killer T cells, natural killer cells, cytotoxic T cells, monocytes, macrophages, dendritic cells, PMNs, mast cells, basophils, and eosinophils.[10-14] Although the function of ST2 is associated with T_H2 response in acute helminthic infection, the role of ST2 in chronic/late-stage schistosomiasis is not well understood.

ST2 is encoded by the *ST2* gene, which is composed of 11 exons and a proximal and a distal promoter on chromosome 2q12.[15] It has been well-documented that single nucleotide polymorphisms (SNPs) on the *ST2* gene are associated with T_H2-mediated diseases such as atopic dermatitis[16,17] and asthma,[15,18] and elevated soluble ST2 (sST2) levels have been observed in childhood asthma.[18] The rs1420101 T allele was linked to an increased risk for asthma in a mixed population of Europe and eastern Asia,[19] while a protective role of the nonsynonymous rs1041973 A allele for asthma was observed in a Mexican population.[20] In addition, the rs654316 A allele in the distal promoter of *ST2* gene significantly correlates with upregulated ST2 transcription and high sST2 levels.[16]

In this study, we investigated the role of ST2 in chronic and late-stage schistosomiasis in a Chinese cohort and aimed to identify *ST2* genetic variants that control severity of disease and expression of ST2. It is thought that helminth infections reduce the risk and severity of allergic disease,[21] therefore an improved understanding of the host immune response to schistosomiasis, including the role of ST2, may also elucidate disease mechanisms relevant to allergic disease.

METHODS

Study populations

This study was approved by the institutional review boards of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, the Federal University of Bahia and was endorsed by the National Commission for Ethics in Human Research in Brazil, and of Johns Hopkins University School of Medicine. The study was conducted under the ethical guidelines of the National Institutes of Health. Informed written consent was provided by all participants in the study.

We recruited 947 participants from the same *S japonicum*–endemic area of Hubei Province, China, as previously described,[22] which included 339 adult subjects diagnosed as late-stage schistosomiasis with liver fibrosis (end stage), 307 adult subjects chronically infected with *S japonicum* but without liver fibrosis (chronic cases), and 301 uninfected adult controls. Clinical characteristics of the cohort are summarized in Table I. Hepatitis B virus (HBV) infection was defined as a positive HBsAg blood test, and alcohol consumption was defined as drinking more than 50 mL of Chinese white wine per day, for more than 2 years.

Fifteen paraffin-embedded *S japonicum*–induced liver fibrosis tissues, 15 paraffin-embedded HBV-induced liver fibrosis tissues, and 15 normal liver tissues from adjacent areas of hepatic hemangioma were collected from the Hepatic Surgery Center, Tongji Hospital, Huazhong University of Science and Technology (Wuhan, China), as described previously.[22] Disease state of the tissues was confirmed by histopathologic diagnosis.

For replication of findings, 812 subjects characterized for *S mansoni* infection were recruited from 5 communities (Buri, Camarao, Genipapo, Sempre Viva, and Cobo) of the district of Conde, Bahia, on the Caribbean coast of Brazil, under a whole population ascertainment scheme, as described previously.[23] The research protocol for these subjects was approved by institutional review boards at Johns Hopkins University School of Medicine and the Federal University of Bahia and was endorsed by the National Commission for Ethics in Human Research in Brazil. Adults provided written consent or verbal consent recorded by a witness. Children gave verbal assent, and written consent for children's participation was also obtained from a parent or guardian.

DNA isolation and SNP selection

Blood donated by participants was preserved in 5-mL EDTA tubes. DNA extraction from peripheral leukocytes was performed using the AutoGen FLEX STAR (AutoGen, Holliston, Mass) using FlexiGene DNA AGF 3000 Kit (catalog no. 51297; AutoGen) according to the standard protocol provided by QIAGEN (Hilden, Germany). DNA qualities were measured by NANODROP 2000 (Thermo Fisher Scientific. Waltham, Mass). Six *ST2* SNPs (rs10173081, rs10206753, rs1041973, rs12712135, rs1420101, rs6543119) were selected based on previous associations with T_H2-related traits in several genome-wide association studies and based on their allele frequencies and haplotype tagging in the HapMap CHB population (http://www.hapmap.org/).[15,18,24,25]

Genotyping

ST2 SNPs were genotyped by TaqMan^T probe-based 5′ nuclease assay with minor groove binder chemistry (Applied Biosystems, Foster City, Calif) in all samples. PCR was conducted using 2.5 μL TaqMan Genotyping Master Mix (part no. 4371357; Applied Biosystems) and 10 ng DNA in a total volume of 5 μL per well in 384 microplates. All assays were performed blindly without knowledge of disease status. Allelic discrimination was detected by ABI 7900 HT Sequence Detection System using software SDS2.4 (Applied Biosystems).

ELISA

The 5 mL venous blood that was collected from the participants was contained in SST Tube with Silica Clot Activator, Polymer Gel, Silicone-Coated Interior (REF367955; Becton Dickinson, Devon, United Kingdom). Serum was separated under centrifugation of 10 minutes, 3200 rpm. Serum sST2 levels were then measured by Presage ST2 Kits (Critical Diagnostics, San Diego, Calif) in 868 samples. The same number of samples from the end-stage group, chronic cases, and controls were plated on each plate, which also included 2 ELISA controls and standards provided by the kits. All measurements were conducted in duplicate on 96-well microplates according to the manufacturer's protocol. Readings were taken within 15 minutes after adding the stop solution at dual absorbance of 450 nm and 570 nm using a plate reader (Reader model no. iMark, Reader serial no. 10894; Bio-Rad, Irvine, Calif). The plate reader software used mean OD values of each standard to generate a standard curve using a 4-Parameter Fit equation. The sST2 concentrations for the samples and ELISA controls were calculated using this standard curve and corresponding sample mean OD values.

TABLE I. Clinical characteristics of the Hubei cohort

Trait	Total	End stage	Chronic cases	Controls	Beta (95% CI); *P* value	OR (95% CI); *P* value
N	947	339	307	301		
Males, n (%)	554 (58.7)	251 (74.3)	178 (58.4)	125 (41.5)		0.35 (0.28-0.45); < 1E-10
Age, mean ± SD	50.1 ± 13.1	55.9 ± 10.2	53.1 ± 11.0	41.7 ± 11.8	1.07 (1.06-1.08); < 10E-10	
HBV infection, n (%)	116 (12.3)	44 (13.0)	47 (15.3)	25 (8.3)		1.39 (0.98-1.96); .063
Alcohol consumption, n (%)	341 (36.0)	106 (31.4)	141 (45.9)	94 (31.2)		1.04 (0.81-1.32); .780

Each of the traits were tested for association with stage of disease (end stage, chronic case, or control) using ordinal regression.
OR, Odd ratio.

Immunohistochemistry

Immunohistochemistry (IHC) was conducted as follows: The paraffin embedded liver sections were incubated in 65°C incubator for deparaffinizing before being transferred into dimethylbenzene (10 minutes), 100% ethanol (20 seconds), 90% ethanol (20 seconds), and 80% ethanol (20 seconds) for fixation and dehydrating. The slides in citrate were put into a microwave on high for 8 minutes and on low for 10 minutes for antigen retrieval. We used 3% H_2O_2 to block endogenous peroxidase. Sections were treated with 5% BSA at room temperature for 20 minutes before being incubated with rabbit anti-human ST2 antibody (diluted in 1:200; catalog no. A1913; ABclonal, Woburn, Mass) at room temperature for 1 hour. The sections were then reacted with horseradish peroxidase–bonded secondary antibody at room temperature for 30 minutes. The slides were stained with diaminobenzidine (DAKO, Glostrup, Denmark) for 40 seconds, followed by hematoxylin counterstaining. Primary antibodies were substituted by IgG control antibodies (A7016; Beyotime, Shanghai, China) as negative controls. Staining intensity of ST2 was scored under a phase contrast microscope (Nikon Digital ECLIPSE C1 system; Nikon Company, Tokyo, Japan) based on intensity of hepatic cell staining as follows: 0 (none), 1 (light brown), 2 (brown), 3 (dark brown). The stained slides were evaluated by 3 independent pathologists without knowledge of the clinicopathologic information of patients.[22]

Statistical analysis

Unless otherwise stated, statistical analyses were conducted using the R programming language (version 3.2.3; R Foundation, Vienna, Austria).

Allele and genotype frequencies, call rates, and departures from Hardy-Weinberg equilibrium ([HWE] expected genotype frequencies assuming a random mating nonevolving population) were calculated using the PLINK version 1.07 software package (www.cog-genomics.org/plink2).

Linkage disequilibrium (a measure of the correlation between genetic variants) between pairs of SNPs was estimated using r^2, and haplotype blocks were defined using the Gabriel algorithm, as implemented in Haploview software version 4.2 (Broad Institute, Cambridge, Mass).

Univariate logistic ordinal regression was used to assess the relevance of possible covariates age, sex, HBV infection, and alcohol consumption in the Hubei cohort, using ordinal outcomes: control, chronic case, and end stage. Ordinal logistic regression was also used to test for association between each of the 6 *ST2* SNPs and disease severity as represented by the 3 ordinal outcomes. Additive allelic encoding was used to represent a SNP (0, 1, or 2 copies of the rare allele). A separate model was fitted for each of the SNPs, and each of the models included sex, age, HBV infection, and alcohol consumption as covariates. These analyses were done using the *polr* function in the *MASS* R package. To assess the possible heterogeneous effect of SNPs (eg, a SNP that is associated with chronic case vs end-stage disease, but is not associated with control vs end-stage disease), logistic regression was also used to test for association with the 3 different pairwise disease stage classifications (control vs chronic case, control vs end stage, and chronic case vs end stage). These analyses were done using the *glm* function in the base R package (using a binomial distribution with a logit link), including sex, age, HBV infection, and alcohol consumption as covariates.

Prior to analyzing sST2 levels in the Hubei cohort, the *ng/mL* values were log10 transformed, and these values were then adjusted for sex, age, HBV infection, alcohol consumption, and plate factors by fitting a linear model (*lm* function in the base R package) and adding the grand mean of the log10 transformed values to the model residuals. These adjusted values were used to estimate the mean and standard error of sST2 levels in the disease severity groups. A *t* test was used to test for differences in mean sST2 levels between end-stage cases and a combined control and chronic cases group, and this test also used the adjusted values (*t.test* function in the base R package). The necessity and effect of adjusting for these factors are illustrated in Fig E1 (in this article's Online Repository at www.jacionline.org); the statistical adjustment removes the variance in sST2 that are due to the factors, allowing for the assessment of differences in sST2 that are caused by other factors such as disease severity and genetic variants.

Linear regression was used to test for association between each of the *ST2* SNPs and adjusted sST2 levels in the Hubei cohort (*lm* function in the base R package). Additive allelic encoding was used to represent the SNP, and the analysis was adjusted for stage of disease.

Haplotype associations between particular combinations of *ST2* alleles and adjusted sST2 levels in the Hubei cohort were tested using sliding windows of 2 to 6 SNPs. Haplotypes were estimated using the expectation-maximization algorithm in the *haplo.stats* R package. Score tests were used to quantify the overall statistical significance of a particular combination of SNPs (*haplo.score* function in the *haplo.stats* R package), and linear models were used to assess the effect of particular haplotypes (combinations of alleles) compared with the most common haplotype (*haplo.glm* function in the *haplo.stats* R package).

To adjust for correlation within families, generalized estimating equation (a statistical method used to solve the parameters of a generalized linear model, accounting for possible unknown correlation between outcomes) regression was used to test for association between each of the *ST2* SNPs and sST2 levels in the Brazilian cohort (*gee* function in the *gee* R package). The sST2 measurements were log10 transformed and used as outcome variable, and the models included sex and age as covariates.

Mann-Whitney *U* tests implemented in Prism (version 5.0, GraphPad) were used to analyze IHC scores of ST2 intensity between the 3 groups of liver tissues.

Replication

The 6 *ST2* SNPs were also genotyped in 822 DNA samples from 318 nuclear families in a Brazilian population from a region endemic for *S mansoni* infection, as previously described.[23,26,27] The sST2 levels were measured as described above.

RESULTS

Call rate, HWE, and minor allele frequency

Genotypic call rates (1 − the proportion of samples with undetermined genotypes) for the 6 *ST2* SNPs ranged from 91% to 98%, and all SNPs were in Hardy-Weinberg equilibrium (HWE) in controls except for rs1041973 ($P = .020$), indicating good genotyping quality. All minor allele frequencies (the frequency of the least common allele) were more than 5%, and thus all SNPs can be considered common. See Table E1 (in this article's Online Repository at www.jacionline.org) for a detailed breakdown of these results.

TABLE II. Association between the 6 *ST2* SNPs typed in this study and sST2 levels

SNP	Base pair position*	Minor/major allele	Function	Hubei			Brazil		
				MAF	Effect size [95% CI]	*P* value	MAF	Effect size [95%CI]	*P* value
rs12712135	102930948	A/G	Intron 1	0.46	0.07 [0.05-0.09]	1E-10	0.44	0.05 [0.03-0.07]	1E-06
rs1041973	102955468	A/C	Missense (exon 3)	0.13	−0.02 [−0.06 to 0.01]	.120	0.36	−0.02 [−0.04 to 0.00]	.020
rs10173081	102957348	T/C	Intron 5	0.09	0.00 [−0.04 to 0.04]	.914	0.21	−0.01 [-0.03 to 0.01]	.530
rs1420101	102957716	T/C	Intron 5	0.41	−0.05 [−0.07 to −0.02]	5E-05	0.31	−0.05 [−0.07 to −0.03]	1E-06
rs6543119	102963072	T/A	Intron 8	0.42	−0.05 [−0.07 to −0.02]	6E-05	0.30	−0.04 [−0.06 to 0.02]	.001
rs10206753	102968362	C/T	Missense (exon 11)	0.13	−0.05 [−0.08 to −0.02]	.002	0.46	−0.01 [−0.03 to 0.01]	.417

Linear regression was used to test the association between a SNP and log10 transformed adjusted sST2 (adjusted for sex, age, HBV infection, alcohol consumption, and plate factors), and stage of disease was included as a covariate in the model.
MAF, Minor allele frequency.
*From hg19.

Pairwise linkage disequilibrium

Four SNPs of ST2 were intronic and the other 2 were missense variants (Table II). The rs1041973 variant allele results in an Ala to Glu substitution, and the rs10206753 variant allele situated in exon 11 results in a Leu to Ser substitution. The SNP rs1041973, reported to be associated with serum ST2 levels,[18] is located in exon 3 of *ST2* gene. The *ST2* gene contains 2 linkage disequilibrium blocks as defined by Gabriel algorithm (see Fig E2 in this article's Online Repository at www.jacionline.org).[28]

Tests for association between SNPs and stage of disease

Ordinal and logistic regression was used to test for association between each of the 6 *ST2* SNPs and stage of disease. None of the associations were statistically significant or suggestive of association (all $P > .1$) (see Table E2 in this article's Online Repository at www.jacionline.org).

sST2 levels in 3 groups

Fig 1 shows sST2 concentrations in the 3 disease stage groups after adjusting for age, sex, plate factors, HBV infection, and alcohol consumption. According to the figure, the distribution of sST2 in the control and chronic case groups appear similar (combined mean *ng/mL* value of 22.7 [95% CI, 22.0-23.4]), whereas relative to these groups, elevated sST2 levels are observed in the end-stage group (mean *ng/mL* value of 36.04 [95% CI, 33.85-38.37]). A statistical test confirmed that this difference is highly significant ($P < 1E-10$).

Association between *ST2* SNPs and sST2

Table II summarizes association test results of the 6 *ST2* SNPs and sST2 levels in the Hubei cohort. The A allele of rs12712135 was associated with increased levels of ST2 (log10 transformed effect size 0.07 [95% CI, 0.05-0.09]), and this association was highly significant ($P < 1E-10$). The T alleles of rs1420101 and rs6543119 were associated with decreased levels of ST2 (log10 transformed effect sizes −0.05 [95% CI, −0.07 to −0.02] and −0.05 [95% CI, −0.07 to −0.02], respectively), and these associations also achieved high significance levels ($P = 5E-05$ and 6E-05, respectively).

Association between *ST2* haplotypes and sST2

The term haplotype refers to a specific combination of ordered alleles on the same chromosome, usually inherited from the same parent. To determine whether a particular haplotype background affects ST2 levels, haplotypes formed using sliding windows of 2 to 6 SNPs were tested for association with ST2 levels (Table E3 in this article's Online Repository at www.jacionline.org). With the exception of the rs1041973-rs10173081 model, all the models fitted were highly significant ($P < 1E-05$), and with the exception of low-frequency haplotypes, most of the haplotypes showed significant negative associations compared with the reference (most common) haplotype. This means that carriers of less common haplotypes are likely to have decreased levels of sST2 compared with carriers of the most common haplotype.

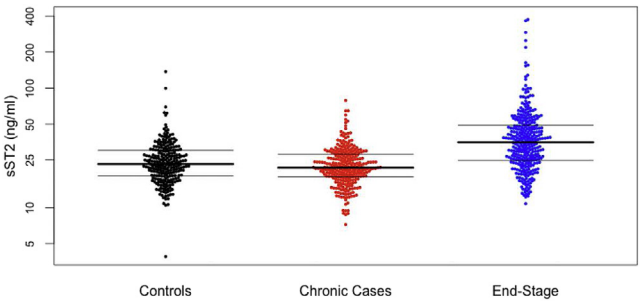

FIG 1. Boxplot of serum sST2 levels in the 3 disease groups. Values expressed in ng/mL were log10 transformed, and the values were then adjusted for sex, age, HBV infection, alcohol consumption, and plate factors by using a linear model.

Association between *ST2* SNPs and sST2 in a Brazilian cohort

The same 6 *ST2* SNPs were assessed in a cohort from Brazil. The SNPs were all common (minor allele frequency > 0.05) (Table II) and in HWE (*P* values ranging between .24 and 1.00). Although the minor allele frequencies of all but rs12712135 were quite different compared with those of the Hubei cohort (Table II), associations among rs12712135, rs1420101, rs6543119, and sST2 levels were also highly significant, and in the same positive/negative direction as in the Hubei cohort.

IHC of *ST2*

To further study the functional effects of *ST2* SNPs, we analyzed ST2 expression in normal liver tissues, HBV-induced liver fibrosis tissues and *Schistosome*-induced liver fibrosis tissues by IHC. ST2 was mainly expressed in the cytoplasm of hepatic cells (Fig 2, *A*). Both the HBV-induced and *Schistosome*-induced

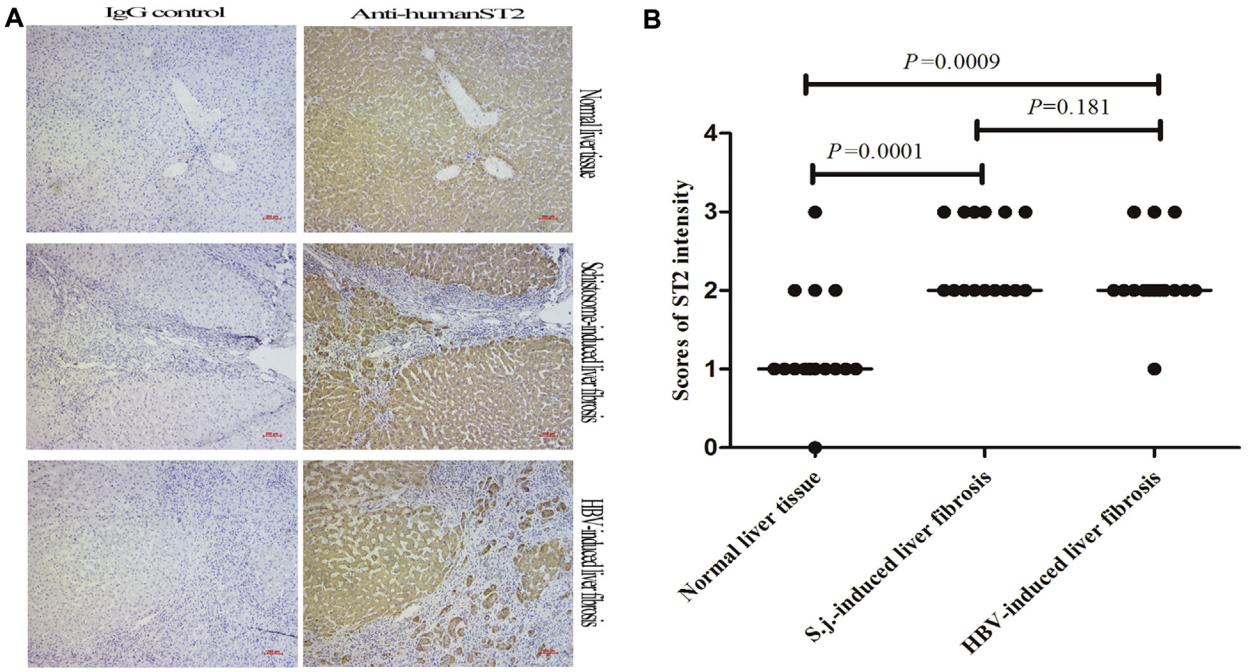

FIG 2. ST2 protein levels were determined by IHC. **A,** Representative images of normal liver tissues, HBV-induced liver fibrosis tissues and *S japonicum* (*Sj*)-induced liver fibrosis tissues (original magnification 100×; bars = 200 μm). **B,** Comparison of scores of ST2 staining intensity among the 3 groups.

liver fibrosis groups showed a stronger ST2 staining compared with that of normal liver tissues (P = .0009 and .0001, respectively). Although the *Schistosome*-induced liver fibrosis group appeared to have a slightly stronger ST2 expression compared with that of the HBV-induced group, the difference was not statistically significant (Fig 2, *B*).

DISCUSSION

Advanced schistosomiasis is typically characterized by hepatomegaly, splenomegaly, portal hypertension, and bleeding esophageal varices.[29,30] Previous studies have demonstrated T_H2-mediated host immune responses contribute to granuloma formation around the *Schistosoma* parasite egg, resulting in portal venous obstruction.[31-34] In most endemic areas, overt disease is generally restricted to a relatively small proportion of the population, and genetic predisposition has been suggested to play a role. Our study is the first to interrogate the role of *ST2* genetic variants in this disease. However, our findings did not support the hypothesis that *ST2* polymorphisms play a role in disease progression.

The *ST2* gene codes for 3 isoforms of ST2: ST2L, a membrane-protein which is expressed on various hematopoietic cell; sST2, a short soluble protein existing in serum; and vST2, a variant-membrane proteins.[25] Associations between *ST2* SNPs and the altered serum levels of ST2 are well documented.[16,18,35] Our study supports the finding that *ST2* SNPs can predict serum ST2 levels in 2 independent *Schistosoma*-infected populations. We also found that carriers of less common *ST2* haplotypes are likely to have decreased ST2 levels in serum than are carriers of the most common *ST2* haplotypes.

Of interest, rs1420101 and rs104973 are thought to be functional SNPs in the *ST2* gene,[16,19,24] and rs1041973 was associated with sST2 levels in Dutch children at age 4 and 8 years.[18] We could not repeat the rs1041793 association results in our adult population. However, the A allele of rs12712135 was positively linked to sST2 levels while the T allele of rs1420101, the T allele of rs6543119, and the C allele of rs10206753 were negatively associated with sST2 levels.

There are 3 potential explanations for the disagreement between the rs1041973 association results in the Dutch study and our Chinese study. The genotype calling quality of rs1041973 may be questionable in our study (HWE P = .02 in controls, indicative of observed genotype proportions deviating from expected proportions, which implies that some of the genotype calls are inaccurate). However, HWE P values in the chronic cases and end-stage disease groups were not suggestive of genotyping errors, and genotype calling errors were not apparent from the TaqMan assay cluster plots. Differences in ethnicity (Dutch cohort vs our Chinese cohort) may also explain the disagreement, although we note that rs1041973 has a missense variant that is thought to have a functional effect. Finally, the disagreement could be due to differences between child and adult immune function (children in the Dutch cohort vs our adult cohort).

Despite the strong associations we observed between genetic variants and sST2 levels, and between stage of disease and sST2 levels, none of the sST2 genetic variants we tested were associated with stage of disease. This points to the possible independent effects of these factors, that is, the genetic variants we tested did not play a role in the increased sST2 levels we observed in end-stage disease. Future study investigating the interaction between *ST2* variants and variants in other genes that may interact with *ST2,* such as *IL-33*, could potentially elucidate the role of genetic variants in disease progression.

ST2 is broadly expressed in various immune cells including T_H2 cells. Numerous studies have demonstrated the role of ST2 in fibrotic diseases.[36-43] $ST2^{-/-}$ mice are protected from the

development of bleomycin-induced lung fibrosis,[36] and ST2 modulates the process of lung fibrosis through limiting inflammatory cells infiltration and collagen deposition.[39] ST2L expression in myofibroblasts is elevated on IFN-γ and IL-4 stimulation.[43] ST2 has also been related with liver fibrosis and cirrhosis because its mRNA is upregulated in abnormal mouse and human liver tissues.[40] Furthermore, recombinant ST2 enhances CCl_4-induced liver fibrosis by stimulating T_H2 immune responses.[37] These studies indicate that ST2 levels may increase, possibly reflecting the development of fibrosis and a T_H2-type immune response in the tissue. We observed elevated sST2 levels in our patients with end-stage schistosomiasis *japonicum*, and all of these patients showed the typical linear echogenic images in liver diagnosed by ultrasound, indicating the cirrhosis caused by *S japonicum*. These results were further verified by our IHC experiments. Future study will focus on the molecular mechanisms mediated by ST2, to precisely understand how the predominance of ST2 influences the T_H2 immune response, leading to granuloma modulation and fibrosis in liver.

In conclusion, this is the first study to investigate the role of *ST2* SNPs in schistosomiasis, particularly in severity of disease induced by *S japonicum*. Although we were not able to show that these polymorphisms play a role in disease progression, we identified a number of SNPs that are strongly associated with sST2 levels. We also demonstrated that elevated ST2 levels in both serum and hepatic tissue are associated with late-stage schistosomiasis.

We thank all the participants in this study and acknowledge field-workers and local Centers for Disease Control and Prevention hospitals providing support for our study. We are grateful to Audrey Grant who performed initial analysis of schistosomiasis in the Brazilian cohort, and Alvaro Cruz, Eduardo Vieria Ponte, Ricardo Riccio Oliveira, and all the field-workers who helped collect data in Conde, Brazil.

Clinical Implications: Genetic variants in the ST2 gene confer a risk for higher levels of ST2 in serum in patients with schistosomiasis, especially patients with end-stage disease.

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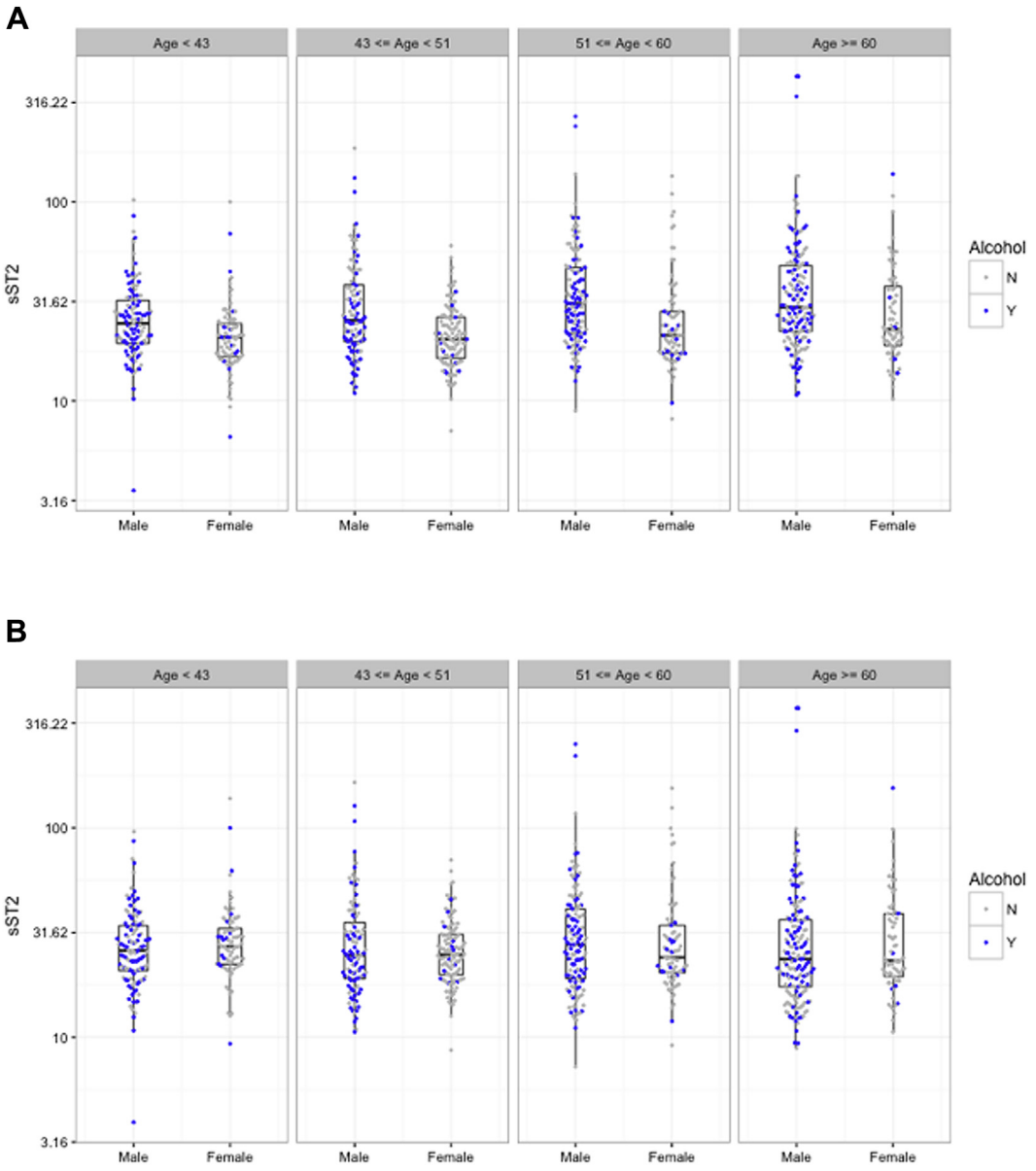

FIG E1. A, The distribution of sST2 ng/mL measurements stratified by age, sex, and alcohol consumption. **B,** The distribution of sST2 ng/mL measurements adjusted for age, sex, and alcohol consumption, stratified by age, sex, and alcohol consumption.

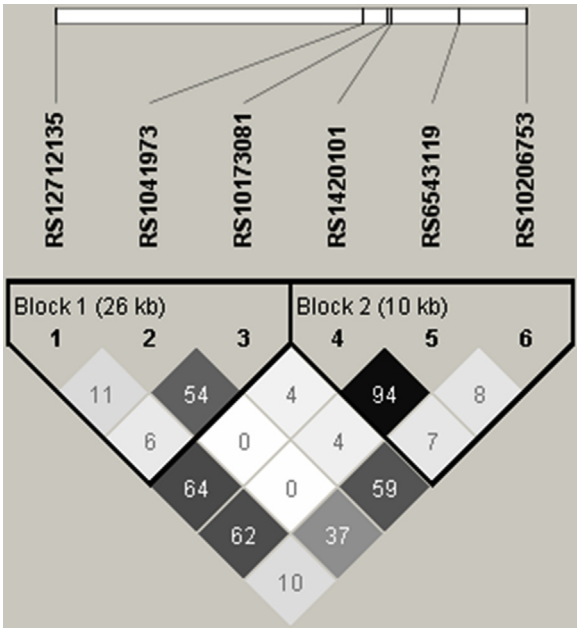

FIG E2. Pairwise linkage disequilibrium (LD) between the 6 ST2 SNPs typed in this study. LD blocks were defined using the Gabriel algorithm. *Darker colors* indicate stronger correlation, and LD was estimated using r^2.

TABLE E1. Frequency distribution of the 6 *ST2* SNPs typed in this study in the Hubei cohort

SNP	Chromosome: position*	Minor/major allele	All samples			HWE *P* value
			MAF	Call rate	Genotype counts	
rs12712135	2:102930948	A/G	0.46	0.92	168/374/231	.47
rs1041973	2:102955468	A/C	0.13	0.95	21/162/630	.02
rs10173081	2:102957348	T/C	0.09	0.98	5/136/690	.67
rs1420101	2:102957716	T/C	0.41	0.91	132/381/272	1.00
rs6543119	2:102963072	T/A	0.42	0.92	135/386/269	.88
rs10206753	2:102968362	C/T	0.13	0.97	13/193/631	.76
			End stage			
rs12712135	2:102930948	A/G	0.44	0.97	59/155/93	.73
rs1041973	2:102955468	A/C	0.12	0.96	8/63/247	.12
rs10173081	2:102957348	T/C	0.09	0.95	3/51/262	.73
rs1420101	2:102957716	T/C	0.43	0.89	59/139/102	.35
rs6543119	2:102963072	T/A	0.43	0.88	57/141/99	.64
rs10206753	2:102968362	C/T	0.13	0.96	6/72/245	.80
			Chronic cases			
rs12712135	2:102930948	A/G	0.45	0.85	52/126/79	.90
rs1041973	2:102955468	A/C	0.13	0.93	7/61/213	.30
rs10173081	2:102957348	T/C	0.10	0.99	3/55/235	1.00
rs1420101	2:102957716	T/C	0.40	0.91	36/145/93	.10
rs6543119	2:102963072	T/A	0.41	0.94	40/151/91	.09
rs10206753	2:102968362	C/T	0.15	0.98	6/79/210	.82
			Controls			
rs12712135	2:102930948	A/G	0.48	0.95	71/134/80	.34
rs1041973	2:102955468	A/C	0.11	0.96	8/50/230	.02
rs10173081	2:102957348	T/C	0.07	1.00	0/41/259	.38
rs1420101	2:102957716	T/C	0.40	0.95	45/141/101	.81
rs6543119	2:102963072	T/A	0.40	0.95	47/135/105	.81
rs10206753	2:102968362	C/T	0.11	0.99	3/60/234	1.00

*From hg19.

TABLE E2. Association between the 6 *ST2* SNPs typed in this study and stage of disease

			P values			
SNP	**Chromosome: position***	**OR (95% CI)**	**Ordinal**	**Control vs chronic case**	**Control vs end stage**	**Chronic case vs end stage**
rs12712135	2:102930948	0.91 (0.75-1.11)	.341	.477	.502	.879
rs1041973	2:102955468	1.04 (0.80-1.37)	.759	.517	.671	.729
rs10173081	2:102957348	1.11 (0.80-1.54)	.536	.125	.613	.421
rs1420101	2:102957716	1.07 (0.88-1.31)	.490	.828	.596	.389
rs6543119	2:102963072	1.13 (0.93-1.38)	.230	.453	.370	.607
rs10206753	2:102968362	1.01 (0.77-1.33)	.941	.197	.809	.252

Each of the SNPs were tested for association with stage of disease (end stage, chronic case, or control) using ordinal or logistic regression, and sex, age, HBV infection, and alcohol consumption were included as covariates in the statistical model.
*From hg19.

TABLE E3. Associations between haplotypes and serum ST2 levels

ST2 haplotypes						Frequency	Effect size	*P* value
rs12712135	rs1041973	rs10173081	rs1420101	rs6543119	rs10206753			2.96E-16
A	C	C	C	A	T	0.45		
G	A	T	C	A	C	0.08	−0.04	1.53E-02
G	C	C	C	A	C	0.04	−0.17	6.03E-12
G	C	C	T	T	T	0.37	−0.07	1.75E-11
G	A	C	T	T	T	0.04	−0.11	1.34E-05
*	*	*	*	*	*	0.02	−0.06	1.33E-01
rs12712135	rs1041973	rs10173081	rs1420101	rs6543119				2.59E-16
A	C	C	C	A		0.45		
G	C	C	C	A		0.04	−0.17	3.37E-12
G	C	C	T	T		0.37	−0.07	2.45E-11
G	A	C	T	T		0.04	−0.11	1.27E-05
G	A	T	C	A		0.09	−0.04	1.26E-02
*	*	*	*	*		0.02	−0.05	2.90E-01
	rs1041973	rs10173081	rs1420101	rs6543119	rs10206753			1.14E-14
	C	C	C	A	T	0.45		
	C	C	T	T	T	0.37	−0.07	1.67E-10
	A	C	T	T	T	0.04	−0.10	2.84E-05
	A	T	C	A	C	0.09	−0.04	2.09E-02
	C	C	C	A	C	0.04	−0.16	6.59E-11
	*	*	*	*	*	0.02	−0.08	6.95E-02
rs12712135	rs1041973	rs10173081	rs1420101					2.15E-15
A	C	C	C			0.45		
G	C	C	C			0.04	−0.16	1.66E-11
G	C	C	T			0.38	−0.07	9.06E-11
G	A	C	T			0.04	−0.11	1.60E-05
G	A	T	C			0.09	−0.04	1.76E-02
*	*	*	*			0.01	−0.04	6.04E-01
	rs1041973	rs10173081	rs1420101	rs6543119				8.71E-07
	C	C	C	A		0.49		
	A	C	T	T		0.04	−0.09	6.34E-04
	A	T	C	A		0.09	−0.02	1.81E-01
	C	C	T	T		0.37	−0.05	3.81E-07
	*	*	*	*		0.02	−0.06	1.92E-01
		rs10173081	rs1420101	rs6543119	rs10206753			3.40E-15
		C	C	A	T	0.45		
		C	C	A	C	0.04	−0.16	1.09E-11
		C	T	T	T	0.41	−0.07	1.63E-12
		T	C	A	C	0.09	−0.04	2.49E-02
		*	*	*	*	0.01	−0.06	2.41E-01
rs12712135	rs1041973	rs10173081						3.58E-13
A	C	C				0.46		
G	A	C				0.04	−0.10	5.96E-06
G	A	T				0.09	−0.04	2.38E-02
G	C	C				0.41	−0.08	2.62E-14
*	*	*				0.00	−0.11	5.18E-01
	rs1041973	rs10173081	rs1420101					4.90E-06
	C	C	C			0.50		
	A	C	T			0.04	−0.08	1.08E-03
	A	T	C			0.09	−0.02	2.03E-01
	C	C	T			0.37	−0.05	2.36E-06
	*	*	*			0.01	−0.07	3.03E-01
		rs10173081	rs1420101	rs6543119				8.22E-07
		C	C	A		0.49		
		C	T	T		0.41	−0.06	1.97E-08
		T	C	A		0.09	−0.02	1.48E-01
		*	*	*		0.01	−0.04	4.44E-01
			rs1420101	rs6543119	rs10206753			3.71E-12
			C	A	T	0.45		
			C	A	C	0.13	−0.08	8.00E-08
			T	T	T	0.41	−0.07	4.63E-12
			*	*	*	0.01	−0.04	3.77E-01

(Continued)

TABLE E3. *(Continued)*

ST2 haplotypes						Frequency	Effect size	*P* value
rs12712135	rs1041973							7.22E-13
A	C					0.46		
G	A					0.13	−0.06	1.28E-05
G	C					0.42	−0.08	1.95E-14
*	*					0.00	−0.12	4.98E-01
	rs1041973	rs10173081						3.90E-02
	C	C				0.87		
	A	C				0.04	−0.06	4.20E-03
	A	T				0.09	0.00	9.29E-01
		rs10173081	rs1420101					1.11E-06
		C	C			0.50		
		C	T			0.41	−0.05	1.24E-07
		T	C			0.09	−0.02	1.58E-01
			rs1420101	rs6543119				4.85E-07
			C	A		0.58		
			T	T		0.41	−0.05	5.81E-08
			*	*		0.01	−0.04	4.64E-01
				rs6543119	rs10206753			4.87E-11
				A	T	0.45		
				A	C	0.13	−0.08	1.61E-07
				T	T	0.42	−0.07	6.49E-11
				*	*	0.00	0.02	9.24E-01

*Rare haplotypes were combined.