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Original Article

Potential association of a functional polymorphism in the *CCL5* gene with susceptibility to spontaneous preterm birth in a Chinese Han population

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Abstract: The etiology of spontaneous preterm birth (SPTB) remains unclear. We investigated the association between the chemokine CCL5 (C-C motif chemokine ligand 5) polymorphism In1.1 T/C (rs2280789) and the risk of SPTB in a Chinese Han population. The *CCL5* In1.1 T/C polymorphism was genotyped in 520 preterm neonates and 650 term neonates. Compared with the T positive genotypes (TT + TC genotypes), the CC genotype was associated with increased SPTB susceptibility [odds ratio (OR), 1.44; confidence intervals (95% CIs), 1.01-2.05; *P* = 0.043]. In the preterm neonates without PROM, a marginal association was observed between SPTB susceptibility and the CC genotype compared to the T-positive genotypes (TT + TC genotypes) (OR, 1.48; 95% CI, 1.00-2.19; P = 0.05). By using the enzyme linked immunosorbent assay, we observed that CCL5 concentration was lower in the preterm neonates (n = 30) than in the term neonates (n = 10) (*P* = 0.035) and that the CC genotype was associated with decreased serum CCL5 levels in preterm neonates (*P* < 0.001). Our findings suggest that the *CCL5* In1.1 T/C polymorphism may contributes to the risk of SPTB in a Chinese Han population.

Keywords: *CCL5*, spontaneous preterm birth, premature rupture of membranes, susceptibility, polymorphism

Introduction

Preterm birth (PTB, < 37 complete weeks of gestation) is a major challenge facing modern obstetrics. Globally, PTB is the leading cause of newborn deaths and the second leading cause of death after pneumonia in children under the age of five [1-3]. Approximate 15 million neunates are born prematurely annually across the globe. In China, about 10% of all births end preterm, affecting nearly 1.1 million neonates every year, second only to India (http://www.who.int/mediacentre/factsheets/fs363/en/). The etiology of spontaneous preterm birth (SPTB) remains unclear; however, numerous studies have demonstrated that genetic factors are contributors to SPTB. In twins, a heritability range for SPTB of 17-40% has been shown [4, 5]. Familial aggregation has been demonstrated for SPTB [4-8]. There is also a higher incidence of preterm deliveries among mothers with a history of previous preterm deliveries [9]. In addition, association studies have identified a number of genetic polymorphisms that are risk factors for SPTB [10-12]. Identifying susceptibility genes conferring increased risks for SPTB would advance the development of solutions to preventing SPTB and would help to clarify the causes and mechanisms of this disease.

The chemokine CCL5 (C-C motif chemokine ligand 5, gene aliase: *RANTES*) is a ligand for the chemokine receptor 5, and a major chemokine in both the acute and chronic phases of inflammation [13, 14]. Athayde *et al*. reported that CCL5 is present in amniotic fluid and that its concentration decreases as pregnancy advances but then increases during labor and during microbial invasions of the amniotic cavi-

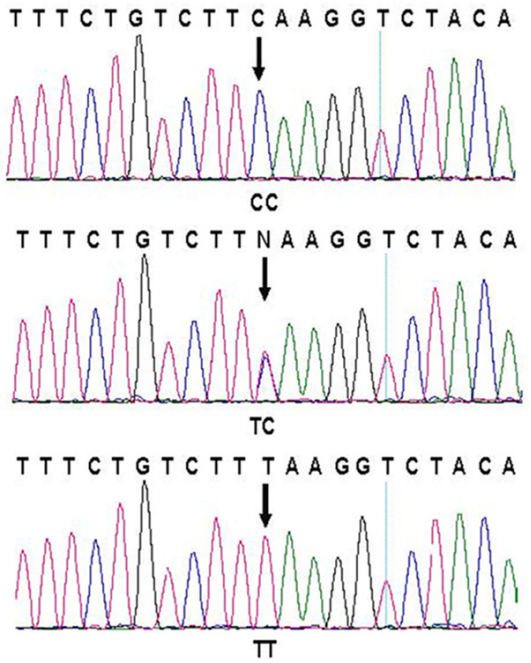

Figure 1. Sequencing analysis of the RANTES In1.1 T/C polymorphism. The 3 charts represent the CC, TC and TT genotypes, respectively. The arrows indicate the nucleotide positions of the base changes.

ty [15]. Furthermore, Brou *et al.* showed that the concentration of CCL5 in amniotic fluid was significantly decreased in SPTB compared to normal term birth and that higher concentrations of CCL5 reduced the risk of SPTB in a combined analysis in African Americans [16]. We hypothesized that *CCL5* may be a susceptibility gene for SPTB. It is expected that single nucleotide polymorphisms (SNPs) within *CCL5* could result in genotype-dependent differences in SPTB susceptibility.

The *CCL5* gene located at 17q12, which composes of 4 exons that spans more than 8000 bp of genomic DNA. The *CCL5* gene is polymorphic and among its validated polymorphisms, a SNP in the first intron of the *CCL5* gene (In1.1 T/C, rs2280789) has been extensively studied and described to be associated with CCL5 expression levels [17, 18]. The In1.1 T/C polymorphism is located within an intronic *CCL5* regulatory element that modulates *CCL5* transcription [18]. Many studies have shown that the *CCL5* In1.1 T/C polymorphism is associated with a variety of diseases, such as human immunodeficiency virus-1 (HIV-1) infection and acquired immune deficiency syndrome (AIDS) progression, severe respiratory syncytial virus (RSV) infection, and mycetoma granuloma formation [17-19]. However, the role of the In1.1 T/C polymorphism in SPTB has never been specifically investigated. In the present study, we examined whether the *CCL5* In1.1 T/C polymorphism has any bearing on the risk or severity of SPTB in a Chinese Han population.

Material and methods

Study subjects

This case-control study consisted of 569 preterm singleton neonates and 673 term neonates. All subjects were unrelated ethnic Chinese Han individuals and were enrolled from Beijing City and its surrounding regions between January 2009 and May 2011. The cases were neonates from pregnancies complicated by SPTB. Two subgroups were distinguished; neonates born between 33 and 36 weeks were considered as moderate PTB and neonates of gestational age < 33 weeks were included in a group of severe PTB. Controls were randomly selected from singleton pregnancies delivered at term from mothers with no prior history of premature rupture of membranes (PROM) or PTB. The diagnosis of cases, the inclusion and exclusion criteria for cases and controls were described previously [20].

From the 569 preterm neonates, 30 were randomly selected for serum sampling (see Additional file 1, Table S1). Sera were also collected from 10 of the 673 term neonates (see Additional file 1, Table S1). For each participant, serum was collected into tubes immediately after delivery. We stored the sera at 4°C upon collection. Then, all sera were stored at -80°C and prepared for the enzyme linked immune sorbent assay (ELISA).

Ethics

This study was performed with the approval of the Ethical Committee of General Hospital of Beijing PLA (BZE-2013005) and conducted according to the principles expressed in the Declaration of Helsinki. At recruitment, demographic factors and medical histories were collected using a structured questionnaire. Written informed consent was obtained from all participants' guardians.

Table 1. Distributions of select characteristics among preterm neonates and controls

Variable[a]	Preterm neonates (n = 569)	Term neonates (n = 673)	x^2	*P* value[b]
Maternal age at delivery (year)	28 (26-30.5)	29 (26-31)	0.932	0.352
Gravidity	1 (1-6)	1 (1-7)	0.670	0.503
Parity	1 (1-3)	1 (1-3)	0.161	0.872
Gestational age at delivery (week)	34.0 (32.0-35.4)	39.0 (38.2-39.6)	30.420	< 0.001
Neonatal birth weight (g)	2060 (1700-2500)	3400 (3045-3650)	27.508	< 0.001
Neonatal sex			0.282	0.595
Boy	318 (55.9)	366 (54.4)		
Girl	251 (44.1)	307 (45.6)		
5 min Apgar score	10 (9-10)	10 (10-10)	12.837	< 0.001
Sub-categories of SPTB				
Severe SPTB (< 32 weeks)	123 (21.6)			
Moderate SPTB (33 to 36 weeks)	446 (78.4)			
PROM	171 (30.1)			

Abbreviations: PROM, premature rupture of membranes. [a]Results are expressed as medians (25th-75th percentile) or as absolute numbers (percentage). [b]χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables.

Genotype analysis

Genomic DNA was extracted from whole blood specimens using the Relax Gene Blood DNA System (TianGen Biotech Co. Ltd.). The *CCL5* In1.1 T/C polymorphism was genotyped using polymerase chain reaction (PCR) direct sequencing. The primers 5'-CCTGGTCTTGACCACCACA-3' and 5'-GCTGACAGGCATGAGTCAGA-3' were used to amplify the target region. The PCR conditions were performed as described previously except for a PCR annealing temperature of 56°C [20]. The persons performing the genotyping were blind to the subjects' case-control status. For quality control, a 15% masked, random sample of cases and controls was tested twice by different persons, and all results showed 100% concordance (**Figure 1**).

ELISA

The serum levels of CCL5 were measured by Human CCL5 ELISA kits (Biolegend, USA). The assay was performed according to the instructions of the manufacturers. Concentrations in pg/ml were calculated from the standard curve of standard substance. The detection limit of standard substance was from 15.60 to 1000 pg/ml. All measurements were performed in duplicate.

Statistical analysis

The genotype and allele frequencies for the *CCL5* In1.1 T/C polymorphism were determined by gene counting. The significance of deviations from Hardy-Weinberg equilibrium was tested using the random-permutation procedure implemented in the Arlequin package (http://lgb.unige.ch/arlequin). The allelic and single frequencies in each of the two groups were analyzed using the χ^2 test using SPSS software (version 15.0; SPSS Inc.). Logistic regression model was adopted to analyze the association of the *CCL5* In1.1 T/C polymorphism with susceptibility to SPTB by online software SNPSpD (http://gump.qimr.edu.au/general/daleN/SNPSpD). Odds ratios (ORs) and 95% confidence intervals (CIs) were used to indicate the strength of all associations. An association was considered significant at a *P* value of < 0.05, and all statistical tests were two-sided.

Results

Demographic characteristics

All subjects were of Chinese Han descent. The baseline characteristics of the study population are shown in **Table 1**. There were no statistically significant differences in maternal age, gravidity, parity or neonatal gender between the preterm neonates and term neonates. Significant differences between preterm neonates and term neonates were observed for gestational age at delivery (week) ($P < 0.001$), birth weight (g) ($P < 0.001$) [20, 21]. The APGAR 5 (5 min after birth) has been shown to have utility in predicting mortality in the preterm neo-

Table 2. Genotype and allele frequencies of the *CCL5* In1.1T/C polymorphism in preterm neonates and controls

	Preterm neonates (n = 520)	Term neonates (n = 650)	OR (95% CI)	*P* value[a]
Genotypes				0.11[b]
TT	208 (40.0)	264 (40.6)	Reference	
TC	237 (45.6)	318 (48.9)	0.98 (0.76-1.25)[a]	
CC	75 (14.4)	68 (10.5)	1.42 (0.97-2.07)[a]	
TC + CC	312 (60.0)	386 (59.4)	1.06 (0.83-1.34)[a]	0.65[a]
Recessive model			1.44 (1.01-2.05)[c]	0.043
$P_{\text{Hardy-Weinberg}}$	0.574	0.052		
Alleles				
T allele	0.63	0.65		0.25[d]
C allele	0.37	0.35		

NOTE: Due to genotyping failure, the actual sample size was 520 for the preterm neonates and 650 for the term neonates. Abbreviations: OR, odds ratio; CI, confidence interval. [a]The ORs and the *P* values were calculated using a multivariate logistic regression analysis and were adjusted for maternal age at delivery (year) and neonatal sex. [b]χ^2 test was used to determine the distribution of the three different genotype frequencies (*df* = 2). [c]The value is from a comparison of the CC genotype with the TT + TC genotype. [d]Atwo-sidedχ^2 test was used to determine the distribution of the allelic frequencies (*df* = 1).

nates [22]. In our study population, the APGAR 5 in cases was also significantly lower than that in controls ($P < 0.001$). Compared with preterm neonates without PROM, significantly lower parity and higher neonatal birth weight were found in preterm neonates with PROM (Table S2). There were no statistically significant differences in maternal age at delivery, gravidity, gestational age at delivery, neonatal sex, 5 min Apgar score and sub-categories of SPTB between the preterm neonates with and without PROM. Compared with moderate SPTB, significantly lower neonatal birth weight, lower 5 min Apgar score and higher gravidity were found in severe SPTB (Table S3). The maternal age at delivery, parity, neonatal sex and PROM status in moderate SPTB were comparable with that in severe SPTB.

The CCL5 In1.1 T/C polymorphism is associated with SPTB susceptibility

The population based frequencies of the *CCL5* In1.1 T/C polymorphism are presented in Figure S1. According to the data derived from the 1000 Genomes Project Phase 1, the allele frequencies of *CCL5* In1.1 T/C polymorphism did vary among Africans (~20%), Europeans (~12%) and Chinese (~32%). The allele frequencies of *CCL5* In1.1 T/C polymorphism in term neonates of our study were similar to those among Chinese derived from the 1000 Genomes Project Phase 1 (35% vs. ~32%).

The genotyping results are presented in **Tables 2** and **3**. The observed genotype frequencies of the *CCL5* In1.1 T/C polymorphism were in Hardy-Weinberg equilibrium for both preterm neonates and term neonates (**Table 2**). For the overall sample, based on the logistic regression analysis with adjustments for maternal age at delivery (year) and neonatal sex, compared with the TT + TC genotypes, the CC genotype was associated with an increased susceptibility for SPTB risk (OR, 1.44; 95% CI, 1.01-2.05; $P = 0.043$).

The association between the *CCL5* In1.1 T/C polymorphism and SPTB susceptibility were further examined by stratifying preterm neonates by PROM status (**Table 4**). There was no association between the *CCL5* In1.1 T/C polymorphism and SPTB susceptibility in preterm neonates with PROM (OR, 1.39; 95% CI, 0.84-2.31; $P = 0.21$). However, in the preterm neonates without PROM, a marginal association was observed between SPTB susceptibility and the CC genotype compared to the T-positive genotypes (TT + TC genotypes) (OR, 1.48; 95% CI, 1.00-2.19; $P = 0.05$).

We also analyzed the association between the term neonates and the two subgroups of preterm neonates: moderate SPTB, and severe SPTB (**Table 5**). There was no association between the *CCL5* In1.1 T/C polymorphism and susceptibility to both moderate SPTB (OR, 1.41; 95% CI, 0.96-2.06; $P = 0.08$) and severe SPTB (OR, 1.57; 95% CI, 0.90-2.73; $P = 0.12$).

The expression of CCL5 in the sera from preterm and term neonates by ELISA

We assessed the expression of CCL5 in the sera from preterm and term neonates by ELISA (**Figure 2**). The genotypes of CCL5 In1.1 T/C

Table 3. Multivariate analysis for susceptible polymorphismin preterm neonates and controls

Variable	Preterm neonates (n = 520)	Term neonates (n = 650)	*P* value	Adjusted OR (95% CI)	Adjusted β (95% CI)
Maternal age at delivery (year), mean ± SD	28.5 ± 4.2	28.9 ± 4.2	0.175	0.981 (0.954-1.009)	-0.019 (-0.047, 0.009)
Neonatal sex, girl, n (%)	204 (39.2)	309 (47.5)	0.006	0.717 (0.568-0.909)	-0.330 (-0.565, -0.096)
CCL5 In1.1T/C polymorphism					
T/T + T/C	445 (85.6)	582 (89.5)		Reference	Reference
C/C	75 (14.4)	68 (10.5)	0.043	1.438 (1.011-2.046)	0.363 (0.112, 0.716)

Note: The model included the variables of age, sex and gene with two groups (T/T + T/C and C/C).

Table 4. Genotype and allele frequencies of the *CCL5* In1.1T/C polymorphism in the controls and in preterm neonates delivered with or without PROM

	Preterm neonates	Term neonates	OR (95% CI)	*P* value
Without PROM	(n = 349)	(n = 650)		
Genotypes				0.13[b]
TT	141 (40.4)	264 (40.6)	Reference	
TC	157 (45)	318 (48.9)	0.95 (0.72-1.26)[a]	
CC	51 (14.6)	68 (10.5)	1.44 (0.95-2.19)[a]	
TC + CC	208 (59.6)	386 (59.4)	1.04 (0.79-1.35)[a]	0.79[a]
Recessive model			1.48 (1.00-2.19)[c]	0.05[c]
Alleles				
T allele	0.63	0.65		0.33[d]
C allele	0.37	0.35		
With PROM	(n = 171)	(n = 650)		
Genotypes				0.42[b]
TT	67 (39.2)	264 (40.6)	Reference	
TC	80 (46.8)	318 (48.9)	1.04 (0.72-1.50)[a]	
CC	24 (14)	68 (10.5)	1.42 (0.83-2.44)[a]	
TC + CC	104 (60.8)	386 (59.4)	1.11 (0.78-1.57)[a]	0.57[a]
Recessive model			1.39 (0.84-2.31)[c]	0.21[c]
Alleles				
T allele	0.63	0.65		0.39[d]
C allele	0.37	0.35		

NOTE: Due to genotyping failure, the actual sample size was 520 for the preterm neonates and 650 for the term neonates. Abbreviations: OR, odds ratio; CI, confidence interval. [a]The ORs and the *P* values were calculated using a multivariate logistic regression analysis and were adjusted for maternal age at delivery (year) and neonatal sex. [b]χ^2 test was used to determine the distribution of the three different genotype frequencies (*df* = 2). [c]The value is from a comparison of the CC genotype with the TT + TC genotype. [d]A two-sided χ^2 test was used to determine the distribution of the allelic frequencies (*df* = 1).

polymorphism among 30 preterm neonates and 10 term neonates measured by CCL5 ELISA are listed in online supplementary Table S4. A significantly lower expression of CCL5 was observed in the sera from preterm neonates as compared to the sera from term neonates (6231.78 ± 498.53 vs. 8206.10 ± 430.04, *P* = 0.035) (**Figure 2A**). Furthermore, there was a significant association between the In1.1 T/C genotype and the expression of CCL5 in the sera from preterm neonates, with the CC genotype carriers having lower expression of CCL5 than the T allele carriers (1970.13 ± 483.92 vs. 7084.11 ± 413.46, *P* < 0.001) (**Figure 2B**).

Discussion

In this study, we investigated the genetic associations of the *CCL5* In1.1 T/C polymorphism with the risk of occurrence of SPTB in a Han population in North China. By case-control study, we found that the *CCL5* In1.1 T/C CC genotype was marginally associated with increased SPTB susceptibility. We also observed that the CC genotype was marginally associated with increased SPTB susceptibility in preterm neonates without PROM. In addition, we found that CCL5 concentration was lower in the preterm neonates than in the controls and that the CC genotype was associated with decreased serum CCL5 levels in preterm neonates. The discovery of genetic factors involved on SPTB may lead to medical breakthroughs and reduction in spontaneous PTB, neonatal morbidity and mortality. These results suggest that *CCL5* In1.1 T/C polymorphism may be used as one of the biomarkers to identify high-risk subgroups of preterm neonates who might benefit from personalized prevention and treatment.

Table 5. Genotype and allele frequencies of the *CCL5* In1.1T/C polymorphism in term neonates and in two sub-groups of preterm neonates

Genotypes and alleles	Cases		Controls (n = 650)	OR (95% CI)		*P* value	
	Severe SPTB (n = 123)	Moderate SPTB (n = 397)		Severe SPTB vs. Controls	Moderate SPTB vs. Controls	Severe SPTB vs. Controls	Moderate SPTB vs. Controls
Genotypes						0.18[b]	0.13[b]
TT	42 (34.1)	166 (41.8)	264 (40.6)	Reference	Reference		
TC	62 (50.4)	175 (44.1)	318 (48.9)	1.29 (0.84-1.98)[a]	0.90 (0.69-1.18)[a]		
CC	19 (15.4)	56 (14.1)	68 (10.5)	1.81 (0.99-3.33)[a]	1.33 (0.89-2.00)[a]		
TC + CC	81 (65.8)	231 (58.2)	386 (59.4)	1.38 (0.92-2.08)[a]	0.98 (0.76-1.26)[a]	0.11[a]	0.86[a]
Recessive model				1.57 (0.90-2.73)[c]	1.41 (0.96-2.06)[c]	0.12[c]	0.08[c]
Alleles							
T allele	0.59	0.64	0.65			0.016[d]	0.57[d]
C allele	0.41	0.36	0.35				

NOTE: Due to genotyping failure, the actual sample size was 520 for the preterm neonates and 650 for the term neonates. Abbreviations: OR, odds ratio; CI, confidence interval. [a]The ORs and the *P* values were calculated using a multivariate logistic regression analysis and were adjusted for maternal age at delivery (year) and neonatal sex. [b]χ^2 test was used to determine the distribution of the three different genotype frequencies (*df* = 2). [c]The value is from a comparison of the CC genotype with the TT + TC genotype. [d]A two-sided χ^2 test was used to determine the distribution of the allelic frequencies (*df* = 1).

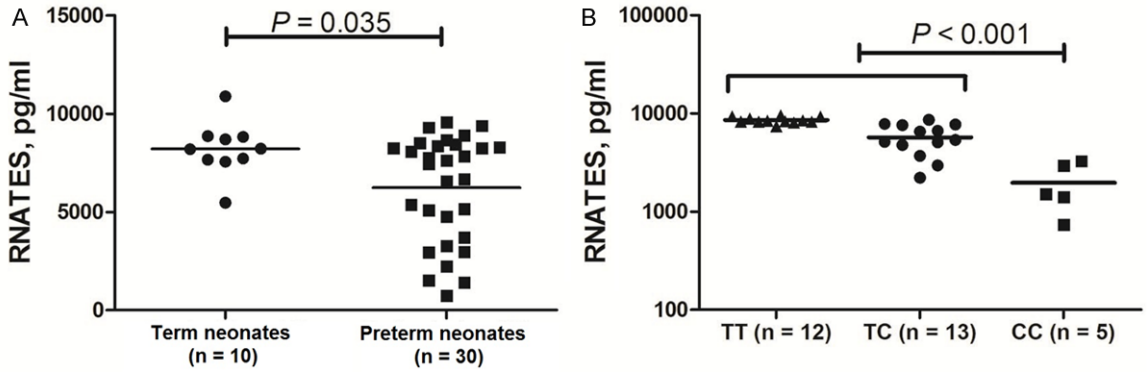

Figure 2. Serum CCL5 levels in preterm neonates and controls. A. Serum CCL5 levels in preterm neonates and term neonates. B. Serum CCL5 levels in preterm neonates with In1.1T/C polymorphism TT, TC and CC genotypes.

The genetic association between the *CCL5* In1.1 T/C polymorphism and SPTB is biologically plausible. Mounting evidence implicates involvement of inflammatory mediators in SPTB [23-25] during which a massive influx of leukocytes selectively invades the uterus; the leukocytes follow chemokine signals to stromal tissues by selectively releasing their array of cytokines, more chemokines, and other effectors [26]. If this cascade of inflammatory cytokines is activated early in pregnancy, preterm labor can occur [27]. CCL5 is a chemokine that has chemotactic activities for leukocytes including T cells, monocytes, eosinophils and basophils [14, 28, 29]. CCL5 has been demonstrated to be apotent chemoattractant for murine monocytes, which are involved in the initiation of preterm labor in mice [30, 31]. A recent study showed that the concentration of CCL5 was significantly decreased in cases compared with controls in amniotic fluid in African Americans, indicating that higher concentrations of CCL5 reduced the risk of SPTB [16]. Hecht *et al*. also reported that newborns who had inflammatory lesions of the placenta had lower levels of CCL5 [32]. Another study showed that a reduced risk of bronchopulmonary dysplasia, the pathogenesis of which is involved in early postnatal inflammation, in a cohort of extreme SPTB was prominently associated with an increased concentration of CCL5 [33]. Consistent with the results of previous studies, we found that CCL5

concentration was lower in the sera from preterm neonates compared to the sera from term neonates. Our results, together with those of previous studies, support the importance of CCL5 in the pathogenesis of SPTB.

Preterm PROM (PPROM) accounts for 30-40% of PTB. PROM and other altered molecular toutes of inflammation have been proposed involving in pathways of PTB [34]. Thus in our study, PROM was analyzed genetic predisposition of carrying the *CCL5* In1.1 T/C polymorphism in two subtypes, preterm neonates with or without PROM. The results showed that the In1.1 T/C was marginally associated with SPTB in preterm neonates without PROM and illustrated that SPTB and PROM have distinct pathway and should be regarded as separate entities. Recently, Capece et al performed a analysis of genes associated with SPTB and PROM and reported that the associated genes with SPTB concentrated on the inflammary/infection related network and genes with PPROM mainly include coagulation function, collagen metabolism and matrix degradation [35].

The In1.1 T/C polymorphism is located in an intronic region of *CCL5* and affects CCL5 serum levels by causing differential binding of CCL5 to nuclear proteins [18]. An *et al.* reported that the presence of the In1.1C allele results in a reduction in the transcription of CCL5 (3-fold) compared to the In1.1T allele [18]. In our study population, the carriers of the CC genotype were overrepresented in preterm neonates compared with term neonates, suggesting that the CC genotype is a risk genotype for SPTB. This hypothesis was confirmed by the ELISA test measurements. By ELISA, we found that the concentrations of CCL5 in the sera from CC genotype carriers were lower than those from T allele carriers. Given the role of CCL5 which plays a protective role in pregnancy, it is reasonable to hypothesize that the individuals who carry the CC genotype, in whom CCL5 serum levels are lower compared to the T allele carriers, have a higher SPTB susceptibility. However, these results warrant further confirmation in future studies.

During recent years, the *CCL5* In1.1 T/C polymorphism has been studied in various diseases. Some studies reported an association of the In1.1 T/C polymorphism with the risks of mycetoma granuloma formation, pulmonary tuberculosis, transplantation and AIDS [18, 36-38]. Several reports showed no associations between the In1.1 T/C polymorphism and severe RSV-induced bronchiolitis and myocardial infarction [17, 19, 39]. To our knowledge, this is the first demonstration of an association between the *CCL5* In1.1 T/C polymorphism and SPTB susceptibility. We provide confirmation for the initial hypothesis that CCL5 may contribute to the pathogenesis of SPTB. However, our initial findings should be independently verified in other populations of different ancestry, such as white subjects and African subjects.

There are some limitations in our research. Firstly, although our results suggest an association of the *CCL5* In1.1 T/C polymorphism with SPTB, we cannot exclude that this polymorphismis in linkage disequilibrium with a nearby causative SNP. Thus, further studies are needed to clarify the relationship between *CCL5* polymorphisms and the risk of SPTB. Secondly, the small sample size of SPTB in different subgroups has hindered our efforts in identifying potential associations between genetic factor and disease outcome, which need further investigation on another case cohort.

In summary, this report describes the first case-control study of the *CCL5* In1.1 T/C polymorphism in relation to SPTB. Our results suggest that the In1.1 T/C polymorphism is marginally associated with increased SPTB susceptibility in Chinese Han population. These findings may provide support for the importance of CCL5 in the pathogenesis of SPTB. If confirmed by other studies, knowledge of genetic factors contributing to the pathogenesis of SPTB as presented here would be important for the assessment of host susceptibility to SPTB and other diseases.

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Disclosure of conflict of interest

None.

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Table S1. Distributions of select characteristics among 30 preterm neonates and 10 controls

Variable[a]	Cases (n = 30)	Controls (n = 10)	*P* value
Maternal age at delivery (year)	30.7 (4.5)	28.8 (3.5)	0.090[b]
Gravidity	2 (1-2)	1 (1-1)	0.033[c]
Parity	1 (1-2)	1 (1-1)	0.128
Gestational age at delivery (week)	30.7 (4.5)	38.4 (1.3)	< 0.001[b]
Neonatal birth weight (g)	1585 (666)	3116 (763)	< 0.001[b]
Neonatal sex			1.00[d]
Boy	15 (50.0)	5 (50.0)	
Girl	15 (50.0)	5 (50.0)	
1 min Apgar score	8 (6-10)	10 (10-10)	0.003[c]
5 min Apgar score	9 (9-10)	10 (10-10)	0.017[c]
Sub-categories of SPTB			
Extremely preterm (< 28 weeks)	10 (33.3)		
Very preterm (28 to < 32 weeks)	10 (33.3)		
Moderate to late preterm (32 to < 37 weeks)	10 (33.3)		
PROM	8 (26.7)		

Abbreviations: PROM, premature rupture of membranes. [a]Continuous variables were summarized as means and standard deviations (SD) or as medians (25th-75th percentile), and categorical variables were summarized as frequencies and proportions. [b]*t* test, two-sided. [c]Mann-Whitney U test, two-sided. [d]χ^2 test, two-sided.

Table S2. Distributions of select characteristics among preterm neonates with and without PROM

Variable[a]	Preterm neonates with PROM (n = 171)	Preterm neonates without PROM (n = 398)	*P* value[b]
Maternal age at delivery (year)	29 (27-31)	29 (26-30)	0.224
Gravidity	1 (1-2)	1 (1-2)	0.797
Parity	1 (1-1)	1 (1-2)	< 0.001
Gestational age at delivery (week)	34.1 (32.3-35.2)	34.0 (32.0-35.6)	0.636
Neonatal birth weight (g)	2210 (1800-2600)	2017.5 (1680-2450)	0.006
Neonatal sex			
Boy	98 (57.3)	220 (55.3)	0.654
Girl	73 (42.7)	178 (44.7)	
5 min Apgar score	10 (10-10)	10 (9-10)	0.120
Sub-categories of SPTB			
Severe SPTB (< 32 weeks)	31 (18.1)	92 (23.1)	0.185
Moderate SPTB (33 to 36 weeks)	140 (81.9)	306 (76.9)	

Abbreviations: PROM, premature rupture of membranes. [a]Results are expressed as medians (25th-75th percentile) or as absolute numbers (percentage). [b]χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables.

Table S3. Distributions of select characteristics among two sub-groups of preterm neonates

Variable[a]	Severe SPTB (n = 123)	Moderate SPTB (n = 446)	*P* value[b]
Maternal age at delivery (year)	28 (25-30)	28 (26-31)	0.292
Gravidity	2 (1-3)	1 (1-2)	0.033
Parity	1 (1-1)	1 (1-1)	0.269
Neonatal birth weight (g)	1400 (1180-1610)	2205 (1910-2650)	< 0.001
Neonatal sex			0.09
Boy	77 (62.6)	241 (54.0)	
Girl	46 (37.4)	205 (46.0)	
5 min Apgar score	9 (8-10)	10 (10-10)	< 0.001
PROM			0.185
With PROM	31 (25.2)	140 (31.4)	
Without PROM	92 (74.8)	306 (68.6)	

Abbreviations: PROM, premature rupture of membranes. [a]Results are expressed as medians (25th-75th percentile) or as absolute numbers (percentage). [b]χ^2 test for categorical variables and the Mann-Whitney U test for continuous variables.

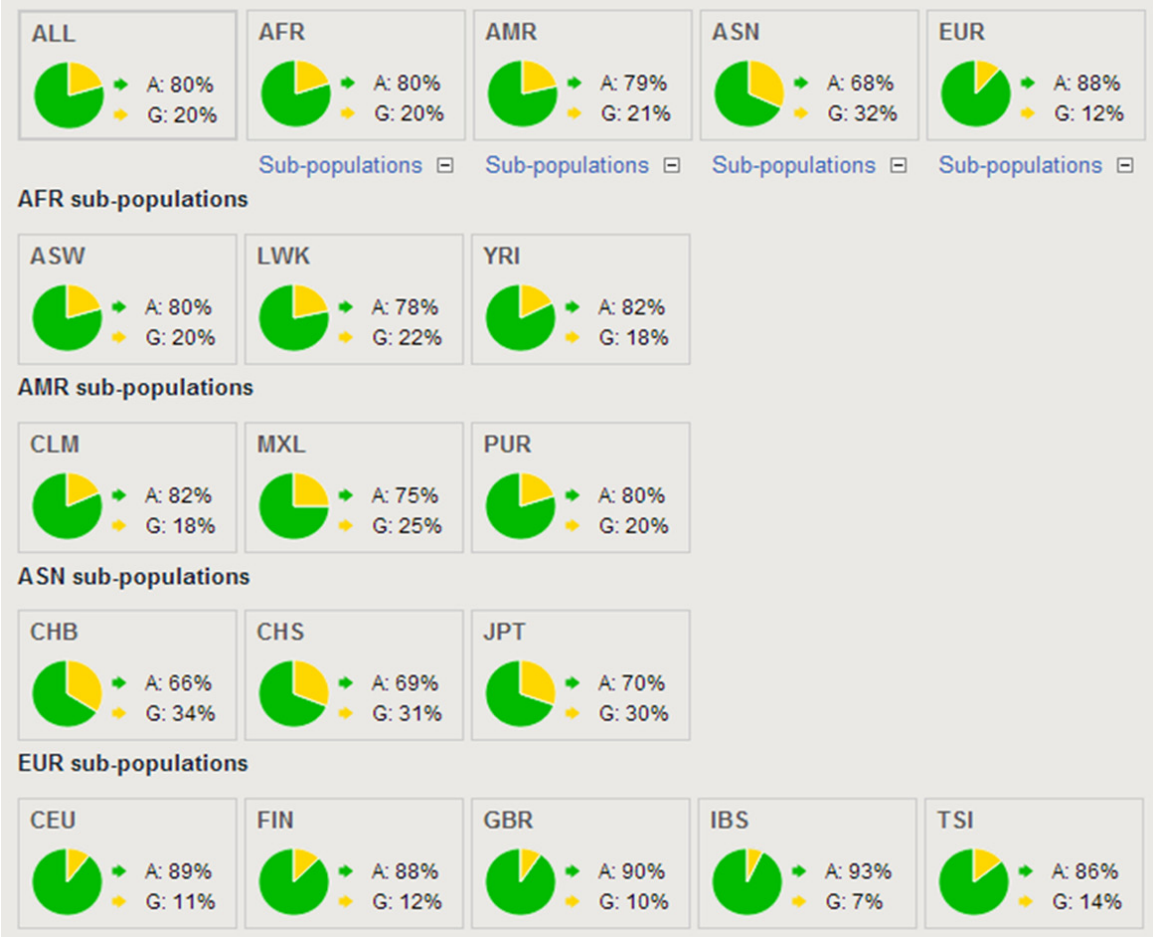

Figure S1. 1000 Genomes Project Phase 1 allele frequencies of *CCL5* In1.1 T/C polymorphism. The allele frequency plots are derived and modified from the website of the 1000 Genomes Project. All, all individuals; AFR, all African individuals (ASW, Americans of African Ancestry in South-West USA; LWK, Luhya in Webuye, Kenya; YRI, Yoruba in Ibadan, Nigera); AMR, all American individuals (CLM, Colombians from Medellin, Colombia; MXL, Mexican Ancestry from Los Angeles, USA; PUR, Puerto Ricans from Puerto Rico); ASN, all East Asian individuals (CHB, Han Chinese in Bejing, China; CHS, Southern Han Chinese, China; JPT, Japanese in Tokyo, Japan); EUR, all European individuals (CEU, Utah Residents [CEPH] with Northern and Western European ancestry; FIN, Finnish in Finland; GBR, British in England and Scotland; IBS, Iberian population in Spain; TSI, population from Tuscany, Italy).

Table S4. The genotypes of RANTES In1.1 T/C polymorphism among 30 preterm neonates and 10 controls

Population cases	Genotype	Population cases	Genotype
Extremely preterm (< 28 weeks)		Moderate to late preterm (32 to < 37 weeks)	
1	C/C	1	T/T
2	T/T	2	T/T
3	C/C	3	T/T
4	T/C	4	C/C
5	T/T	5	T/T
6	T/C	6	T/C
7	T/C	7	T/C
8	T/C	8	T/C
9	T/T	9	T/C
10	T/T	10	C/C
Very preterm (28 to < 32 weeks)		Controls	
1	T/C	1	T/T
2	T/T	2	C/C
3	T/C	3	T/C
4	T/C	4	T/C
5	T/T	5	T/T
6	C/C	6	C/C
7	T/C	7	T/T
8	T/T	8	T/C
9	T/T	9	T/T
10	T/C	10	T/T