Polymorphisms in the *FAM167A-BLK*, but not *BANK1*, are associated with primary Sjögren's syndrome in a Han Chinese population

F. Sun, J. Xu, Z. Wu, P. Li, H. Chen, J. Su, X. You, M. Li, Y. Zhao, X. Tian, Y. Li, F. Zhang

Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing, China.

Abstract

Objectives

Primary Sjögren's syndrome (pSS) is an autoimmune disease with a complex genetic background. Single nucleotide polymorphisms (SNPs) in the BANK1 and FAM167A-BLK genes have been associated with multiple autoimmune diseases. In this study, we investigated whether SNPs in the BANK1 (rs4522865, rs17266594, and rs10516487) and in the FAM167A-BLK region (rs2736340, rs13277113) could be associated with pSS in Chinese Han.

Methods

Blood DNA was extracted from 540 patients with pSS and 577 healthy controls, and genotyped using the Sequenom MassArray system.

Results

There was no significant association between the polymorphisms of BANK1 and pSS. However, the frequency of pSS patients with the T allele (rs2736340) and A allele (rs13277113) of the FAM167A-BLK region was higher than that in the controls (p=0.034; p=0.026 respectively). Genotype and haplotype frequencies of these two SNPs (rs2736340 and rs13277113) between the patients and healthy controls were also significantly different. In addition, associations were observed between the two SNPs and the patients negative for anti-LA/SSB antibodies (p=0.036 and p=0.031 respectively). There was no epistatic interaction between the SNPs in the BANK1 and FAM167A-BLK region.

Conclusion

Our results indicated that the SNPs (rs2736340, rs13277113) of the FAM167A-BLK region, but not the BANK1 SNPs (rs4522865, rs17266594, and rs10516487), were associated with the development of pSS in Han Chinese.

Key words

Primary Sjögren's syndrome, single nucleotide polymorphisms, BANK1, FAM167A-BLK

Fei Sun, Juanjuan Xu*, Ziyan Wu*, Ping Li, Hua Chen, Jinmei Su, Xin You, Mengtao Li, Yan Zhao, Xinping Tian, Yongzhe Li, Fengchun Zhang*

**These authors made an equal contribution to this study.*

Please address correspondence and reprint requests to: Yongzhe Li and/or Fengchun Zhang, Department of Rheumatology and Clinical Immunology, Peking Union Medical College and Chinese Academy of Medical Sciences, 41 Damucang Hutong, Xicheng District, 100032 Beijing, P.R. China. E-mail: yongzhelipumch@126.com and: zhangfccra@yahoo.com.cn

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Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterised by lymphocytic infiltration into exocrine glands, leading to the development of xerostomia and keratoconjunctivitis. The disease primarily affects women with a ratio of 9:1 female to male, and patients usually present during their fourth or fifth decade of life (1). The prevalence of pSS ranges from 0.33% to 0.77% among the Chinese (2), similar to that in European populations (3-5). Although pSS has been considered as a common rheumatic disorder, the etiology of pSS currently is still unclear (6). Like other systemic autoimmune diseases, pSS is generally regarded as a complex disease involving interactions between genetic and environmental factors. Therefore, understanding the genetic factors contributing to the pathogenesis of pSS is of great significance.

Aberrant B cell response is a hallmark of the pathogenesis of pSS. Indeed, high levels of autoantibody production, hypergammaglobulinaemia, circulating immune complexes, oligoclonal B cell expansion, formation of ectopic lymphoid structures within the involved tissues, and increased risk of developing B-cell lymphoma are common in pSS patients (1, 7, 8). Accumulated evidence has suggested that auto-reactive B cells play an important role in the pathogenesis of pSS and that therapies targeting B cells have been proposed as a modality for the treatment of pSS (9). However, it is unknown which factors initiate B cell autoimmunity in pSS.

Currently, there is no report of the genome-wide association studies (GWAS) of pSS. The potential genetic factors contributing to the development of pSS are estimated, mainly according to other autoimmune diseases (10). Numerous studies have suggested that pSS is a polygenic disorder, which may share common genetic determinants with related autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc) (6). Recently, the polymorphisms of the B-cell scaffold protein with ankyrin repeats 1 (*BANK1*) gene and the family with sequence similarity 167 member A-B-lymphoid tyrosine kinase (*FAM167A-BLK*) region have been well confirmed as susceptibility factors for multiple autoimmune diseases. Indeed, the branch-point-site single nucleotide polymorphism (SNP) rs17266594 and nonsynonymous SNP rs10516487 (R61H) in the *BANK1* gene have been shown to be associated with SLE (11-15), SSc (16, 17), RA (18), and psoriasis (19). In addition, the SNP rs4522865 in the first intron of *BANK1* is also associated with SLE in GWAS of Asian populations (20). The *BANK1* gene interacts with the *FAM167A*(C8orf13)-*BLK* (21, 22). Two variants rs2736340 and rs13277113 in the intergenic region between the *FAM167A* and *BLK* genes are common risk factors for SLE (23-25), RA (26-28), SSc (29, 30), and pSS in a Caucasian population (31).

However, whether these SNPs in the *BANK1* and *FAM167A-BLK* genes could contribute to the development of pSS in Asians has not been explored. Given that these two genes are involved in the activation and function of B cells, the major players in the pathogenesis of pSS, we analysed the potential associations of three SNPs (rs4522865, rs17266594, and rs10516487) in the *BANK1* gene and two SNPs (rs2736340, rs13277113) in the *FAM167A-BLK* region with the susceptibility to pSS in a Chinese Han population. Our data indicate that the two SNPs (rs2736340, rs13277113) in the *FAM167A-BLK* region, but not the SNPs in the *BANK1*, are risk factors of the development of pSS in this population. We discuss the implications of our findings.

Subjects and methods

Patients and controls

A total of 540 patients with pSS and 577 healthy controls were included in this study, and all subjects were unrelated and self-reported as Han Chinese. The patients with pSS were consecutively recruited from the outpatient department and ward in Peking Union Medical College Hospital between October 2008 and December 2011, according to the American-European consensus group classification criteria for pSS (32). The

positivity of serum anti-Ro/SSA and anti-LA/SSB antibodies were examined by enzyme linked immunosorbent assay (Euroimmun AG, Lübeck, Germany). The controls were healthy subjects attending the same hospital for routine physical examination, with no history or family history of autoimmune diseases. The study protocol was approved by the Ethics Committee of the Peking Union Medical College Hospital, and informed consent was obtained from individual participants.

DNA extraction and genotyping

Peripheral blood sample (2 ml) was collected from each participant, and DNA was extracted using DNA isolation kit (Bioteke, Beijing, China). Individual DNA samples were genotyped using Sequenom technology (San Diego, CA, USA) of MassArray analysis with matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry (MALDI-TOF MS). The primers for the multiplex polymerase chain reaction (PCR) and for locus-specific extension of these SNPs were designed using the accompanying MassArray Assay Design 3.0 software. The DNA samples were subjected to multiplex PCR in duplicate in 384-well plates, and subsequently the locus-specific extension reaction. The final products were desalted and spotted onto the 384-SpectroCHIP array (Sequenom). The specific allele of individual samples was analysed using MALDI-TOF MS, and the resultant mass spectrograms and genotype data were analysed by the accompanying MassArray Typer software.

Statistical analysis

The 5 SNPs were first tested for Hardy-Weinberg equilibrium (HWE) in control populations, and any SNPs with significant deviation from HWE ($p<0.05$) were excluded from subsequent analysis. The genetic power for this case-control study was calculated using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/). The potential associations of pSS with allele, genotype, and epistasis frequencies were tested using the PLINK v1.07 toolset (http://pngu.mgh.harvard.edu/~purcell/plink/). The odds ratios (ORs) and 95% confidence interval (95% CI) were calculated, and a *p*-value less than 0.05 was considered statistically significant. Genotype frequencies were further analysed by three genetic models: additive, dominant, and recessive. Haplotype analysis was carried out with Haploview software v4.2 (http://www.broadinstitute.org/haploview). For multiple comparisons, a Bonferroni correction was used.

Table I. Characteristics of the subjects.

	Patients	Controls
Gender (Male/Female)	21/519	22/555
Age, years (mean ± SD)	47.9±12.0	49.1±10.63
Ocular symptoms	313	Negative
Oral symptoms	392	Negative
Ocular signs	364	Negative
Positive for salivary gland histopathology[a]	190	Negative
Oral signs	340	Negative
Auto-antibodies		
Anti-Ro/SSA	540	Negative
Anti-LA/SSB	283	Negative

[a] Salivary gland histopathology was available for 214 patients.

Results

Characterisation of subjects and the quality control of the data

To determine the potential association of these SNPs, a total of 540 Chinese Han patients with pSS and 577 ethnically- and geographically-matched healthy controls were recruited. The baseline characteristics of the study groups are summarises in Table I. In order to meet the classification criteria for pSS, all of the patients had positive detection of serum anti-Ro/SSA. Because salivary gland biopsy is an invasive procedure and only some patients were subjected to histological examination of salivary gland, which is another important diagnosis basis for pSS. There was no deviation from HWE in the healthy control group, and genotyped success rate for the five SNPs (rs4522865, rs17266594, rs10516487 rs2736340, rs13277113) were 98%, 93.0%, 97.9%, 98.2% and 98.2% respectively. Accordingly, all SNPs were further analysed. The power analysis revealed more than 80% power ($\alpha=0.05$) for detecting association with an OR of 1.1–1.6 for both heterozygotes and homozygotes, based on an assumption of 0.33% pSS prevalence in a Chinese population and 0.78 for the risk allele frequency, similar to the allele frequencies of the tested SNPs in studies of Asian population.

Association of the SNPs with pSS in the Han population

There was no statistically significant difference in the allelic or genotypic frequencies in the SNPs (rs4522865, rs17266594 and rs10516487) in the *BANK1* between the pSS patients and healthy controls (all, $p>0.05$; Table II). Further analysis with three genetic models indicated that there was no significant association of these genotypes with pSS in this Chinese Han population (all, $p>0.05$; Table III). In contrast, the percentages of pSS patients with T allele of rs2736340 or A allele of rs13277113 in the *FAM167A-BLK* were significantly higher than that in the controls ($p=0.034$ or $p=0.026$, respectively, in Table II). The percentages of pSS patients with the "TT" genotype of rs2736340 or "AA" genotype of rs13277113 were significantly higher than that in the controls ($p=0.026$ or $p=0.041$, respectively). Further analysis with three genetic models revealed statistically significant difference in the genotypic distribution in this population when using the additive and dominant models, particularly using the dominant model (Table III). A similar significant association between the SNPs of the *FAM167A-BLK* was observed in this population of females (data not shown).

Table II. Allele and genotype distribution of the *BANK1* and *FAM167A-BLK* gene markers in primary SS patients and controls.

Gene	SNP	Group	Allele, n (%)		*p*-value	OR (95%CI)	Genotype, n (%)			χ2	*p*-value
			G	A			GG	AG	AA		
BANK1	rs4522865	control	437 (38.7)	691 (61.3)	0.963	1	85 (15.1)	267 (47.3)	212 (37.6)	0.24	0.887
		case	414 (38.8)	652 (61.2)		0.99 (0.84-1.18)	77 (14.4)	260 (48.8)	196 (36.8)		
			C	T			CC	CT	TT		
BANK1	rs17266594	control	129 (12.4)	913 (87.6)	0.305	1	6 (1.2)	117 (22.5)	398 (76.4)	1.1	0.577
		case	144 (13.9)	892 (86.1)		0.88 (0.68-1.13)	8 (1.5)	128 (24.7)	382 (73.7)		
			T	C			TT	CT	CC		
BANK1	rs10516487	control	154 (13.7)	968 (86.3)	0.706	1	9 (1.6)	136 (24.2)	416 (74.2)	0.15	0.927
		case	152 (14.3)	912 (85.7)		0.96 (0.75-1.21)	9 (1.7)	134 (25.2)	389 (73.1)		
			C	T			CC	TC	TT		
FAM167A-BLK	rs2736340	control	319 (28.3)	809 (71.7)	0.034	1	41 (7.3)	237 (42.0)	286 (50.7)	7.32	0.026
		case	259 (24.3)	807 (75.7)		1.23 (1.02-1.49)	38 (7.1)	183 (34.3)	312 (58.5)		
			G	A			GG	AG	AA		
FAM167A-BLK	rs13277113	control	348 (30.9)	780 (69.1)	0.026	1	50 (8.9)	248 (44.0)	266 (47.2)	6.41	0.041
		case	283 (26.5)	783 (73.5)		1.23 (1.03-1..48)	42 (7.9)	199 (37.3)	292 (54.8)		

Table III. Analysis of the five SNPs based on three genetic models.

	geneSNP	Additive model		Dominant model		Recessive model	
		p-value	χ^2	*p-value*	χ^2	*p*-value	χ^2
BANK1	rs4522865	0.963	0.00	0.780	0.08	0.771	0.08
BANK1	rs17266594	0.297	1.09	0.324	0.97	0.583	0.30
BANK1	rs10516487	0.702	0.15	0.698	0.15	0.910	0.01
FAM167A-BLK	rs2736340	0.035	4.42	0.009	6.77	0.929	0.01
FAM167A-BLK	rs13277113	0.026	4.93	0.012	6.37	0.556	0.35

Table IV. Association of the five SNPs with the production of anti-SSB antibody were analysed.

	rs4522865		rs17266594		rs10516487		rs2736340		rs13277113	
Group	*p*-value	OR(95%CI)	*p*-value	OR(95%CI)	*p*-value	OR(95%CI)	*p*-value	OR(95%CI)	*p*-value	OR(95%CI)
P *vs*. N	0.298	0.88 (0.69-1.12)	0.704	0.93 (0.66-1.33)	0.892	1.02 (0.76-1.37)	0.49	1.10 (0.83-1.46)	0.525	1.09 (0.83 1.43)
P *vs*. C	0.574	0.94 (0.76-1.16)	0.531	1.10 (0.81-1.51)	0.764	0.95 (0.67-1.34)	0.183	0.85 (0.68-1.08)	0.147	0.85 (0.67-1.06)
N *vs*. C	0.516	1.07 (0.87-1.33)	0.288	1.18 (0.87-1.61)	0.629	1.08 (0.80-1.45)	0.036	0.77 (0.61-0.98)	0.031	0.77 (0.61-0.98)

Group P: patients positive for anti-LA/SSB antibody; Group N: patients negative for anti-LA/SSB antibody; Group C: Healthy controls.
Group P (n=283); Group N (n=257); Group C (n=577).

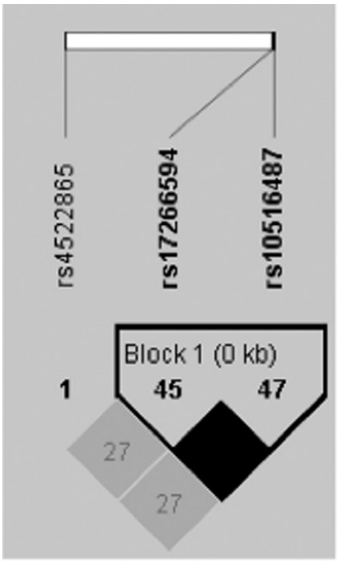
Table V. Haplotype analysis of BANK1 and FANM167A-BLK SNPs.

Gene	SNPs	Haplotype	Total frequency	Case	Control	χ^2	*p*-value
BANK1	rs17266594- rs10516487	TC	0.86	0.85	0.86	0.26	0.608
BANK1	rs17266594- rs10516487	CT	0.14	0.14	0.13	0.22	0.642
FAM167A-BLK	rs2736340-rs13277113	TA	0.71	0.73	0.69	4.72	0.030
FAM167A-BLK	rs2736340-rs13277113	CG	0.26	0.24	0.28	4.72	0.030
FAM167A-BLK	rs2736340-rs13277113	TG	0.03	0.02	0.03	0.11	0.743

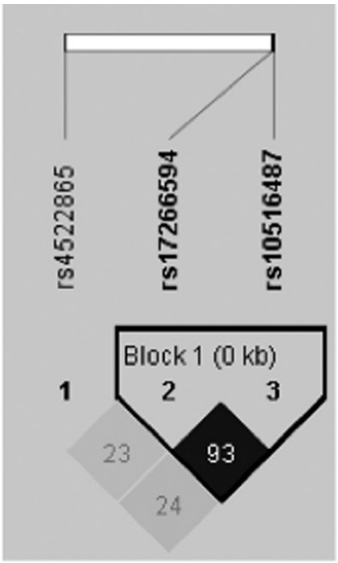
Correlation between the SNPs and the clinical characteristics *of pSS*

Next, we performed the χ^2 test on the basic *clinical* characteristics (ocular symptoms, oral symptoms, ocular signs, positive for salivary gland histopathology, oral signs and anti-LA/SSB) of pSS. For each characteristic of pSS, comparisons were conducted in three pairs as follows: patients positive for a certain characteristic *versus* negative patients; patients positive for a certain characteristic *versus* healthy controls; patients negative for a certain characteristic *versus* healthy controls. Surprisingly, there was a statistically significant difference in the FAM167A-BLK (rs2736340 and rs13277113) between the anti-SSB negative patients and controls in this population (p=0.036 and p=0.031, respectively) (Table IV). However, no association signal was detected when other characteristics were analysed (data not shown).

A. LD analysis of the SNPs in the *BANK1*

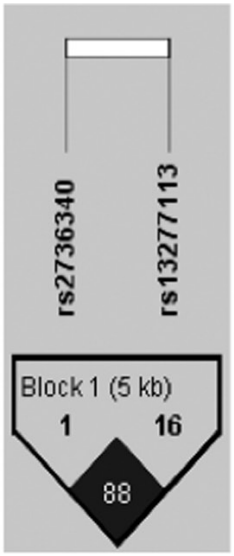


Data from Hapmap

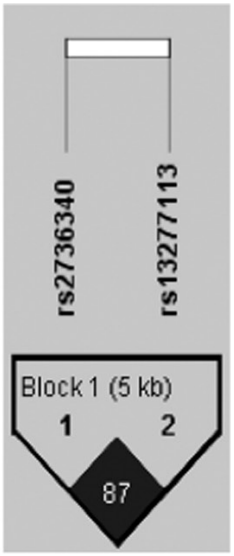


Data from our study

B. LD analysis of the SNPs in the *FAM167A-BLK*



Data from Hapmap



Data from our study

Fig. 1. Linkage disequilibrium (LD) analysis of the SNPs in the *BANK1* and *FAM167A-BLK* region. The LD plots were generated by Haploview software v4.2 and data from our study were similar to that from the HapMap CHB population. The number (divided by 100) in the small square represents r^2 value and ranges from 0 to 1. (**A**) The 2 SNPs (rs17266594 and rs10516487) in the *BANK1* reside in an LD block, and they had a strong LD (r^2=1 in HapMap CHB population; r^2=0.93 in our study). The rs4522865 is in another LD block, which is far from rs17266594 and rs10516487. (**B**) The rs2736340 has a strong LD with rs13277113 (r^2=0.88 in HapMap CHB population; r^2=0.87 in our study).

Analysis of the haplotype and epistatic interaction among the SNPs

The results from the linkage disequilibrium (LD) analysis of these SNPs and the data from the HapMap CHB population are shown in Table V and Figure 1. The SNPs rs17266594 and rs10516487 in the *BANK1* had strong LD (r^2=0.93) (Fig. 1A), but they did not show significant difference in the haploptype frequency between the pSS patients and controls. However, there was significant difference in the haplotype frequency of the SNPs (rs2736340 and rs13277113) in the *FAM167A-BLK* between the pSS patients and controls, and these two SNPs are located in the same LD block (r^2=0.87) (Fig. 1B) in our study. Moreover, there was a significant difference in the "TA" or "CG" haplotype frequency between the pSS patients and controls (Table V). Finally, there was no significant epistatic interaction among the SNPs of the *BANK1* and *FAM167A-BLK* in this population (Table VI).

Bonferroni correction was used multiple comparisons

Following Bonferroni correction, all *p*-values were multiplied by five which was the number of the total SNPs. Then the SNP rs2736340, but not the SNP rs13277113, of the *FAM167A-BLK* remained significantly associated with pSS tested only using the dominant model (*p*-adjusted for Bonferroni = 0.045) in this population.

Discussion

This was the first study in Han population to examine the potential association of these SNPs in the *BANK1* (rs4522865, rs17266594, and rs10516487) and in the *FAM167A-BLK* region (rs2736340, rs13277113) with the susceptibility to pSS. We found that SNPs (rs2736340 and rs13277113) in *FAM167A-BLK* region, but not those (rs4522865, rs17266594 and rs10516487) in the *BANK1* gene, were associated with an increased risk for the development of pSS in Han Chinese.

The *BANK1* encodes a B-cell-specific scaffold protein and is predominantly expressed in B cells. The *BANK1* regulates B-cell receptor-induced calcium mobilisation from intracellular stores by promoting inositol 1,4,5-trisphosphate receptor phosphorylatin (33). Currently, the association of SNPs in the *BANK1* gene with SLE and SSc have been well established in multiple populations, but the influences of different variants of the *BANK1* gene on B-cell signalling and function have not been clarified. In 2008, Sergey V Kozyrev *et al*. (11) first found that the SNPs (rs17266594 and rs10516487) were associated with SLE affected regulatory sites and key functional domains of the *BANK1*, contributing to sustained BCR signaling and B-cell hyperactivity. Furthermore, the rs17266594 had a negligible effect on the *BANK1* splicing or expression, while the rs10516487 had a dual role in increasing gene expression and gen-

Table VI. Analysis of gene-gene interaction for *BANK1* and *FAM167A-BLK* variants.

Gene	SNP	*BANK1*		
		rs4522865	rs17266594	rs10516487
FAM167A-BLK	rs2736340	0.167	0.802	0.600
	rs13277113	0.237	0.895	0.796

erating a protein variant more prone to multimerisation (34). In addition, the rs10516487 and rs17266594 variants are not only associated with higher susceptibility to SLE, but are also significantly associated with certain subphenotypes of SLE with high-titer antinuclear antibodies (ANA) and anti-Ro/SSA antibodies in Han Chinese (15). The SNP rs4522865 selected in our study is an independent factor for increased susceptibility to SLE in Asians (20). However, we did not observe any significant association between the frequency of these variants and patients with pSS in this population. Our data were consistent with a previous report that showed no significant association of *BANK1* polymorphisms with pSS in a Caucasian population (31). These findings suggest that the rs4522865, rs17266594, and rs10516487 variants may be related to a range of autoimmune diseases, but not to pSS. It is possible that there may be fundamental differences in the *BANK1*-related genetic susceptibility and pathogeneses between pSS and other autoimmune diseases or that the *BANK1*-related genetic susceptibility conferred to pSS might be attributed to other SNPs, at least in Han Chinese.

The function of the *FAM167A* remains a mystery; however, the BLK is known to encode a non-receptor tyrosine kinase in the src family that is mainly expressed by B cells (35). The *BLK* regulates the BCR-related signaling and B cell development by activating the nuclear factor (NF)-kappaB (36, 37). In the present study, we found that the rs13277113 and rs2736340 variants were associated with the development of pSS in this population and extended previous findings in other autoimmune diseases, such as SLE (23, 25), RA (27, 28), and SSc (29, 30). The rs2736340 and rs13277113 resided in the same LD block, and they are the most common susceptibility loci in the *FAM167A* for other autoimmune diseases. However, it is recognised that after Bonferroni correction, these variants only had a weak association with pSS in this population. Indeed, G Nordmark *et al*. (31) also found that neither rs13277113 nor rs2736340 was strongly associated with pSS in Europeans. Accordingly, the rs13277113 and rs2736340 variants may be not crucial contributors to the genetic susceptibility to pSS as to other autoimmune diseases. We are interested in further investigating the importance of other SNPs in the *FAM167A-BLK* gene region in the development of pSS in a Han Chinese population.

We also found that the rs13277113 and rs2736340 variants had a weak association with pSS in patients with negative serum anti-LA/SSB antibody. These data support the notion of genetic diversity of autoimmune diseases, such as pSS (38-40). It is possible that the rs13277113 and rs2736340 variants may increase the susceptibility to pSS not by increasing the antibodies' production but by another unknown signal pathway in this population. A previous study shows that the rs13277113 variant is not only associated with an increased risk for the development of SLE, but also with reduced BLK expression and increased *FAM167A* expression (23). So we are interested in further exploring whether the reduced BLK expression and the rs13277113 variant can shape B cell development in pSS patients.

Although both the *BANK1* and *BLK* have potential role in B cell signaling and recent studies have indicated that the *BLK* can interact with *BANK1* during the pathogenesis of SLE (21, 22). However, we did not find any potential epistatic interaction between the examined SNPs in these two genes and pSS in this population. This finding may be due to the lack of association between *BANK1* and pSS. It is possible that other genes associated with B-cell signaling may play important roles in the pathogenesis of pSS.

We recognised that our study had limitations of a small sample size that resulted in an insufficient power and difficulty for further stratification. Accordingly, we might have missed the opportunity to analyse the potential association of these genetic variants with some special subtypes of pSS in this population. Indeed, our previous study has revealed that some SNPs are associated with increased risk for the development of certain clinical subtypes of SLE in the Chinese (41). In addition, we did not test the function of these genetic variants in the development of antigen-specific B cells in these patients. Therefore, further studies with a bigger population and functional studies of B cells are warranted.

In summary, our data indicated that the SNPs (rs2736340, rs13277113) of the *FAM167A-BLK* region, but not the *BANK1* SNPs (rs4522865, rs17266594, and rs10516487), were associated with the development of pSS in Han Chinese. Our findings suggest that the pathogenesis of pSS is different from other autoimmune diseases, such as SLE, and that the rs2736340 and rs13277113 variants may be used as biomarkers for predicting the development of pSS in Han Chinese.

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