Concise Report

Title: Association of *PHRF1-IRF7* region polymorphism with clinical manifestations of systemic lupus erythematosus in a Japanese population.

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Running head: *PHRF1-IRF7* polymorphism in Japanese SLE

Abstract

Interferon regulatory factor 7 (IRF7) has an essential role in the production of type I interferon. Although recent studies detected association of a single nucleotide polymorphism (SNP) rs4963128 in *PHRF1/KIAA1542,* located closely to *IRF7,* and *IRF7* rs1131665 (Gln412Arg) with SLE, causal variants have not been established. In this study, we resequenced exons and introns of *IRF7* to screen for all common polymorphisms, and examined whether they were associated with SLE in 416 Japanese patients with SLE and 505 healthy controls. We also tested whether the association of *PHRF1* rs4963128 with SLE was replicated in Japanese. None of the *IRF7* polymorphisms was associated with SLE. *PHRF1* rs4963128T was not significantly associated with occurrence of SLE either; however, this allele was significantly increased in SLE with anti-Sm antibodies (6.8%) as compared with healthy controls (3.1%, *P*=0.014, odds ratio [OR] 2.31) and SLE without anti-Sm antibodies (3.3%, *P*=0.041, OR 2.12). This allele was also increased in SLE with renal disorder (5.1%) as compared with those without renal disorder (2.4%, *P*=0.047, OR 2.17). These results confirmed recently reported association of *PHRF1* rs4963128T with anti-Sm antibody positive SLE in African-American populations, and supported the role of *PHRF1-IRF7* region in the genetics of SLE.

Keywords systemic lupus erythematosus, genetic studies, polymorphism, interferons

Introduction

Interferon regulatory factor 7 (IRF7) is a transcription factor essential for type I interferon (IFN) induction[1]. IRF7 is activated by toll-like receptor (TLR) 7 and TLR9 in plasmacytoid dendritic cells and induces a large amount of type I IFN[1]. Type I IFN plays a crucial role in systemic lupus erythematosus (SLE). Serum levels of IFN are elevated in SLE patients, and patients treated with IFNα sometimes develop features of SLE[2]. *IRF7* is also one of the IFN-inducible genes, whose expression in peripheral blood mononuclear cells (PBMCs) is upregulated in SLE[2,3].

In 2008, a genome-wide association study (GWAS) in Caucasians identified association of rs4963128 located in *PHD and ring finger domains 1* (*PHRF1*), also known as *KIAA1542*, with SLE[4]. Subsequently, the association was confirmed by a replication study in Caucasians[5] but not by a GWAS in Chinese[6].The SNP rs4963128 is located at 23 kb downstream of *IRF7* in chromosome 11p15.5, and linkage disequilibrium (LD) was observed between *PHRF1* and *IRF7* SNPs in the HapMap samples. Because the function of PHRF1 was unknown, the association of rs4963128 was interpreted to be caused by LD with *IRF7*.

Recently, association of *IRF7* SNP rs1131665 (Gln412Arg) with SLE was reported in multiple ethnic groups[7]. In that study, the association of *PHRF1* rs4963128 was not replicated in Asians mainly consisting of Chinese and Koreans. Association of *IRF7-PHRF1* region SNPs with autoantibody profile has also been reported [8].

Given the functional significance of IRF7 in the type I IFN induction, IRF7 is considered to be a strong candidate gene for autoimmune diseases. However, although approximately 50 *IRF7* polymorphisms are deposited in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), the genotype frequency

and LD data are not available for many of them. Moreover, to our knowledge, resequencing of entire exons and introns of *IRF7* has not been reported. In this study, we performed polymorphism screening of all exons and introns of *IRF7* gene, followed by an association study of the detected *IRF7* polymorphisms as well as *PHRF1* rs4963128, in Japanese patients with SLE and healthy controls.

Materials and methods

Patients and controls

An association study was conducted in 416 Japanese patients with SLE (26 males and 390 females, mean ± SD 43.1 ± 14.1 years) and 505 healthy individuals (234 males and 271 females, 34.1 ± 9.8 years), recruited at University of Tsukuba, Juntendo University, Sagamihara National Hospital and the University of Tokyo. All patients fulfilled the criteria for SLE proposed by the American College of Rheumatology (ACR)[9].

Association with clinical characteristics such as renal disorder, presence of anti-dsDNA antibody and anti-Sm antibody was also examined. Renal disorder was defined according to the ACR criteria[9] (proteinuria or cellular cast). Anti-dsDNA antibody was measured by ELISA, and the cut off value was determined according to the normal range at each institute. Anti-Sm antibody was detected using Ouchterlony immunodiffusion. Appearance of precipitin band was considered positive.

This study was reviewed and approved by the research ethics committees of University of Tsukuba, Sagamihara National Hospital and Juntendo University. Informed consent has been obtained from all participants.

Polymorphism screening and genotyping

Screening of *IRF7* polymorphisms was performed by direct sequencing of genomic DNA from 16 SLE patients and 16 healthy controls. Genomic DNA sequence was obtained from the GenBank database (accession number: NT_009237.18). To amplify the entire *IRF7* gene by polymerase chain reaction (PCR), 13 primer sets were designed (Supplementary Table 1). Direct sequencing was conducted using a BigDye Terminator v3.1 Cycle Sequencing kit on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City,

CA).

Three polymorphisms, rs10902179A>G, rs3832720 GGAGGC repeats (3 or 4 repeats), and rs55917943 A indel, were selected as tag polymorphisms based on the screening data with the criteria of minor allele frequency (MAF) ≥0.05 and an r^2 threshold of 0.8 (Figure 1a). These three polymorphic sites as well as two *IRF7* nonsynonymous SNPs rs1061502A>G (Lys179Glu) and rs1131665A>G (Gln412Arg) were tested for association in 416 SLE patients and 505 healthy controls. In addition, *PHRF1* rs4963128 was also included in the analysis.

Genotyping of the SNPs rs10902179 and rs55917943 were genotyped using Custom TaqMan SNP Genotyping Assays (ABI). Genotyping of rs1061502 was performed by TaqMan SNP Genotyping Assay (C___1611549_10) (ABI). The SNPs rs3832720, rs1131665 and rs4963128 were genotyped by direct sequencing using primers IRF7-2F-2R, IRF7-11F-11R and PHRF1-F-R (Supplementary Table 1), respectively.

Statistical analysis

Statistical significance of differences in allele frequencies between cases and controls was tested using χ^2 test with 2×2 contingency tables. When one or more cells in the contingency tables contained the variables of 5 or less, Fisher's exact test was applied. Pairwise r^2 values were calculated using Haploview version 4.0 software (Broad Institute, Cambridge, MA).

Power calculation was conducted based on the allele frequencies in Japanese and the sample size in this study (416 cases and 505 controls)[10]. This study had >80% power to detect association with the genotype relative risk of ≥2.31 for rs1061502 and rs1131665, 1.71 for rs10902179, 1.37 for rs3832720, 1.33 for rs55917943, and 1.92 for rs4963128.

Results

Polymorphism screening and an association study of *IRF7* gene

Due to lack of publicly available genotype frequency and LD data of *IRF7* polymorphisms, we screened polymorphisms in all exons and introns of *IRF7* by resequencing genomic DNA from 16 Japanese SLE patients and 16 healthy individuals. The polymorphisms detected by resequencing and LD structure are demonstrated in Figure 1a. Three tag polymorphisms selected based on the allele frequency and LD status in the screening samples, rs10902179A/G, rs3832720 GGAGGC repeats (3 or 4 repeats) and rs55917943A indel, as well as two low-frequency nonsynonymous SNPs, Lys179Glu (rs1061502A>G) and Gln412Arg (rs1131665A>G), were tested for association in 416 Japanese SLE patients and 505 healthy controls.

Call rate was 100% for these five polymorphisms, and none of the genotype distribution was significantly deviated from Hardy–Weinberg equilibrium in the cases and controls (P>0.05). LD between each *IRF7* polymorphism in 505 healthy individuals is shown in Figure 1b. The two nonsynonymous SNPs, rs1061502 and rs1131665, were in absolute LD with each other (r^2=1). A novel 30 bp indel was detected in intron 8 (position 553742-553713 [NT_009237.18]), whose call rate was 92.5% and was excluded from the analysis.

As shown in Table 1, association of *IRF7* SNPs with occurrence of SLE was not detected in Japanese.

An association study of *PHRF1* rs4963128

We next examined whether the previously reported association of *PHRF1* rs4963128 with SLE in Caucasians was replicated in Japanese. The allele frequency of rs4963128T in our Japanese 505 healthy controls (3.1%) was substantially lower than in the Caucasians (33.7%)[4], which was consistent with

the frequency data of rs4963128T in the HapMap database (CEU: 32.9%, JPT: 2.2%, CHB: 4.5%). The SNP rs4963128 was in moderate LD (r^2=0.54) with *IRF7* nonsynonymous SNPs (rs1061502/rs1131665) (Figure 1b). In agreement with the previous reports from Asian populations[6,7], no significant association of rs4963128 with SLE was observed in Japanese (Table 1).

Association of SLE subset with polymorphisms in *IRF7* and *PHRF1*

We next investigated whether polymorphisms in *IRF7* and *PHRF1* were associated with SLE subsets such as presence of renal disorder, anti-Sm antibodies and anti-dsDNA antibodies (Table 2). When the allele frequencies were compared between each subset of SLE and healthy controls, significant association of rs4963128T was observed in SLE with anti-Sm antibodies (case-control analysis). The association was confirmed when the SLE patients with anti-Sm antibodies and those without were compared (case-only analysis). Association of rs4963128T with renal disorder was also detected in case-only comparisons. On the other hand, association of *IRF7* with the SLE subsets was not detected.

Discussion

IRF7 plays a crucial role in the induction of type I IFN. Given the significance of type I IFN in SLE, *IRF7* has been thought to be a strong candidate susceptibility gene to SLE, which gained strong support by the recent studies that reported the association of *PHRF1* rs4963128 located closely to *IRF7*[4,5], as well as *IRF7* rs1131665 (Gln412Arg), with SLE[7]. To examine whether *IRF7* primarily contributes to susceptibility to SLE and the association of rs4963128 can be explained by LD with common polymorphisms in *IRF7*, we performed polymorphism screening of *IRF7* by resequencing, and conducted an association study of *IRF7* polymorphisms as well as *PHRF1* rs4963128 in Japanese. However, evidence for association with the occurrence of SLE was observed neither for rs4963128 nor *IRF7* SNPs. Analysis for gene-gene interaction between rs4963128 and each SLE risk alleles established in Japanese (*HLA-DRB1, IRF5, STAT4, BLK, FCGR2B, TNFAIP3, TNIP1* and *TNFSF13*) failed to show significant interaction (data not shown).

With respect to *PHRF1* rs4963128, our observation was consistent with the report by Fu et al.[7] and GWAS in Chinese[6], which failed to detect the association between rs4963128 and occurrence of SLE in Asians. On the other hand, in contrast to our observation, association of *IRF7* rs1131665 (Gln412Arg) has been reported in Asian populations, the majority of which were Chinese and Koreans[7]. This lack of agreement does not appear to be explained by the difference in the population allele frequency of 412Arg, which was 1.7% in this study and 2.9% in the previous study of the Asian populations[7]. Another possibility is the lack of sufficient detection power for the low frequency variants. In rs1131665, the sample size of this study provided 80% power to detect associations only when the genotype relative risk was ≥2.31. However, in this study, although not statistically significant, opposite tendency towards increase

of 412Arg rather than the previously reported 412Gln[7] was observed in SLE (412Arg, SLE: 1.9%, control: 1.7%). Thus, it appears unlikely that the lack of association is explained only by the insufficient statistical power. At this point, it appears that the difference in the association reflects slight differences in the genetic background among the Japanese, Korean and Chinese populations[11]. In the future, association of *IRF7* with SLE should be examined using an independent larger sample set.

Although the association of *PHRF1* rs4963128 with occurrence of SLE was not detected, we found that rs4963128 was associated with the presence of anti-Sm antibodies and renal disorder in SLE patients. In agreement with our results, Salloum et al. reported the association of anti-Sm antibodies with rs4963128 in African Americans[8]. In addition, they showed that among the SLE patients with anti-Sm antibodies, only the patients carrying rs4963128T exhibited higher IFNα activity. Their observations suggested that rs4963128T is not only involved in induction of anti-Sm antibodies, but also in upregulation of IFNα under the presence of anti-Sm antibodies. Preferential association of *PHRF1* with patients with renal disorder in our study is in line with the previously reported association between renal disorder and presence of anti-Sm antibody[12] and IFN signature[13]. Anti-Sm antibodies are directed against small nuclear ribonucleoproteins. It is assumed that interaction of the RNA-protein complex with specific B cell receptors on the surface of B cells may lead to the transport of the complex to endosomes, where RNA binds to TLR7 and may induce activation of IRF7, which eventually leads to B cell activation and production of anti-Sm antibodies[14]. Therefore, the lack of causative variations within *IRF7* was an unexpected finding. Because our current study which resequenced 16 cases and 16 controls focused on common variants, this study does not exclude the possibility that rare variants of *IRF7* might play a role. In addition, according to the HapMap

database, LD with rs4963128 encompasses the entire *IRF7* gene and extends to the upstream region of *IRF7,* which has the opposite direction of transcription to *PHRF1*. Therefore, although association of polymorphisms in *IRF7* was not detected in this study, the possibility that rs4963128T, or other allele(s) in the regulatory region of *IRF7* which is in LD with rs4963128T, may be associated with anti-Sm antibodies through the effects on *IRF7* expression cannot be excluded.

Finally, it is also possible that *PHRF1* might be causally involved in the pathogenesis of SLE. *PHRF1* was initially identified by screening of an adult brain cDNA library, and its expression was observed in heart, brain, lung, liver, skeletal muscle, kidney, pancreas, spleen, testis and ovary[15]; however, the function of *PHRF1* has not been elucidated yet. Further studies are required to elucidate the molecular mechanisms of *PHRF1-IRF7* region association with SLE with anti-Sm antibody and renal diseases.

In conclusion, resequencing of the Japanese SLE and controls did not detect common polymorphisms associated with SLE within *IRF7* exons and introns. However, we independently replicated the association of *PHRF1* SNP rs4963128T with anti-Sm antibody recently reported in the African-American populations, and also detected association with the presence of renal disorder. These results supported the role of *PHRF1-IRF7* region in the genetics of clinical manifestations of SLE.

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Declaration of Conflict of Interest

The Authors declare that there is no conflict of interest.

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Table 1. An sssociation study of polymorphisms in *IRF7* and *PHRF1/KIAA1542* with susceptibility to SLE in a Japanese population.

	SLE (n=416)	Controls (n=505)	*P*	OR (95%CI)
IRF7				
rs10902179 G	34 (4.1)	50 (5.0)	0.38	0.82 (0.52-1.28)
rs3832720 $(GGAGGC)_3$	169 (20.3)	209 (20.7)	0.84	0.98 (0.78-1.23)
rs1061502G(179Glu)	16 (1.9)	17 (1.7)	0.70	1.15 (0.58-2.28)
rs1131665G (412Arg)	16 (1.9)	17 (1.7)	0.70	1.15 (0.58-2.28)
rs55917943 A ins	253 (30.4)	304 (30.1)	0.89	1.01 (0.83-1.24)
PHRF1/KIAA1542				
rs4963128T	32 (3.8)	31 (3.1)	0.36	1.26 (0.76-2.09)

OR: odds ratio, 95%CI: confidence interval. Allele frequencies are shown in parentheses (%).

Association was tested by chi-square analysis using 2×2 contingency tables.

Table 2. An association study of *PHRF1/KIAA1542 -IRF7* region SNPs with clinical characteristics of SLE in a Japanese population.

		Case-control analysis		Case-only analysis (+ versus -)	
	allele frequency n (%)	*P*	OR (95%CI)	*P*	OR (95%CI)
IRF7 rs1061502G(179Glu), rs1131665G (412Arg)					
SLE					
anti-Sm Abs + (n=88)	5 (2.8)	0.36[a]	1.71(0.62-4.69)	0.38[a]	1.57(0.54-4.57)
anti-Sm Abs - (n=300)	11 (1.8)	0.82	1.09(0.51-2.34)		ref
anti-dsDNA Abs + (n=324)	16 (2.5)	0.26	1.48(0.74-2.95)	0.092[a]	7.54(0.45-126.4)
anti-dsDNA Abs - (n=72)	0 (0.0)	0.25[a]	0.20(0.01-3.28)		ref
renal disorder + (n=224)	12 (2.7)	0.21	1.61(0.76-3.40)	0.13[a]	2.52(0.81-7.88)
renal disorder - (n=185)	4 (1.1)	0.62[a]	0.64(0.21-1.91)		ref
Control (n=505)	17 (1.7)		ref		
PHRF1/KIAA1542 rs4963128T					
SLE					
anti-Sm Abs + (n=88)	12 (6.8)	0.014	2.31(1.16-4.59)	0.041	2.12(1.02-4.43)
anti-Sm Abs - (n=300)	20 (3.3)	0.77	1.09(0.61-1.93)		ref
anti-dsDNA Abs + (n=324)	29 (4.5)	0.13	1.48(0.88-2.48)	0.24[a]	2.20(0.66-7.33)
anti-dsDNA Abs - (n=72)	3 (2.1)	0.79[a]	0.67(0.20-2.23)		ref
renal disorder + (n=224)	23 (5.1)	0.054	1.71(0.98-2.97)	0.047	2.17(0.99-4.75)
renal disorder - (n=185)	9 (2.4)	0.53	0.79(0.37-1.67)		ref
Control (n=505)	31 (3.1)		ref		

OR: odds ratio, 95%CI: confidence interval, Abs: antibodies. Allele frequencies are shown in parentheses (%).
Association was tested by chi-square analysis or Fisher's exact test using 2×2 contingency tables.
[a] Fisher's exact test was employed.

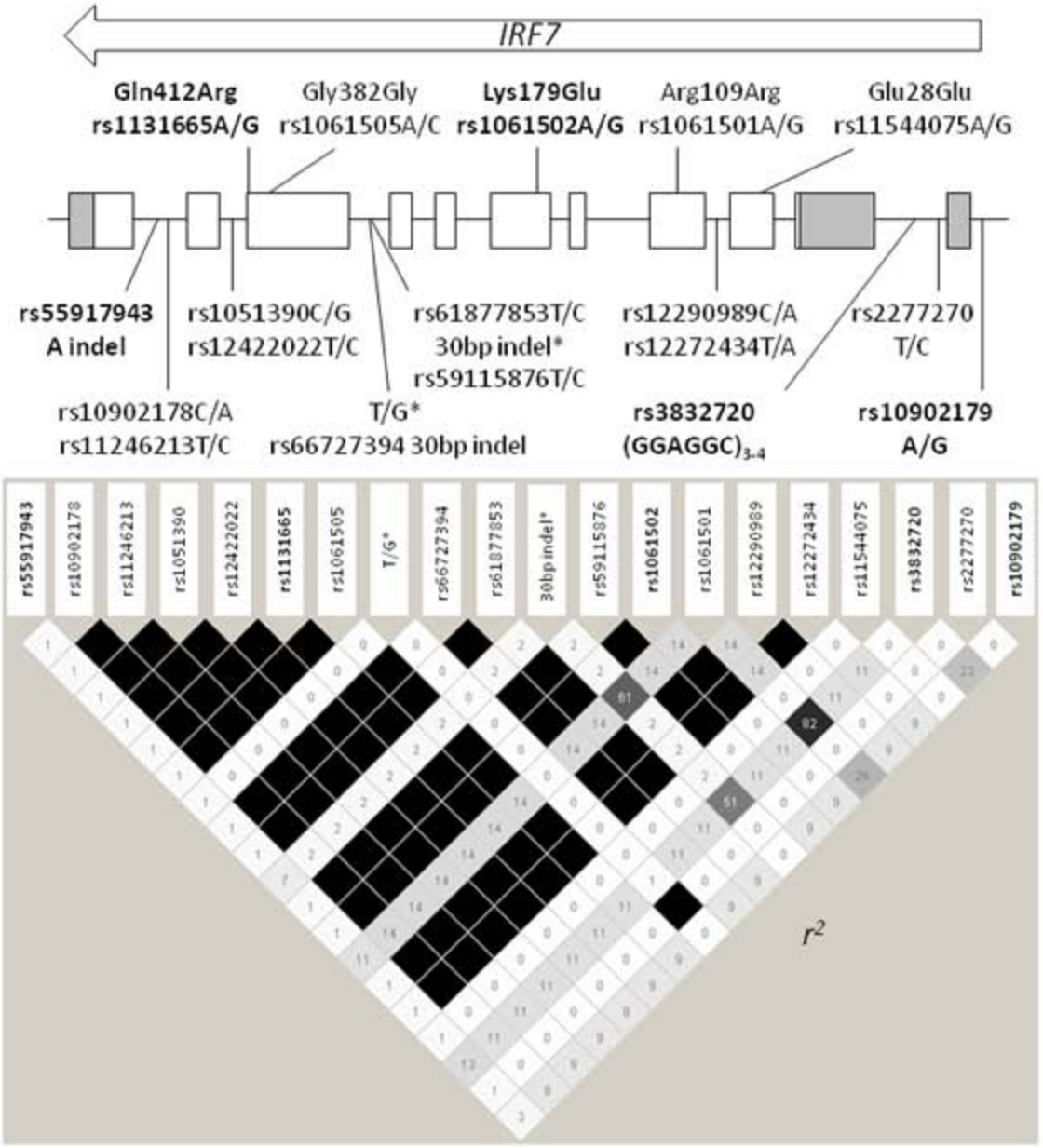
Figure Legends

Figure 1. Polymorphisms and linkage disequilibrium status of *PHRF-IRF7* region in Japanese.

(a) *IRF7* polymorphisms detected by resequencing in the screening samples and pairwise r^2 values based on data in the 16 SLE and 16 healthy individuals used for resequencing. The polymorphisms genotyped for association study are shown in boldface. Two novel polymorphisms, T/G SNP at position 553672 and 30bp indel polymorphism at position 553742-553713 of chromosome 11 (NT_009237.18), were identified and shown by asterisk.

(b) Pairwise r^2 values among the five *IRF7* polymorphisms and *PHRF1* rs4963128 based on data in 505 Japanese healthy individuals.

a


IRF7
Gln412Arg
rs1131665A/G
Gly382Gly
rs1061505A/C
Lys179Glu
rs1061502A/G
Arg109Arg
rs1061501A/G
Glu28Glu
rs11544075A/G
rs55917943
A indel
rs1051390C/G
rs12422022T/C
rs61877853T/C
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rs59115876T/C
rs12290989C/A
rs12272434T/A
rs2277270
T/C
rs10902178C/A
rs11246213T/C
T/G*
rs66727394 30bp indel
rs3832720
(GGAGGC)3-4
rs10902179
A/G
rs55917943
rs10902178
rs11246213
rs1051390
rs12422022
rs1131665
rs1061505
T/G*
rs66727394
rs61877853
30bp indel*
rs59115876
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rs12290989
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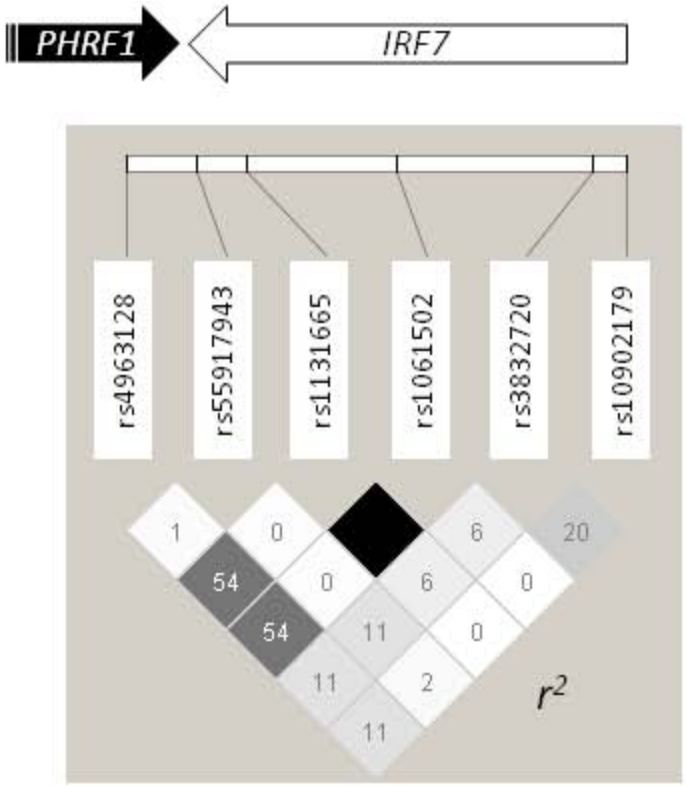
rs11544075
rs3832720
rs2277270
rs10902179
r2

b


PHRF1
IRF7
rs4963128
rs55917943
rs1131665
rs1061502
rs3832720
rs10902179
1
0
6
20
54
0
6
0
54
11
0
11
2
11
r2

Supplementary Table 1. Primers used in this study.

Fragment (chromosomal position*)	Primer name	Primer sequence
PHRF1 (529375 - 529709)	PHRF1 F	5’-CAGGGTGCTTGATGTGTTGT-3’
	PHRF1 R	5’-CACAGAGCTACCCATGCTTTC-3’
IRF7-1 (556087-555820)	IRF7-1 F	5’-CCTCCACTCCTCCCTACTCC-3’
	IRF7-1 R	5’-CCCAGCTCTTGGCTCTACC-3’
IRF7-2 (555990-555583)	IRF7-2 F	5’-GCCTGGCCACCATAAAAGC-3’
	IRF7-2 R	5’-CGCACACATGAAGTCACAGG-3’
IRF7-3 (555721-555334)	IRF7-3 F	5’-GAAGCGCCACTGTTTAGGTTT-3’
	IRF7-3 R	5’-CGGGCTCTTACCTCTCAGGA-3’
IRF7-4 (555480-555087)	IRF7-4 F	5’-CTGTGACACCTGGCCACAC-3’
	IRF7-4 R	5’-GGGTCCCCACCTTGAAGA-3’
IRF7-5 (555192-554753)	IRF7-5 F	5’-CTGCAGTGGCTGGACGAG-3’
	IRF7-5 R	5’-AAGCAGGACGAATGCCAAC-3’
IRF7-6 (554875-554452)	IRF7-6 F	5’-GTGATGCTGCGGGATAACTC-3’
	IRF7-6 R	5’-AGAGGCAGGCAGAGAGAAGG-3’
IRF7-7 (554575-554167)	IRF7-7 F	5’-CTCTGGTGTCGGGGCCTA-3’
	IRF7-7 R	5’-CCCACACCTCCAGCACACG-3’
IRF7-8 (554268-553866)	IRF7-8 F	5’-TCTGCTGACAGCGTCATGG-3’
	IRF7-8 R	5’-CTGCATCCGGAAGGGAAT-3’
IRF7-9 (553965-553456)	IRF7-9 F	5’-CGGCACTAACGACAGGTGA-3’
	IRF7-9 R	5’-TACCTGCTGGGGGTCTGTG-3’
IRF7-10 (553616-553243)	IRF7-10 F	5’-CACAGCTTGGTCTCCACACA-3’
	IRF7-10 R	5’-ACAGTTCCGAGGCAGCAG-3’
IRF7-11 (553412-553003)	IRF7-11 F	5’-GCTACACGGAGGAACTGCTG-3’
	IRF7-11 R	5’-ACCAGGACCAGGCTCTTCTC-3’
IRF7-12 (553130-552721)	IRF7-12 F	5’-TCCCCTTCTTCAGAGCTGGT-3’
	IRF7-12 R	5’-CTGAGGCTGCTGCTATCCAG-3’
IRF7-13 (552907-552424)	IRF7-13 F	5’-GGCCATACACCGGGTCAC-3’
	IRF7-13 R	5’-TTGGCTGTGATGTGTGTGGT-3’

*Chromosomal positions were based on the chromosome 11 genomic sequence obtained from the GenBank database (accession number: NT_009237.18).