The combination of polymorphisms within interferon-γ receptor 1 and receptor 2 associated with the risk of systemic lupus erythematosus

Hitoshi Nakashima*, Hisako Inoue, Mitsuteru Akahoshi, Yosuke Tanaka, Kunihiro Yamaoka, Eiichi Ogami, Shuuji Nagano, Yojiro Arinobu, Hiroaki Niiro, Takeshi Otsuka, Yoshiyuki Niho

First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

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Abstract Genetic factors seem to play a significant role in susceptibility to systemic lupus erythematosus (SLE). We previously described the amino acid polymorphism (Val14Met) within the IFN-γ receptor 1 (*IFN-γR1*), and that the frequency of the Met14 allele in SLE patients was significantly higher than that of the healthy control population [Tanaka et al. (1999) Immunogenetics 49, 266–271]. We also found an amino acid polymorphism (Gln64Arg) within IFN-γ receptor 2 (*IFN-γR2*). Since the IFN-γ receptor is a complex consisting of IFN-γR1 and IFN-γR2, we searched for the particular combination of two kinds of amino acid polymorphisms found within the IFN-γ receptor which plays a prominent role in susceptibility to SLE. The greatest risk of the development of SLE was detected in the individuals who had the combination of *IFNGR1* Met14/Val14 genotype and *IFNGR2* Gln64/Gln64 genotype.

Key words: Interferon-γ receptor 1 (IFN-γR1); Interferon-γ receptor 2 (IFN-γR2); Genetic polymorphism; Susceptibility; Systemic lupus erythematosus (SLE)

1. Introduction

Systemic lupus erythematosus (SLE) is characterized by multisystem inflammation and the production of autoantibodies by activated B lymphocytes and by decreased cellular immune responses related to a dysregulation of T lymphocytes. Autoantibodies can generate immune complexes and may cause tissue damage through the recognition of an autoantigen. Although many factors have been proposed, such as genetic factors, environmental factors, hormonal action, viruses and dysregulation of cytokine production, the cause of this disease is not well understood.

Interferon-γ (IFN-γ) is a secretory protein produced by activated T lymphocytes and natural killer (NK) cells. On binding to a specific cell surface receptor complex consisting of IFN-γ receptor ligand-binding chain (IFN-γR1) and IFN-γ receptor signal-transducing chain (IFN-γR2) [2,3], IFN-γ induces antiviral activity, upregulation of class II major histocompatibility complex (MHC) molecule expression, B cell maturation, and release of mediators of inflammation [4–6]. The predominance of either Th1 or Th2 immune response can have great significance for many disease conditions, and IFN-γ as well as interleukin-2 is mainly responsible for the Th1 response, cell-mediated immunity [7].

We have reported that the frequency of the amino acid polymorphism (Val14Met) in *IFN-γR1* in SLE patients was significantly higher than in the healthy control population, which suggested that this polymorphism influenced the susceptibility to SLE as one of the genetic factors[1]. We found an amino acid polymorphism (Gln64Arg) in *IFN-γR2*, and clarified that the particular combination of these two kinds of amino acid polymorphism is associated with the susceptibility to SLE.

2. Materials and methods

2.1. Patients and healthy controls

This study included 96 outclinic patients with SLE (13 males and 83 females, mean age 40.9, S.D. 12.65, range 16–78 years old), and 91 healthy control volunteers (10 males and 81 females, mean age 40.1, S.D. 13.01, range 17–76 years old). All the patients met more than four of the American Rheumatism Association revised criteria for the diagnosis of SLE.

2.2. RT-PCR SSCP analysis of the IFNGR2 cDNA sequence

Total RNA was extracted from peripheral mononuclear cells (2×10^7 cells) of 200 unrelated Japanese individuals by the guanidinium isothiocyanate/phenol extraction method (Isogen, Nippon Gene Ltd., Tokyo, Japan). One-tenth of the total RNA preparation was used in first-strand cDNA synthesis (RNA PCR Kit; PE Biosystems Japan Ltd., Urayasu, Japan). One-fifth of the cDNA reaction mixture was used as a template in polymerase chain reaction (PCR), and cycling conditions for PCR were an initial 2 min at 94°C, followed by 1 min at 94°C, and 1 min at 60°C for 35 cycles, with an extension time of 7 min at 72°C. Three pairs of synthetic oligonucleotides, which were about 400 bp apart, were selected to amplify three segments, which were named YF-AFI, YF-AFII and YF-AFIII (Table 1). The whole segments almost covered the *IFNGR2* open reading frame (1012 nucleotides) starting from nucleotide position 649 and ending at position 1660, which is a part of the published cDNA clone sequence [2]. A mixture of 2 μl of PCR products and the same amount of denaturing solution (95% formamide, 0.1% BPB, 0.1% xylene cyanol) was heated for 5 min at 94°C and chilled on ice before being applied to a 10% polyacrylamide gel plate (acrylamide:bis = 49:1, 0.8 mm × 8.0 cm × 9.0 cm). Electrophoresis was performed at a constant voltage of 45 V for 12 h. After electrophoresis, the DNA bands were visualized by the silver staining method [8] with a commercially available reagent kit (Daiichi Pure Chemicals, Tokyo, Japan). DNAs showing variations on electrophoresis were cloned into the pT7blue T vector (Novagen Ltd., Madison, WI, USA) and then sequenced. Silver staining of PCR products yielded consistent results.

2.3. Detection of polymorphism within the IFNGR2 cDNA sequence

We detected a polymorphism within the *IFNGR2* cDNA sequence as described in Section 3. Since the polymorphism did not create or destroy any restriction enzyme sites, detection by mismatch-PCR/RFLP was performed [9,10]. The sequence of the forward primer (named HNQ64R-U678) is 5′-CTG CTG CTG CTC GGA GTC

*Corresponding author. Fax: (81) (92) 642 5247.
E-mail: hnakashi@intmed1.med.kyushu-u.ac.jp

Abbreviations: IFN-γR1, interferon-γ receptor 1; IFN-γR2, interferon-γ receptor 2; SLE, systemic lupus erythematosus

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Table 1
Sequence of oligonucleotides for PCR

Primer	Sequence	Sequence region (nt)	Size of the amplified fragment (bp)
YF-AFI	Forward: 5′-GACCTGAGCCGCCGCCGAGCG-3′	619–639	342
	Reverse: 5′-GCCTGCTGAGGGACTGGCGGC-3′	940–960	
YAF-AFII	Forward: 5′-CAGAGTGTGACTTCACTGCCG-3′	923–943	375
	Reverse: 5′-GCAGTTGTGCCTGGACTTGTA-3′	1277–1297	
YF-AFIII	Forward: 5′-AAAACCCTCCAGAGTGTACTG-3′	1254–1274	428
	Reverse: 5′-CTAGGCCCATGCTTTGGTTCA-3′	1660–1681	

TTC-3′. The reverse primer, HNQ64R-L866 (sequence: 5′-ACT GTC GGT GTA TTT AAA CTG AGC T-3′), contains a 2 bp mismatch (underlined) just proximal to its 3′ end such that the 189 bp amplified product incorporates a restriction site for the endonuclease *Sac*I in the presence of guanine at nucleotide 839, but not in the presence of adenine (Fig. 1). DNAs digested with 5 units of *Sac*I under the conditions recommended by the manufacturer (New England Biolab, Inc., Beverly, MA, USA) were size-separated by 4% agarose gel electrophoresis. *Sac*I digestion of the PCR amplicon produced 170 bp and 19 bp products for the G839 allele, and an intact 189 bp product for the A839 allele.

2.4. Detection of the polymorphism within the IFNGR1 cDNA sequence

The determination of the genotypes for *IFNGR1* cDNA was performed by the method previously described [1]. Briefly, RT-PCR was performed with the reverse primer (YT-1: 5′-GTA AAA ACA GGG ACC TGT GGC ATG-3′) and the forward primer (YT-2: 5′-CTC CTA CCC CTT GTC ATG CAG GAT-3′). The forward primer YT-2 contains a 1 bp mismatch (underlined) just proximal to its 3′ end such that the 164 bp amplified product incorporates a restriction site for the endonuclease *Fok*I in the presence of guanine at nucleotide 88, but not in the presence of adenine. *Fok*I digestion of the PCR amplicon produced 130 bp and 34 bp products for the G88 allele (Val14 allele), and an intact 164 bp product for the A88 allele (Met14 allele). We were not able to detect any individual homozygous for the Met14 allele (Fig. 2).

2.5. Statistical analysis

Crude odds ratios (OR) and 95% confidence intervals (CI) were calculated from logistic regression models by the PC-SAS version 6.04 (SAS Institute, Inc., Cary, NC, USA). The reported P values are two-sided and considered significant at $P \leq 0.05$.

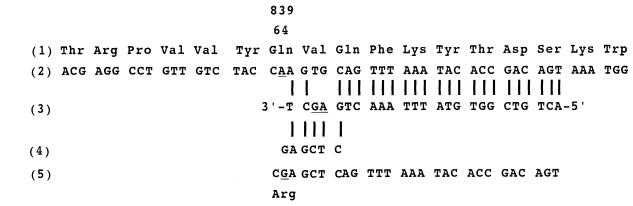

Fig. 1. Mismatch-RT-PCR primer for detection of polymorphism within the *IFNGR2* cDNA sequence. (1) A part of the IFN-γR2 amino acid sequence. (2) A part of the nucleotide sequence of the *IFNGR2* cDNA open reading frame. The DNA polymorphism is located at nucleotide 839 from the starting of cDNA. The polymorphism A839G (underlined) leads to an amino acid substitution at position 64 (Gln64Arg). (3) Reverse primer for mismatch-RT-PCR/RFLP analysis. This primer, named HNQ64R-L866, contains a 2 bp mismatch (underlined) just proximal to its 3′ end. (4) Endonuclease *Sac*I recognition site sequence. (5) The 3′ terminal sequence of PCR product in the presence of guanine at nucleotide 839 (underlined) incorporates a recognition site for the endonuclease *Sac*I, but not in the presence of adenine.

3. Results

DNA variants observed in RT-PCR SSCP analysis with YF-AFI primers were sequenced and the sole single base substitution of guanine for adenine at nucleotide 839 (A839G) was identified. This substitution occurred at the second position of codon 64 (CAA to CGA), and led to an amino acid substitution of arginine for glutamine (Gln64Arg). This polymorphism can be detected by the mismatch-PCR/RFLP method. The endonuclease *Sac*I digestion of the PCR amplicon discriminated between the Gln64 allele and Arg64 allele, resulting in a 189 bp product, and 170 bp and 19 bp products, respectively (Fig. 2). We could determine the genotype for *IFNGR2* as well as *IFNGR1*.

The distribution of *IFNGR1* and *IFNGR2* genotypes is presented in Table 2, and we calculated ORs based on single genotype in SLE cases and healthy controls in Table 3. There was a significant association between *IFNGR1* genotypes and SLE. The *IFNGR1* Met14 genotype increased the risk of SLE (OR 4.0, 95% CI 1.3–12.6, $P = 0.02$). We have already reported that the proportion of the *IFNGR1* genotype was statistically significantly different between SLE cases and healthy controls [1]. But there was no significant association between *IFNGR2* genotypes and SLE.

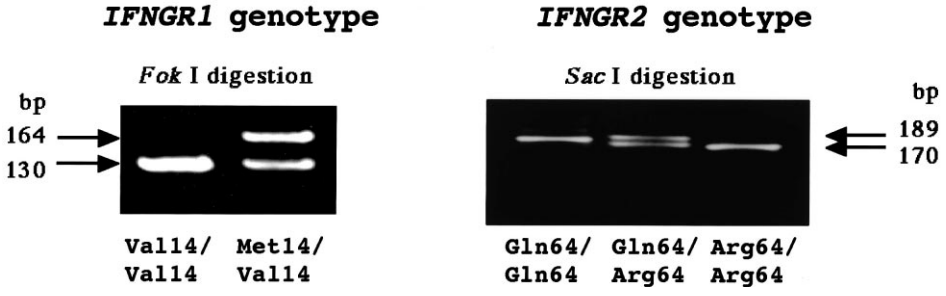

Fig. 2. Genotyping for *IFNGR1* and *IFNGR2* by mismatch-RT-PCR/RFLP. *IFNGR1* genotype: The amplified products from RNAs using primers YT-1 and YT-2 were digested with endonuclease *Fok*I and electrophoresed on a 4% agarose gel. Ethidium bromide-stained DNAs show RFLP. *Fok*I digestion of the PCR amplicon produced 130 bp and 34 bp products for the Val14 allele, and an intact 164 bp product for the Met14 allele. We were not able to detect any homozygous for the Met14 allele. *IFNGR2* genotype: The amplified products from RNAs using primers YF-AFII-U678 and HNQ64R-L866 were digested with endonuclease *Sac*I and electrophoresed on a 4% agarose gel. *Sac*I digestion of the PCR amplicon produced 170 bp and 19 bp products for the Arg64 allele, and an intact 189 bp product for the Gln64 allele.

Table 2
Distribution of *IFNGR1* and *IFNGR2* genotypes in SLE and healthy controls

Genotype	Cases ($n=96$)	Healthy controls ($n=91$)
IFNGR1		
Val14/Val14	0.84 (81)	0.96 (87)
Met14/Val14	0.16 (15)	0.04 (4)
IFNGR2		
Arg64/Arg64	0.19 (18)	0.21 (19)
Arg64/Gln64	0.49 (47)	0.55 (50)
Gln64/Gln64	0.32 (31)	0.24 (22)

Table 3
Odds ratio based on single genotype in SLE cases and healthy controls

Genotype	Crude OR (95% CI)	*P*
IFNGR1		
Val14/Val14	1.0	
Met14/Val14	4.0 (1.3–12.6)	0.02
IFNGR2		
Arg64/Arg64	1.0	
Arg64/Gln64	1.0 (0.5–2.1)	0.98
Gln64/Gln64	1.5 (0.6–3.5)	0.36

In Table 5, we show ORs based on genotype combination in SLE cases and healthy controls. The greatest risk was detected in individuals having the combination of the *IFNGR1* Met14/Val14 genotype and the *IFNGR2* Gln64/Gln64 genotype (OR 9.6, 95% CI 1.1–85.7, $P=0.04$). The other combinations did not have significantly increased risk.

4. Discussion

We found a genetic polymorphism of A839G within *IFNGR2*. This polymorphism led to an amino acid substitution of arginine for glutamine (Gln64Arg) within *IFN-γR2*. In fact, this substitution had already been described in the report of the *IFN-γR2* cloning work [2]. A genetic polymorphism (Val14Met) within *IFN-γR1*, which is another component of the IFN-γ receptor, was also reported [1].

In this work we studied the association between the genotype combination of these *IFN-γR* polymorphisms and SLE. Our data show that there was no association between *IFN-γR2* genotype and SLE (Table 3). However, the risk of SLE associated with the *IFN-γR1* Met14/Val14 genotype was confined to individuals whose *IFN-γR2* genotypes were Gln64/Gln64 (OR = 9.6 in Gln64/Gln64 vs. OR = 3.6 in Arg64/Arg64). This result suggests the possibility of an interaction between the two polymorphisms (Table 5). Although the direct tests for interaction between these genotypes showed no statistically significant effect, it was probably due to small sample sizes. The *IFN-γR2* Gln64/Gln64 genotype was not risk factor among the individuals whose *IFN-γR1* genotype was Val14/Val14 (Table 4).

Table 5
Odds ratio based on genotype combination in SLE cases and healthy controls

Genotype combination		Crude OR (95% CI)	*P*
IFNGR1	*IFNGR2*		
Val14/Val14	Arg64/Arg64	1.0	
	Arg64/Gln64	1.1 (0.5–2.4)	0.86
	Gln64/Gln64	1.3 (0.5–3.3)	0.55
Met14/Val14	Arg64/Arg64	3.6 (0.3–38.3)	0.29
	Arg64/Gln64	2.4 (0.4–15.0)	0.35
	Gln64/Gln64	9.6 (1.1–85.7)	0.04

The physiological mechanism behind these associations is not clear. Although there was no difference in the receptor function concerning HLA-DR expression induction of B cells between Gln64 IFN-γR2 and Arg64 IFN-γR2 [2], the function of B cells bearing variant IFN-γR1 (Met14/Val14) was significantly reduced compared with that of normal receptor B cells (Val14/Val14) [1]. The combination of IFN-γR1(Met14/Val14) and IFN-γR2(Gln64/Gln64) may induce synergistic dysfunction of the receptor. But we consider that these polymorphisms do not play a direct role in pathogenesis of SLE, but are members of genetic factors which may induce the development of this disease by combination with other loci or other circumstances. In fact, there was a normal individual carrying these polymorphisms (Table 4). Further accumulation of knowledge on genetic polymorphisms concerning the cytokine network would deepen our understanding of the genetic factors involved in SLE.

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Table 4
Distribution of genotype combinations between *IFNGR1* and *IFNGR2* in SLE cases and healthy controls

	IFNGR1: Val14/Val14		*IFNGR1*: Met14/Val14			
	Controls ($n=87$)	Cases ($n=81$)	Controls ($n=4$)	Cases ($n=15$)		
IFNGR2						
Arg64/Arg64	0.21 (18)	0.16 (15)	0.25 (1)	0.20 (3)		
Arg64/Gln64	0.55 (48)	0.53 (43)	0.5 (2)	0.27 (4)		
Gln64/Gln64	0.24 (21)	0.28 (23)	0.25 (1)	0.53 (8)		
	IFNGR2: Arg64/Arg64		*FNGR2*: Arg64/Gln64		*FNGR2*: Gln64/Gln64	
	Controls ($n=19$)	Cases ($n=18$)	Controls ($n=50$)	Cases ($n=47$)	Controls ($n=22$)	Cases ($n=31$)
IFNGR1						
Val14/Val14	0.95 (18)	0.83 (15)	0.96 (48)	0.91 (43)	0.95 (21)	0.74 (23)
Met14/Val14	0.05 (1)	0.17 (3)	0.04 (2)	0.09 (4)	0.05 (1)	0.26 (8)

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