

가

IL-6

promoter

polymorphism

가

IL-6 promoter

polymorphism

2000 12

가

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가 IL-6 promoter polymorphism

가

IL-6 가

IL-6 가 ,

가 가 E-selectin, T 가 IL-2R

가 가 CRP

가 , 가 IL-6가

megakaryocyte , 가

가 IL-6가 keratinocyte

.

IL-6 가가 IL-6 polymorphism

1999 1 12

가 10 10

. IL-6 ELISA kit .

genomic DNA IL-6 DNA 5'-flanking region

GenBank primer

, PCR genomic DNA sequencing

mutation site . mutation site site-directed

mutagenesis IL-6 promoter-luciferase plasmid

CV-1 cell transfection mutation .

가 IL-6 136.9 pg/ ml 가 가

20.9 pg/ ml (p<0.01) 5

pg/ml . 가 IL-6 promoter
 IL-6 가 -174bp
 G/C polymorphism 가
 promoter gene .
 IL-6 promoter wild type
 7 . -55bp A G
 substitution -810bp GGGCTG insert -816bp G
 deletion . , -66bp T insert,
 -142bp C insert, -579bp G deletion, -666bp C insert,
 -770bp GC insert . 가 IL-6
 -55bp G substitution ,
 -810bp GGGCTG insert -816bp G deletion IL-6
 .
 , 가
 IL-6 promoter polymorphism ,
 polymorphism
 polymorphism IL-6 ,
 IL-6
 가 .

: 가 , IL-6, promoter, polymorphism

가 IL-6 promoter polymorphism
< >

I

가 , , .
 , , .
 , .
 1967 가 ¹ , ,
 가 , 6 5 .
 가 가
 가 , , .
 , 1) 5 , 2) , 3)
 , 4) , 5) , 6) 6
 5가 가
 ^{2,3} ,

가 ,

^{4,5} 10-14

가 가

E-selectin, intercellular adhesion molecule-1(ICAM-1) lymphocyte function associated antigen -1(LFA-1) , Kim ⁶

가 가 E-selectin 가 가

가 가

interleukin-1(IL-1), tumor necrosis factor- (TNF-)

가 ^{7,8}

^{9,10} IL-1, TNF-

¹¹

가 가

T , B

cytokine 가 IL-1 ,

IL-4, TNF- , p60 sTNF-R, interferon- (IFN-), IL-6, IL-10, MCP-1, MIP-1 ,

RANTES 가 ¹²⁻²⁰ transforming growth

factor-beta 1 가 ²¹

IgM IgG 가 가 ²² 가

가

²³ 가 T 가

²⁴ IL-2 II-2 가 가 ^{25,26} T
 가 , , ,
 가 가
 가 Epstein-Barr(EB) virus virus capsid
 가가 EB virus ²⁷,
 BCG 65kDa heat shock protein
 가가 ²⁸.
²⁹ streptococcal pyrogenic
 exotoxin (SPE) T 가
 SPE ³⁰.
³¹.
 가 staphylococcal toxic shock syndrome
 toxic shock syndrome toxin-1(TSST-1) ,
 SPE . TSST-1 SPE superantigen T
³²⁻³³ Superantigen
 T V family
 T 가
 V 2 V 8 family T ³⁴
 superantigen 가 가
 TSST-1 ³⁵ TSST-1 superantigen
 가 .
 Choi ³⁶ Pietra ³⁷ 가 superantigen
 , conventional antigen
 T , 가

T B 가
 B ,³⁸ Kim³⁹ 가
 Humkv325 derived kappa chain CDR3 11 codon
 가 B 가
 ,⁴⁰ V_H
 가 B
 가
 가 가
 가 IL-6가 가⁴¹ 가
 IL-6가 가⁴² Kim²⁶
 가 IL-6
 IL-6 가
 가
¹⁸ ⁴³
 IL-6 가가
 Fishman⁴⁴
 IL-6 가 , IL-6 가
 IL-6 -174 G/ C polymorphism
 가 가 IL-6가
 ,⁴²
 IL-6 가 , Kim
⁶ 가 IL-6 가
 가 E-selectin 가 , Kim²⁶ 가
 T 가 IL-2R IL-6
 가 가 가

가 CRP 가 ,^{26,41,42}
 가 IL-6가 megakaryocyte
 , 가 가 IL-6가 keratinocyte
 IL-6 가
 가 cytokine .
 가 가 IL-6가
 , IL-6 가 Fishman⁴⁴
 IL-6 polymorphism 가
 가 IL-6 polymorphism
 .

II.

1.

1999 1 1999 12 가
10 . 6
45 5 가
10
2
1

2.

가. IL-6

-70 가
IL-6 R & D System Inc. (Minneapolis,
MN, USA) ELISA kit manual
standard curve

. Sequencing of IL-6 promoter

Genomic DNA

Kawasaki (), EDTA vacutainer tube
whole blood 5 ml , 200 μ l blood E.Z.N.A. Blood DNA kit

(Omega Bioteck, Doraville, GA, USA) DNA DNA
 UV Spectrophotometer 260nm
 -70

Primer

IL-6 DNA 5'-flanking region GenBank
 (Fig. 1). *KpnI* *Hind* 가
 primer , DNA
 primer (Seoul, Korea)
 . Primer Table 1 .

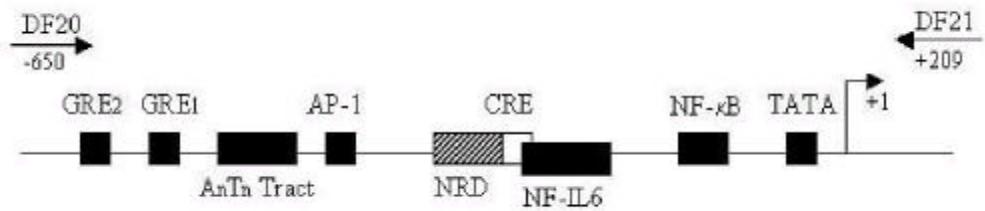


Fig. 1. A schematic representation of the 5' flanking region of IL-6 gene identifying transcription binding sites and the orientation of the oligonucleotide pairs used in this study. Locations of the binding sites are relative to the major transcription start site: TATA box -27 to -24; NF- B -73 to -64; NF-IL-6(C/EBP) -158 to -145; CRE(cAMP responsive element) -163 to -158; NRD (negative regulatory domain) -225 to -164; AP-1 -283 to -277; AnTn Tract -392 to -373; GRE₁ and GRE₂ (glucocorticoid responsive elements 1 and 2) -466 to -461 and -557 to -552, respectively.

Table 1. Sequences of primers used to generate PCR products for IL-6 promoter analysis

DF20	5'-GGA GTC ACA CAC TCC ACCT-3'
DF21	5'-GTG ACT GAC AGC ACA GCT-3'
IL6 <i>KpnI</i>	5'-GATCGGTACCGGAGTCACACACTCCACCTGGAGACGCC-3'
IL6 <i>Hind</i>	5'-GCTAGAAGCTTGTGACTGACAGCACAGCTGGGAGCCTGC-3'

PCR genomic DNA

PCR genomic DNA 10x PCR buffer[20mM Tris-HCl(pH 8.0), 100mM KCl, 20mM Mg²⁺], 2.5mM dNTP mix, 20 pmol primer set, 2.5 Units Taq DNA polymerase (Takara Ex Taq, Shiga, Japan) 가 . PCR Perkin Elmer DNA Thermocycler (Perkin Elmer, Norwalk, CT, USA) 94 3 가 , 94 30 , 66 1 , 72 1 35 PCR . 72 1 4 . PCR product 20% polyacrylamide gel (Fig. 2).

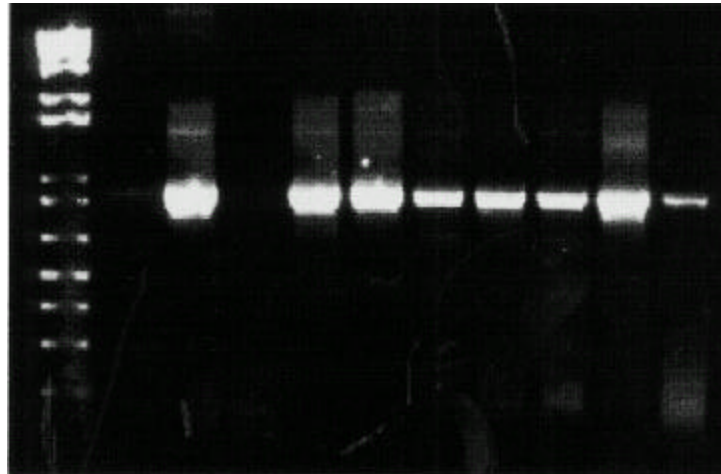


Fig. 2. Gel electrophoresis of the PCR products of the genomic DNA isolated from patients with Kawasaki diseases.

Sequencing

PCR product QIAquick gel extract kit (Quiagen Co, Valencia, CA, USA)
 sequencing primer set(Table 2) inner primer(Table
 1 IL-6 *KpnI* IL-6 *Hind*) automatic DNA sequencer (ALF
 express, Amhersham Pharmacia, Uppsala, Sweden) .

Table 2. Sequences of primers used for manual sequencing

MIS	5'-AGAGTAAAGCTGAAGTCATGCACGAAG-3'
MIR	5'-CTTCGTGCATGACTTCAGCTTTACTCT-3'
MS	5'-ATATTTATTGGGGTTGAGACTCTAAT-3'
MR	5'-CAAATGTGGGATTTCCCATGAGTCTCAA-3'

PCR cloning

Agarose gel 7.5% 858 bp
 Qiagen gel extraction kit (Qiagen Co., Valencia, CA,
 USA) gel . PCR pT7-blue script
 KS(Novagen, Madison, WI, USA) DNA *KpnI* *Hind*
 . plasmid vector DNA(insert)
 T4 DNA ligase 16 4 ligation .

transformation

Hanahan competent cell . agar
 plate(LB) *E.coli* DH5 colony SOB medium 3ml
 . 1-2ml 250ml SOB medium 37
 O.D. 600 0.6 shaking incubator . Transformation
 buffer, DMF solution washing , DNA .

LB(Mg) . LB(with ampicillin)
 plate . X-gal IPTG
 . 37 plate
 . 12-16 white colony 2xTY 2.5ml 37
 . QIAprep miniprep kit (Qiagen Co, Valencia,
 CA, USA) plasmid DNA . DNA 2 µl
 EcoR Hind 0.8% gel (858 bp)
 (Fig. 3) automatic DNA sequencer
 (ALF express, Amhersham Pharmacia, Uppsala, Sweden) .

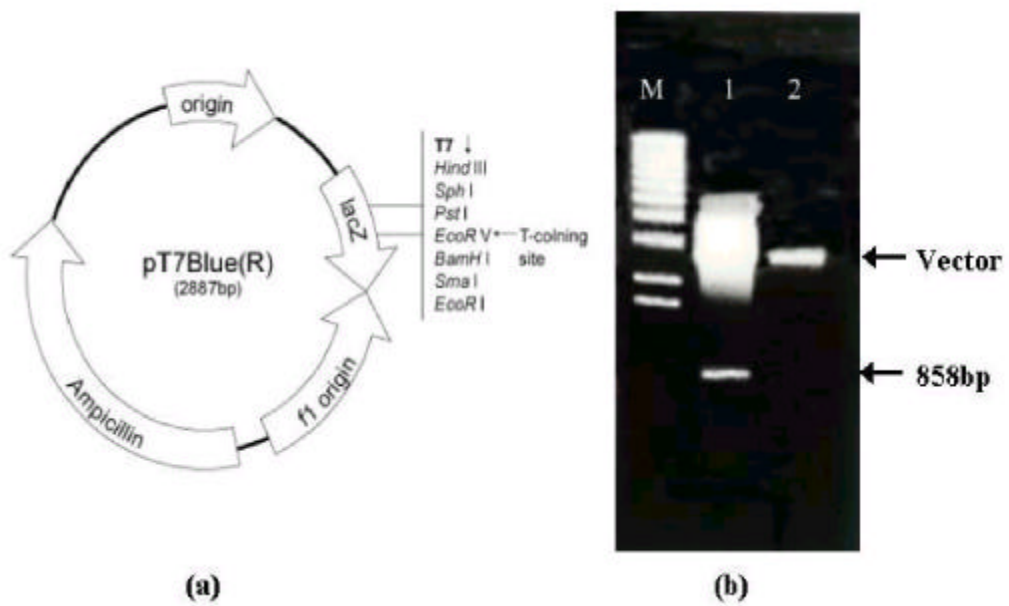


Fig. 3. Cloning of pT7Blue-IL-6 promoter plasmid

- (a) Map of pT7Blue T-vector
- (b) Restriction enzyme digestion of a recombinant clone
 M : 1Kb ladder, 1 : Recombinant clone, 2 : Vector only

. Site-directed mutagenesis

가 genomic DNA Quick Change Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) mutagenesis . PCR 10x reaction PCR buffer 5 µl, DNA template 5-50 ng, primer 125 ng (Table 3), dNTP mix 1 µl, PfuTurbo DNA polymerase (2.5 U/ µl) 1 µl Perkin Elmer DNA Thermocycler (Perkin Elmer, Norwalk, CT, USA) 95 30 가 95 30 , 55 1 , 68 7 , 18 PCR . DpnI , 37 1 supercoiled DNA digestion 1 µl DpnI DNA 50 µl Epicurian Coil XL1-Blue Supercomponent cell transformation . DNA premate kit (Bioneer, Taejeon, Korea) miniprep T7 primer(Table 4) automatic sequencer mutation . Mutation template plasmid DNA

Table 5 .

Table 3. Sequences of primers used for the confirmation of site-directed mutagenesis

Mut 810F	5'- GAG GGG TGT GTG GCC CAG GGA TGC GGG GCG CCA GCA GAG GCA GGC TC-3'
Mut 810L	5'- GAG CCT GCC TCT GCT GCT GGC GCC CCG CAT CCC TGG GCC ACA CAC CCC TC-3'
Mut 60F	5'- GCA CGA AAT TTG AGG ATG GCC AGG CAG TCT AC-3'
Mut 60L	5'- GTA GAC TGC CTG GCC ATC CTC AAA TTT CGT GC-3'

Table 4. Sequences of primers used for site-directed mutagenesis by manual sequencing

T7 promoter within pT7 blue vector	5'-TCT AAT ACG ACT CAC TAT AGG-3'
U-19 mer within pT7 blue vector	5'-GGT TTT CCC AGT ACG CGA CG-3'

Table 5. Sequences of mutated region compared with published IL-6 promoter(desired). See () and dark italic.

Region(bp)	Wild	Mutation	
50-55-60	5'-TGAGGATGGCC-3'	5'-TGAGG(G)TGGCC-3'	Substitution
806-810-816-818	5'-GGGATGCGGGGCG-3'	5'-GGGA (GGGCTG) GCGGG(- G)CG-3'	Insertion and Deletion

. Reporter gene constructs

plasmid DNA *Hind* Klenow dNTP
 blunt , *Bam*HI . Digestion DNA gel
 electrophoresis IL-6 promoter region (858 bp) DNA prepmate kit
 (Bioneer, Taejeon, Korea) gel extraction . *Sma*I *Bg*III
 digestion pGL3 Basic Luciferase vector ligation . Hanahan
 transformation colony plasmid *Hind*
*Kpn*I insert (Fig. 4).

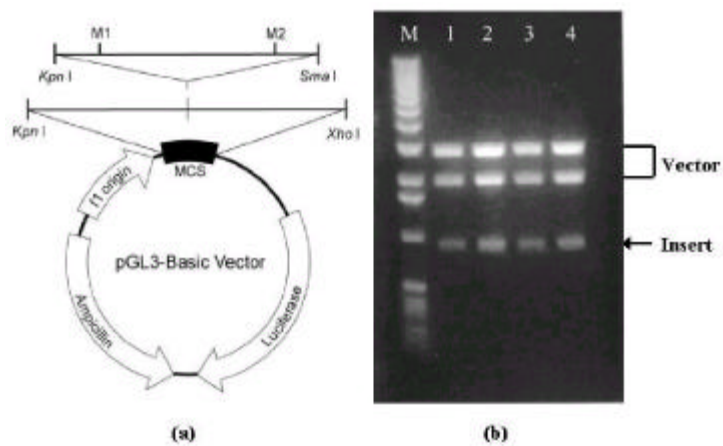


Fig. 4. Cloning of pGL3-Basic-IL-6 promoter plasmid

(a) Map of pGL3-Basic vector

(b) Restriction enzyme digestion of putative recombinant clones

M : 1Kb ladder, 1 : Wild type, 2 : M1, 3 : M2, 4 : M1 + M2

. Transient transfections

6 well tissue culture plate 5x10⁵ CV-1 cell(African green monkey kidney cell) HeLa cell 10% FCS DMEM medium(GIBCO BRL, Gaithersburg, MD, USA) 16 well wild mutation IL-6 promoter luciferase fusion pGL3 -IL-6 plasmid 0.6 μg -galactosidase 50 ng(CMV-gal) lipofectamin plus (GIBCO BRL, Gaithersburg, MD, USA) transfection transfection . 24 10 U/ml IL-1(R&D Systems Inc., Minneapolis, MN, USA) 10 μg/ml LPS (Sigma Co., St. Louis, MO, USA) 가 24 harvest luciferase assay kit (Promega, Madison, WI, USA) luciferase -galactosidase activity . Transfection 3

SAS program 가 IL-6 paired t-test CV-1 HeLa IL-1 LPS Kruskal-Wallis test . 95% .

1. IL-6

가 IL-6 136.9 pg/ml 가 가
 20.9 pg/ml (p<0.01). 5 pg/ml
 (Fig. 5).

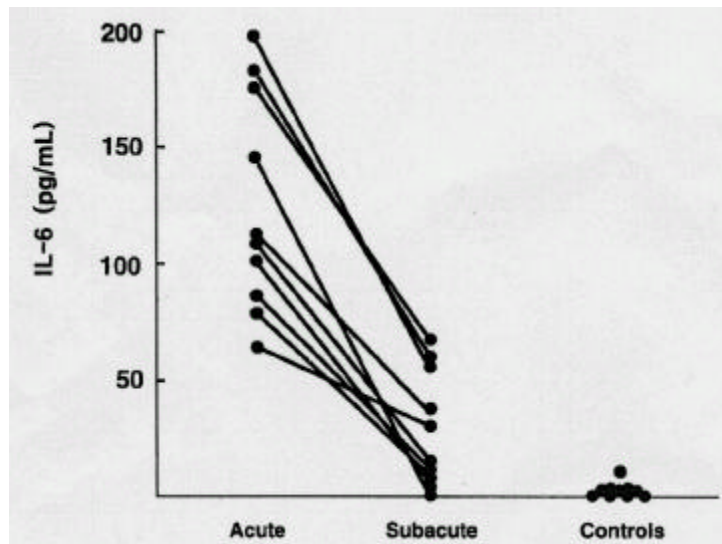


Fig. 5. Levels of serum interleukin-6 in serial samples isolated from the patients with Kawasaki disease during acute and subacute phase of illness and normal controls.

2. IL-6 promoter polymorphism

10 가 IL-6 promoter
 Fishman⁴⁴ -174bp G/C polymorphism

2 Fishman⁴⁴ -174 G/C polymorphism

IL-6 promoter

10 가 IL-6 promoter 858bp

mutation 가

IL-6 promoter wild type

7

-55bp A G substitution -810bp GGGCTG insert -816bp G deletion (Table 5).

, -66bp T insert, -142bp C insert, -579bp G deletion, -666bp C insert, -770bp GC insert

(Table 6).

Table 6. Sequences of other polymorphism compared with published IL-6 promoter(maybe). See () and dark italic.

Region(bp)	Wild	Mutation	
62- 66 -70	5'-GGCAGTCTA-3'	5'-GGCAG(T)TCTA-3'	Insertion
140- 142 -150	5'-AA T CCAGGCTT-3'	5'-AAT(C)CCAGGCTT-3'	Insertion
575- 579 -580	5'-ATGTGG-3'	5'-ATGT(G)G-3'	Deletion
662- 666 -670	5'-CGAGCCACC-3'	5'-CGAGC(C)CACC-3'	Insertion
765- 770 -774	5'-CCAGCCGGTC-3'	5'-CCAGCC(G)GGTC-3'	Insertion

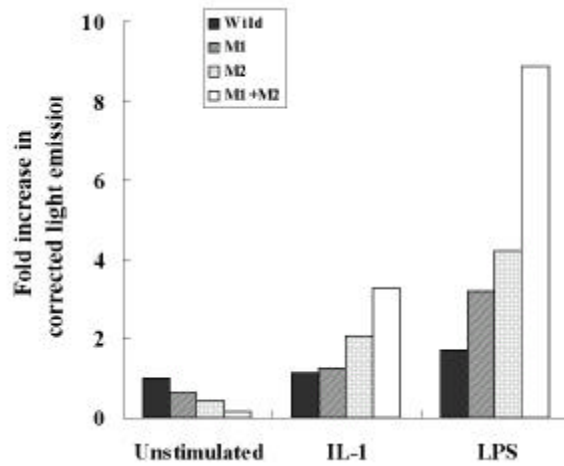


Fig. 6. Luciferase activities of CV-1 cell extracts transfected with wild type, M1, M2, M1+M2 constructs under various stimulated conditions. Results are the mean of three transfections, corrected for protein content and efficiency of transfection by β -galactosidase cotransfection.

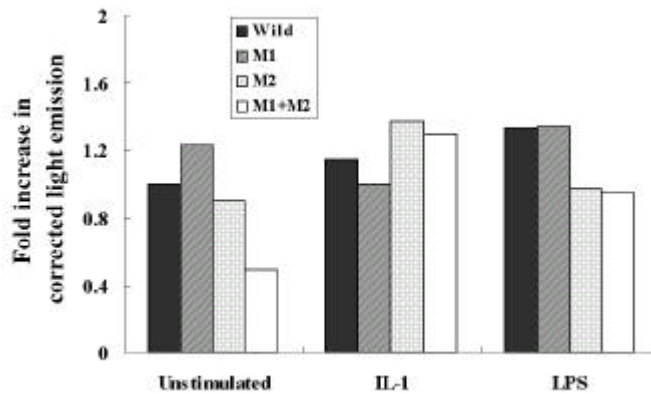


Fig. 7. Luciferase activities of HeLa cell extracts transfected with wild type, M1, M2, M1+M2 constructs under various stimulated conditions. Results are the mean of three transfections, corrected for protein content and efficiency of transfection by β -galactosidase cotransfection.

•

polymorphism

가 .

IL-1 promoter polymorphism 가 ,⁴⁶ IL-1 receptor antagonist

IL-1 polymorphism 가 .⁴⁷

mucocutaneous leishmaniasis -308 TNF- promoter polymorphism

polymorphism .^{48,49} TNF- .⁵⁰

IL-6 polymorphism 가 . Bowcock⁵¹

MspI *BglII* 5 exon 5' flanking region base 가

BstNI 9가 3'-flanking region

RFLP .⁵² IL-6

polymorphism

.⁵³ 57 9

IL-6 3' flanking region polymorphism

IL-6 가 가

. Tsukamoto⁵⁴ IL-6 locus CA repeat polymorphism

, , 가

IL-6 locus CA repeat polymorphism

.⁵⁵ Alzheimer IL-6 polymorphism

polymorphism 3' flanking region .⁵⁶ IL-6 , *MspI* *BglII* RFLP

.⁵⁷ 5' flanking region polymorphism

Ray ⁵⁸ -225 -164bp G/ C
 polymorphism reporter gene expression negative regulatory effect가
 . 5' flanking region polymorphism
 1998 Fishman ,
 IL-6 -174 G/ C
 polymorphism , polymorphism
 IL-6 가 가 , IL-6
 promoter polymorphism
 가 .
 가
 ,
 .
 IL-6가 가 , 가 가 IL-6
 , 가 IL-6가 가
 가 IL-6가 가
 Fishman ⁴⁴ IL-6 polymorphism
 가 가 .
 가
 IL-6 promoter polymorphism .
 Fishman ⁴⁴
 IL-6 promoter 가
 가 가
 가
 . 가 Bowcock ⁵¹
 -174 polymorphism Guarati Indians Afro-Caribbeans
 polymorphism .
 polymorphism TNF- polymorphism

Caucasian TNF- polymorphism
 South African blacks ⁵⁹ 가
⁶⁰
 가
 가 가 가
 가 가
 , 가 ⁶¹
 가
 가 ⁶²
 T
 , 가 가 가 가 가
 가
 ,
 (genetically predisposed) 가
⁶³
 IL-6 polymorphism 가
 가
 IL-6 가 , IL-6
 가
 IL-6
 wild type
 가 가

HLA-B*22:02가 ⁶³가 ⁶⁴ New England
 Israel Caucasian HLA-B*51
 가 ^{65,66} Kaslow ⁶⁷ HLA-A*2, B*44,
 CW5 가
 MHC class I MHC class II 가
⁶⁸
 가
 2.1% 0.19%
⁶⁹
 14.1% 13.3% , 가
⁷⁰
 가
 가 6 , IL-6 가 가 ⁵¹
 IL-6 가 가
 가 , 가
 mutation . 가
 mutation
 mutation IL-6
 A G substitution, -810bp GGGCTG insertion -816bp G
 deletion polymorphism IL-6 가
 , IL-6 가
 가

HeLa CV-1 가
 가
 가 CV-1 M1 M2
 가 IL-6
 IL-1 promoter IL-6 가
 M1 M2 , 가
 IL-6 가 LPS
 mutation repression
 promoter가 IL-1 LPS IL-6
 가 mutation
 가 가
 M1
 mutation wild type GCF-CS, T-Ag-SV40
 CTCF-RS, AP-2, APRT-mouse, Sp1 가
 M2 mutation T-Ag-SV40, CTCF-RS, PuF-RS
 가 Pamela ⁷¹ T-Ag-SV40 early RNA
 가 repressor
 AP-2 AP-2
 T-Ag-SV40 IL-6
 AP-2 Sp1 IL-1
 LPS IL-6
 가 가 가
 가
 LPS promoter IL-1
 IL-6 가 LPS가 NF B I
 B ⁷² LPS가 IL-6 promoter
 NF- B
 가

CV-1 HeLa IL-1 LPS 가
 IL-1 LPS 가 , HeLa
 CV-1 가 가 , IL-6 cytokine HeLa
 가 .
 , 가 IL-6
 polymorphism ,
 IL-6 polymorphism polymorphism
 IL-6 . Kawasaki disease가
 6 polymorphism
 가 .

•
 가 E-selectin, T 가 IL-6 가 , 가 가
 가 가 IL-2R 가
 가 IL-6 ,
 IL-6 가가 IL-6 polymorphism
 . 가 ,
 IL-6 ELISA kit
 genomic DNA IL-6 DNA
 5'-flanking region GenBank
 primer , PCR genomic DNA
 sequencing mutation site . mutation site
 site-directed mutagenesis reporter plasmid construction
 CV-1 cell transfection

1. 가 IL-6 136.9 pg/ ml 가 가
 20.9 pg/ ml (p<0.01). 5
 pg/ ml .
2. 가 IL-6 promoter
 -174bp G/ C polymorphism .
3. 가 IL-6 promoter
 가 .
4. IL-6 promoter wild type
 IL-6 promoter 7 .
 -55bp A G substitution -810bp GGGCTG

insert -816bp G deletion . ,
 -66bp T insert, -142bp C insert, -579bp G deletion,
 -666bp C insert, -770bp GC insert .
 5. -55bp G substitution , -810bp GGGCTG insert -816bp
 G deletion IL-6

, 가 IL-6
 promoter polymorphism ,
 polymorphism polymorphism
 IL-6 , IL-6
 가 .

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Abstract

**The polymorphisms in the promoter region
of the IL-6 gene of the patients with Kawasaki disease**

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During the acute phase, patients with Kawasaki disease(KD) demonstrate a drastical increase in serum interleukin-6 (IL-6) that parallels the duration of fever. To investigate the possibility that this cytokine profile results from a difference in the control of IL-6 expression, the promoter of the IL-6 gene was examined for polymorphisms.

Genomic DNAs from the patients with KD and normal controls were extracted from blood samples collected at our hospital. The promoter region was amplified by PCR and sequenced. We detected several interesting promoter polymorphisms: GGGCTG insertion at -810 bp and G deletion at -816 bp positions (M1), A to G substitution at -55 bp (M2) of the reported IL-6 gene promoter sequence. And other consistent sequence variations different from the reported sequence were also observed at several positions: -66 bp (T insertion), -142 bp (C insertion), -579 bp (T deletion),

-666 bp (C insertion), and -770 bp (GC insertion), which may suggest a sequencing error of the reported sequence. There were no significant differences in the nucleotide sequence between the KD patients and normal control groups.

By preparing IL-6 gene promoter-luciferase reporter fusion plasmid constructs [pGL3-IL-6-wild type, -M1, -M2, -(M1+M2)] and transient transfection assays in HeLa and CV-1 cells, we tested the functional significances of the polymorphisms (M1 and M2). Mutations at these positions significantly decreased luciferase expression ($p < 0.05$), suggesting the promoter elements at which the mutations were introduced are important in transcriptional activation of IL-6 gene. IL-1 and LPS potently stimulated luciferase expression on the wild type and mutant reporter constructs [M1, M2, (M1+M2)], suggesting that the mutation may affect the interaction with transcription factors critical in the transcription of IL-6.

These polymorphisms in IL-6 may be manifested in different pathophysiology or symptoms of disease believed to be related to IL-6.

Key words : Kawasaki disease, IL-6, promoter, polymorphism