□ 원 저 □

Association between Promoter Polymorphism (rs2243115, −570 T/G) of Interleukin 12A and Dyslipidemia and Obesity in Korean Population

College of Nursing, Sungshin Women's University, Seoul, Korea

Seung-Ae Yang

Interleukin 12 (IL12) is a key modulator of the immune function and is composed of IL12A and IL12B. To investigate the association between IL12A polymorphism and dyslipidemia, hypertension, and obesity in Korean population, a total of 263 subjects were analyzed. Single nucleotide polymorphism (SNP) (rs2243115, −570 T/G) in the promoter region of the IL12A gene was selected and genotyped by direct sequencing. SNPStats and SPSS 18.0 were used to obtain the odds ratio (OR), 95% confidence interval (CI), and p value. Multiple logistic regression models were performed for the analysis of genetic data. The rs2243115 SNP was associated with the triglyceride levels (p=0.040 in the codominant2 model and p=0.042 in the recessive model), HDL-cholesterol levels (p=0.003 in the codominant1 model, p=0.0017 in the dominant model, p=0.0018 in the log-additive model, and p=0.009 in allele frequencies), and overweigh/obesity (p=0.032 in the codominant1 model, p=0.017 in the dominant model, p=0.013 in the log-additive model, and p=0.019 in allele frequencies). These results suggest that IL12A promoter SNP (rs2243115, −570 T/G) may be associated with dyslipidemia and obesity in Korean population. (**Korean J Str Res 2012;20:133 ~138**)

Key Words: Dyslipidemia, Interleukin 12A, Obesity, Promoter, Single nucleotide polymorphism

INTRODUCTION

Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) (IL12A) is a subunit in 2 different cytokines, IL12 and IL35. IL12 is composed of IL12A and interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40) (IL12B). IL12A is important for the differentiation of both T helper 1 (Th1) and Th2 cells, and is required for the T-cell-independent induction of interferon gamma (IFNG). IL12 acts on T and natural killer (NK) cells, and is a key activator of both innate and acquired immunities against infectious agents and malignancies (Wolf *et al.,* 1994; Liu *et al.,* 2005). IL12 may mobilize NK cells of the secondary lymphoid tissues to mediate natural killing during immune reactions (Ferlazzo *et al.,* 2004). IL12 inhibits apoptosis induced by ultraviolet B (UVB) radiation, but not apoptosis induced by gamma irradiation. IL12 also reduces UVB-induced DNA damage and the number of sunburn cells in mouse skin (Schwarz *et al.,* 2002). They proposed that IL12 may be useful in preventing UV-induced skin cancer. IL12 activates through a transcription factor, signal transducer and activator of transcription 4 (STAT4). Nitric oxide synthase 2A (NOS2A/

Corresponding author: Seung-Ae Yang, College of Nursing, Sungshin Women's University, 76ga-gil, Dobong-ro, Kangbuk-gu, Seoul 136-742, Korea
Tel: +82-2-920-7728, E-mail: ewha63@sungshin.ac.kr
Received May 15, 2012, Revised June 10, 2012
Accepted June 30, 2012

NOS2) is required for the signaling processes of IL12A (Diefenbach *et al.,* 1999).

Navaglia *et al.*(2005) found that −504T/T homozygosity of IL12A was associated with noncardia gastric cancer, but not with intestinal metaplasia. They suggested that IL12A polymorphism may affect the final steps in gastric carcinogenesis in patients with *H. pylori* infection. Recently, some researchers have reported the relationship between single nucleotide polymorphisms (SNPs) of the IL12A gene and several diseases including hepatitis B (Pan *et al.,* 2011), asthma (Chen *et al.,* 2011), Graves' disease (Guo *et al.,* 2010), pulmonary tuberculosis (Wang *et al.,* 2010), hepatocellular carcinoma (Liu *et al.,* 2011), and cervical cancer (Chen *et al.,* 2009).

Considering a variety of biological and genetic actions of IL12A, we expect that IL12A SNP may contribute to the pathogenesis of adult diseases such as dyslipidemia, hypertension, and obesity. Therefore, we investigated the association between a common SNP (rs2243115, -570 T/G) in the promoter region of the IL12A gene and dyslipidemia, hypertension, and obesity in Korean population.

MATERIALS AND METHODS

1. Study subjects

In this study, a total of 263 subjects were analyzed. Subjects were recruited among participants that examined a general health check-up program. Subjects with severe diseases such as stroke, psychiatric disorders, and cancers were excluded. The biochemical characteristics of individuals were measured such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose levels. Body mass index (BMI, kg/m^2) was also calculated. Subjects were divided into subgroups in accordance with the biochemical features (TC; normal, ≤250 mg/dl and abnormal, >250 mg/dl) (TG; normal, ≤150 mg/dl and abnormal, >150 mg/dl) (HDL-C; normal, ≥45 mg/dl in male/ ≥50 mg/dl in female and abnormal, <45 mg/dl in male/<50 mg/dL in female) (fasting plasma glucose; normal, ≤126 mg/dl and abnormal, >126 mg/dL) (HBA1c; normal, ≤6.5% and abnormal, >6.5%) (hypertension; normal, <140 mmHg in systolic blood pressure (SBP)/<90 mmHg in diastolic blood pressure (DBP) and abnormal, ≥140 mmHg in SBP/≥90 mmHg in DBP). According to the classification of Korean Society for the Study of Obesity (underweight, BMI<18; normal, BMI 18 to <23; moderately obese, BMI 23 to<25; obesity I, BMI25 to <30; obesity II, BMI≥30), subjects were divided into two subgroups, the abnormal (overweight/obese) group (BMI≥23) and the normal group (18≤BMI<23). This study was approved by the ethics review committee of Medical Research Institute, School of Medicine, Kyung Hee University, Seoul, Republic of Korea. Informed consent was obtained from each individual. Our study was conducted according to the guidelines of the Helsinki Declaration.

2. SNP genotyping

Peripheral bloods of all subjects were collected in EDTA or heparin tube. Genomic DNAs were extracted by QIAamp® DNA mini kit (QIAGEN, Valencia, CA, USA). We selected a promoter SNP in the IL12A gene. Genotypes of the SNP (rs2243115, −570 T/G) were performed by direct sequencing (MACROGEN, Seoul, Republic of Korea). Polymerase chain reaction (PCR) was employed using the following primers: for rs2243115 (sense, 5'-CCTCCCAGGACTCTGTGTATTC-3'; antisense, 5'-CTGCCGACGTAGAGAGAGGAGT-3'; product size, 344 bp). Conditions of PCR were 35 cycles at 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec, and 1 cycle at 72°C for 5 min for the final extension reaction. SeqManII software (DNASTAR, Madison, WI, USA) was used to determine the genotyping.

3. Statistical analysis

SNPStats (http://bioinfo.iconcologia.net/index.php) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) were used to obtain the odds ratio (OR), 95% confidence interval (CI), and p value. Multiple logistic regression models (codominant1, codominant2, dominant, recessive, and log-additive models) were applied and age and gender as covariables were adjusted. When the numbers of subject were below 5, the p values were recalculated by Fisher's exact test. The p value with below 0.05 was considered significant.

RESULTS

The demographic and biochemical characteristics of subjects are

shown in Table 1. The age of subjects was 38.7±13.2 (mean±standard deviation) years and the study subjects comprised 135 male and 128 female. However, we did not analyze the TC, fasting plasma glucose, and HBA1c groups, because the numbers of those groups were insufficient. The genotype distributions of the three tested SNPs were consistent with the Hardy-Weinberg equilibrium (data not shown).

Firstly, we analyzed the correlation between TG and IL12A SNP (rs2243115, −570 T/G). Table 2 shows the genotype and allele frequencies of the examined SNP in two groups according to the TG levels (normal, ≤150 mg/dl and abnormal, >150 mg/dl). The promoter SNP rs2243115 was associated with the TG levels [Fisher's exact p=0.040 in codominant2 model (T/T vs G/G), Fisher's exact p=0.042 in recessive model (T/T+T/G vs G/G)]. The G allele frequency was higher in the abnormal (TG> 150 mg/dl) group (9.3%) than in the normal (TG≤150 mg/dl) group (4.5%), although the allele frequency did not show significant difference between two groups (p=0.06).

Secondly, Table 3 displays the genotype and allele frequencies of the examined SNP in two groups according to the HDL-C levels (normal, ≥45 mg/dl in male/≥50 mg/dl in female and abnormal, <45 mg/dl in male/<50 mg/dl in female). The promoter SNP rs2243115 was associated with the HDL-C levels [p=0.003, OR=4.12, 95% CI=1.63～10.40 in codominant1 model (T/T vs T/G), p=0.0017, OR=4.30, 95% CI=1.76～ 10.53 in dominant model (T/T vs T/G+G/G), p=0.0018, OR=3.66, 95% CI=1.63～8.23 in log-additive model (T/T vs T/G vs G/G)]. The allele frequency of rs2243115 showed significantly difference between two groups (p=0.009, OR=2.76, 95% CI=1.29～5.91).

Finally, Table 4 represents the genotype and allele frequencies of the examined SNP in two groups according to BMI (normal, 18≤BMI≤23 kg/m^2 and abnormal, BMI>23 kg/m^2). The promoter SNP rs2243115 was associated with BMI [p=0.032, OR=2.92, 95% CI=1.10～7.75 in codominant1 model (T/T vs T/G), p=0.0017, OR=3.12, 95% CI=1.19～8.16 in dominant model (T/T vs T/G+G/G), p=0.013, OR=3.08, 95% CI=1.20 ～7.87 in log-additive model (T/T vs T/G vs G/G)]. The allele frequency of rs2243115 was also associated with BMI (p=0.019,

Table 1. Demographic and biochemical characteristics of study subjects.

	Total subject	Normal	Abnormal
Total number (n)	263		
Age (mean±SD, year)	38.7±13.2		
TG		≤150 mg/dl	>150 mg/dl
n		209	54
HDL-C		≥45 mg/dl in male, ≥50 mg/dl in female	<45 mg/dl in male, <50 mg/dl in female
n		200	63
Hypertension		<140 mmHg in SBP or <90 mmHg in DBP	≥140 mmHg in SBP or ≥90 mmHg in DBP
n		216	47
BMI		18≤BMI≤23 kg/m^2	BMI>23 kg/m^2
n		125	121

n: number of subjects, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index.

Table 2. Genotype and allele frequencies of IL12A promoter SNP according to TG levels.

SNP		Type	TG≤150 mg/dl		TG>150 mg/dl		Model	OR (95% CI)	p	Fisher's exact p
			n	%	n	%				
rs2243115 −570 T/G	Genotype	T/T	190	90.9	46	85.2	Codominant1	1.60 (0.56～4.60)	0.38	
		T/G	19	9.1	6	11.1	Codominant2	NA (0.00～NA)	NA	0.040
		G/G	0	0.0	2	3.7	Dominant	2.04 (0.77～5.42)	0.16	
							Recessive	NA (0.00～NA)	NA	0.042
							Log-additive	2.24 (0.94～5.37)	0.07	
	Allele	T	399	95.5	98	90.7		1		
		G	19	4.5	10	9.3		2.14 (0.97～4.76)	0.06	

IL12A: interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35), SNP: single nucleotide polymorphism, TG: triglyceride, n: number of subjects, OR: odds ratio, CI: confidence interval, NA: not applicable.

Table 3. Genotype and allele frequencies of IL12A promoter SNP according to HDL-C levels.

SNP		Type	HDL-C≥45 mg/dl in male, ≥50 mg/dl in female		HDL-C<45 mg/dl in male, <50 mg/dl in female		Model	OR (95% CI)	p	Fisher's exact p
			n	%	n	%				
rs2243115	Genotype	T/T	185	92.5	51	81.0	Codominant1	4.12 (1.63～10.40)	0.003	
−570 T/G		T/G	14	7.0	11	17.5	Codominant2	7.16 (0.40～127.99)	0.18	0.39
		G/G	1	0.5	1	1.6	Dominant	4.30 (1.76～10.53)	0.0017	
							Recessive	5.83 (0.34～100.99)	0.25	0.42
							Log-additive	3.66 (1.63～8.23)	0.0018	
	Allele	T	384	96.0	113	89.7		1		
		G	16	4.0	13	10.3		2.76 (1.29～5.91)	0.009	

IL12A: interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35), SNP: single nucleotide polymorphism, n: number of subjects, HDL-C: high-density lipoprotein cholesterol, OR: odds ratio, CI: confidence interval.

Table 4. Genotype and allele frequencies of IL12A promoter SNP according to BMI index.

SNP		Type	Normal range 18≤BMI≤23 kg/m^2		Overweigh/obese BMI>23 kg/m^2		Model	OR (95% CI)	p	Fisher's exact p
			n	%	n	%				
rs2243115	Genotype	T/T	117	93.6	103	85.1	Codominant1	2.92 (1.10～7.75)	0.032	
−570 T/G		T/G	8	6.4	16	13.2	Codominant2	NA (0.00～NA)	NA	0.22
		G/G	0	0.0	2	1.6	Dominant	3.12 (1.19～8.16)	0.017	
							Recessive	NA (0.00～NA)	NA	0.24
							Log-additive	3.08 (1.20～7.87)	0.013	
	Allele	T	242	96.8	222	91.7		1		
		G	8	3.2	20	8.3		2.73 (1.18～6.31)	0.019	

IL12A: interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35), SNP: single nucleotide polymorphism, n: number of subjects, BMI: body mass index, OR: odds ratio, CI: confidence interval, NA: not applicable.

OR=2.73, 95% CI=1.18～6.31).

We evaluated the examined SNP in two groups according to the blood pressure levels. However, rs2243115 was not associated with hypertension (data not shown).

DISCUSSION

IL12, a proinflammatory cytokine, plays crucial roles that promotes the differentiation of Th1 cells, enhances the cytotoxic activity of NK cells, and induces the production of IFNG (Del Vecchio *et al.,* 2007). Some prior studies were investigated the relationship between IL12A polymorphisms and certain diseases including multiple sclerosis, pulmonary tuberculosis, and brain tumor (International Multiple Sclerosis Genetics Consortium, 2010; Wang *et al.,* 2010; Sima *et al.,* 2012). However, there was no study about the relationships between a promoter SNP of IL12A (rs2243115, −570 T/G) and adult diseases such as hypertriglyceridemia, hypercholesterolemia, hypertension, and obesity.

To our knowledge, we firstly investigated the relationships between a promoter SNP (rs2243115, −570 T/G) of IL12A and hypertriglyceridemia, hypercholesterolemia, hypertension, and obesity in Korean population. Our results revealed that rs2243115 SNP may be associated with hypertriglyceridemia, hypercholesterolemia, and obesity.

The G and T allele frequencies of rs2243115 have been reported to be 0.021 and 0.979 in European, 0.085 and 0.915 in Chinese, 0.064 and 0.936 in Japanese, and 0.075 and 0.925 in Sub-Saharan African, respectively. In this study (n=263), the G and T allele frequencies of rs2243115 were 0.06 and 0.94, which are most similar to those seen in Japanese.

To find whether the T/G alleles of rs2243115 (−570 T/G)

bind to the transcription factors, the online program AliBaba 2.1 (http://www.gene-regulation.com/pub/programs/alibaba2) was used. The T allele in rs2243115 has a CCAAT/enhancer-binding protein gamma (C/EBP gamma) binding site, while the G allele has not any transcription factor binding site. C/EBP gamma is a protein that is encoded by the CEBPG gene (Williams *et al.,* 1991). C/EBPs are a family of transcription factors, composed of six members called C/EBP alpha to C/EBP zeta. These proteins are found in hepatocytes, adipocytes, hematopoietic cells, spleen, kidney, brain, and many others organs. C/EBPs are involved in different cellular responses like in the control of cellular proliferation, growth and differentiation, metabolism, immunology, and many others. Interestingly, in the C/EBP family, alpha, beta and zeta have roles in normal adipocyte function and adipogenesis (Lacasa *et al.,* 2001; Díaz-Delfín *et al.,* 2012). In light of these actions, rs2243115 SNP may affect the energy and adipocyte metabolism.

In conclusion, we firstly observed that rs2243115 (−570 T/G) of IL12A was related to TG levels, HDL-C levels, and BMI. These results suggest that IL12A promoter SNP (rs2243115, −570 T/G) may be associated with dyslipidemia and obesity in Korean population.

REFERENCES

Chen T, Liang W, Gao L *et al.* (2011) Association of single nucleotide polymorphisms in interleukin 12 (IL-12A and -B) with asthma in a Chinese population. *Hum. Immunol.* 72:603-606.

Chen X, Han S, Wang S *et al.* (2009) Interactions of IL-12A and IL-12B polymorphisms on the risk of cervical cancer in Chinese women. *Clin. Cancer Res.* 15:400-405.

Del Vecchio M, Bajetta E, Canova S *et al.* (2007) Interleukin-12: biological properties and clinical application. *Clin. Cancer Res.* 13:4677-4685.

Diaz-Delfin J, Domingo P, Mateo MG *et al.* (2012) Effects of rilpivirine on human adipocyte differentiation, gene expression, and release of adipokines and cytokines. *Antimicrob. Agents Chemother.* 56:3369-3375.

Diefenbach A, Schindler H, Rollinghoff M *et al.* (1999) Requirement for type 2 NO synthase for IL-12 signaling in innate immunity. *Science* 284:951-955.

Ferlazzo G, Pack M, Thomas D *et al.* (2004) Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc. Natl. Acad. Sci. U. S. A.* 101:16606-16611.

Guo T, Yang S, Liu N *et al.* (2010) Association study of interleukin-12A gene polymorphisms with Graves' disease in two Chinese populations. *Clin. Endocrinol.* 74:125-129.

International Multiple Sclerosis Genetics Consortium (2010) IL12A, MPHOSPH9/CDK2AP1 and RGS1 are novel multiple sclerosis susceptibility loci. *Genes Immun.* 11:397-405.

Lacasa D, Garcia Dos Santos E, Giudicelli Y (2001) Site-specific control of rat preadipocyte adipose conversion by ovarian status: Possible involvement of CCAAT/enhancer-binding protein transcription factors. *Endocrine* 15:103-110.

Liu J, Cao S, Kim S *et al.* (2005) Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression. *Curr. Immunol. Rev.* 1:119-137.

Liu L, Xu Y, Liu Z *et al.* (2011) IL12 polymorphisms, HBV infection and risk of hepatocellular carcinoma in a high-risk Chinese population. *Int. J. Cancer* 128:1692-1696.

Navaglia F, Basso D, Zambon CF *et al.* (2005) Interleukin 12 gene polymorphisms enhance gastric cancer risk in H pylori infected individuals. *J. Med. Genet.* 42:503-510.

Pan L, Zhang W, Liang Z *et al.* (2011) Association between polymorphisms of the cytokine and cytokine receptor genes and immune response to hepatitis B vaccination in a Chinese Han population. *J. Med. Virol.* 84:26-33.

Sima X, Xu J, Li Q *et al.* (2012) Gene-gene interactions between interleukin-12A and interleukin-12B with the risk of brain tumor. *DNA Cell Biol.* 31:219-223.

Schwarz A, Stander S, Berneburg M *et al.* (2002) Interleukin-12 suppresses ultraviolet radiation-induced apoptosis by inducing DNA repair. *Nat. Cell Biol.* 4:26-31.

Wang J, Tang S, Shen H (2010) Association of genetic polymorphisms in the IL12-IFNG pathway with susceptibility to and prognosis of pulmonary tuberculosis in a Chinese population. *Eur. J. Clin. Microbiol. Infect. Dis.* 29:1291-1295.

Williams SC, Cantwell CA, Johnson PF (1991) A family of C/EBP-related proteins capable of forming covalently linked leucine zipper dimers in vitro. *Genes Dev.* 5:1553-1567.

Wolf SF, Sieburth D, Sypek J (1994) Interleukin 12: a key modulator of immune function. *Stem Cells* 12:154-168.

= 국문초록 =

비만은 여러 질환의 위험요소로 알려져 있으며, 최근 비만에 대한 후보 유전자의 단일염기다형성(single nucleotide polymorphism, SNP)에 관한 연구가 급격히 증가하고 있다. 인터루킨 12A는 전신 면역반응에 관여하며. 염증시 발현이 증가하는 싸이토카인 인터루킨 12의 구성물로 지방세포와 췌장 베타세포에 작용하여 체중 조절과 지방 대사의 조절에 관여한다. 본 연구의 목적은 이러한 IL12A 유전자의 다형성이 고지혈증, 고혈압 및 비만에 미치는 영향을 알아보고자, 263명의 실험 참여자를 분석하였다. IL12A 유전자의 프로모터(promoter)영역에서 1개(rs2243115, −570 T/G)의 SNP을 선정하고 염기서열 분석기를 이용하여 유전자형(genotype)을 분석하였다. SNPStats와 SPSS 18.0 소프트웨어를 이용하여 데이터 분석한 결과, rs2243115의 유전자형이 중성지방 혈중 농도와 관계가 있었다 (p=0.040 in codominant2 model, p=0.042 in recessive model). 그리고 rs2243115의 유전자형과 대립형질(allele)은 HDL-cholesterol의 혈중 농도와 관계가 있었다(p=0.003 in codominant1 model, p=0.0017 in dominant model, p=0.0018 in log-additive model, p=0.009 in allele frequencies). 또한 BMI로 환산한 과체중/비만도와 rs2243115의 유전자형과 대립형질 사이에도 연관이 확인되었다 (p=0.032 in codominant1 model, p=0.017 in dominant model, p=0.013 in log-additive model, p=0.019 in allele frequencies). 이러한 결과는 IL12A 유전자의 다형성이 이상지질혈증 및 비만 발생과 관련이 있음을 시사한다.

중심단어: 이상지질혈증, 인터루킨 12A, 비만, 단일염기다형성