

Lack of association between Single Nucleotide Polymorphism (rs1400986) in Interleukin-20 Gene and Chronic Hepatitis B Virus Infection

F.S. Mirfakhhar (MSc)¹, S.R. Mohebbi (PhD) *¹, S.M. Hosseini (PhD)², P. Azimzadeh (MSc)³,
Sh. Derakhshani (MSc)⁴, M.R. Sarbazi (MD)⁵, A. Sharifian (MD)¹, M.R. Zali (MD)¹

1. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

2. Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

3. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

4. Foodborne and Waterborne Diseases Research Center, Gastroenterology and Liver Diseases Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

5. Department of Disease Control and Prevention, Deputy of Health, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran.

J Babol Univ Med Sci; 19(4); Apr 2017; PP: 28-35

Received: Sep 13th 2016, Revised: Nov 26th 2016, Accepted: Feb 22th 2017.

ABSTRACT

BACKGROUND AND OBJECTIVE: Infection with hepatitis B virus is a major cause of chronic liver diseases and variations within cytokine genes may affect the host immune response to hepatitis B infection. This study was designed to investigate whether IL-20 rs1400986 C/T single nucleotide polymorphism is involved in the progression of chronic hepatitis B infection.

METHODS: In this case-control study a total of 150 chronic hepatitis B patients (Anti-HBc Ab positive and HBsAg positive for more than 6 months) and 146 healthy subjects (Anti-HBc Ab and HBsAg negative) among people who were referred to Tehran Taleghani hospital during 2003 to 2005, were examined for differences in genotype and allele frequencies in this case-control study. Polymerase Chain Reaction- Restriction Fragment length Polymorphism (PCR-RFLP) method was applied for analyzing the polymorphism site.

FINDINGS: Genotypes Frequency in patients group were CC 32.0% and TT 4.0% in comparison to CT 28.1% and TT 2.7% in control group; however no statistically significant difference was observed in the frequency of IL-20 gene polymorphism (rs1400986) between chronically infected patients and healthy controls for neither allele ($P=0.549$) nor genotype ($p=0.599$) frequencies.

CONCLUSION: No association was detected between rs1400986 single nucleotide polymorphism within IL-20 gene and chronic hepatitis B infection; thus, this polymorphism appears to have no influence on susceptibility to chronic hepatitis B.

KEY WORDS: Cytokine, Hepatitis B virus, Polymorphism, Single nucleotide, Interleukin 20.

Please cite this article as follows:

Mirfakhhar FS, Mohebbi SR, Hosseini SM, Azimzadeh P, Derakhshani Sh, Sarbazi MR, Sharifian A, Zali MR. Lack of association between Single Nucleotide Polymorphism (rs1400986) in Interleukin-20 Gene and Chronic Hepatitis B Virus Infection. J Babol Univ Med Sci. 2017;19(4):28-35.

* Corresponding author: S.R. Mohebbi (PhD)

Address: Gastroenterology and Liver Diseases Research Institute, Ayatollah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

Tel: +98 21 22432525

E-mail: sr.mohebbi@sbum.ac.ir

Introduction

Hepatitis B is one of the most common chronic infections in the world. It is estimated that 2 billion people worldwide are infected with hepatitis B (HBV), which may be a large group of people lose their life as a result of complications caused by chronic infection with HBV, including cirrhosis and liver cancer (1,2). HBV, is a member of the Hepadna viridae family is cause of acute or chronic Hepatitis B. When infected with HBV, patients may develop chronic infection, which can result in several stages. Most adults with acute hepatitis B, the virus is cleared from the body by itself; while most infants and children, there is a high probability of progression to chronic infection (1,3).

Mechanisms that clearing the virus or survive infection in patients is yet unknown; however, it seems that the difference in clinical outcome of disease is associated with major factors of host (such as age, gender and ability and response of the immune system person).

It seems that the Human Genome Diversity affects the individual immunological status (3). In case of infection with the hepatitis B, induced inflammatory response causes regeneration and tissue repair in acute phase and prolonged liver damage (due to poorly designed responses of CD8+ and CD4+T cells) ends to fibrosis, cirrhosis and to eventually liver cancer (5 and 4). These inflammatory processes strongly influenced by balance between pro- inflammatory cytokines and anti-inflammatory cytokines (6,7).

In patients with chronic hepatitis B the ratio of Th2 to Th1 is dominant, which leads to increased production of anti-inflammatory cytokines. IL-20 is a primary pro-inflammatory cytokine belonged to IL-10 cytokine family. The region of gene cluster that encodes this interleukin is 195 kb on 1q32 chromosome, and has 28% similarity with human IL-10 gene (5, 8, 9). IL-20 causes the balance of Th1/Th2 and acts with shared IL-10 receptors (10). The signaling of this interleukin occurs by 2 type receptor complex IL-20R1/IL20-R2 and IL-22R1/ IL-22R2 (10) and STAT3 is as a key oppressed transcription factor (5, 8,10,11). IL-20 is produced mainly by stimulating

myeloid cells and epithelial cells (6 and 9). Overexpression of IL-20 in transgenic mice causes skin lesions similar to psoriasis (6).

IL-20 plays role in inflammation, angiogenesis, atherogenesis and chemotaxis, and all factors are involved in the pathogenesis of many inflammatory diseases and autoimmune like Atherosclerosis, psoriasis, rheumatoid arthritis, inflammatory bowel disease (IBD) and lupus nephritis, and is a factor of maintaining the integrity of epithelial tissues during inflammatory responses (9 and 12-15). IL-20 induces production of IL-6 and TNF in monocytes also stimulates proto-oncogene tyrosine kinase ROS in CD8+T cells. This IL targets, keratinocytes, endothelial cells, mesenchymal cells and a variety of tumor cells (16, 17).

This IL highly expresses on hepatocytes of damaged liver tissues in patients with fibrosis, cirrhosis and liver cancer. Single nucleotide polymorphisms in the genes of cytokines and their receptors genes can affect their performance through changing in gene expression, mRNA stability and protein structure (2, 17).

In several studies to date have been trying to determine association between nucleotide changes in different genes with certain diseases and their impact on disease progression and response to treatment (2, 18-20).

The importance and correlation of genetic changes and in particular single nucleotide polymorphism in genes that involved in immune response and cytokine genes with different diseases, including cancers (21,22) inflammatory diseases (23) and viral diseases (24-26) Have been evaluated and new studies are in progress to determine the effect of host genetic factors on the pathogenesis and persistence of hepatitis B infection and to develop new drugs and diagnostic and therapeutic procedures (27).

The results of several studies proved that single nucleotide polymorphisms in genes could affect the susceptibility to chronic infection (29,28) or clearing the hepatitis B virus from the body by the immune response (30,31) or progression of disease (32). So checking on the different polymorphisms in various

genes and their association with disease has become more valuable. The aim of this study was to evaluate the relationship and the role of single nucleotide polymorphism rs1400986 C/T of IL-20 gene in susceptibility to chronic hepatitis B infection.

Methods

This study was cross-sectional and case-control study. After approval by the Digestive Disease Research Center of Shahid Beheshti University of Medical Sciences, a total of 296 healthy volunteers and patients with chronic hepatitis B among patients referred to Taleghani Hospital in Tehran during the years 1392 to 1394 were selected for this study, including 150 chronic hepatitis B patients with positive HBsAg ELISA and AntiHBc-Ab (ELISA kit bioprobes srl Diapro diagnostics, Italy) for more than 6 months (33), and blood samples were also collected as control group from 146 individuals who were healthy (serum HBsAg negative and AntiHBc Ab negative).

This project approved by the Digestive Disease Research Center of Shahid Beheshti University of Medical Sciences and has approved project code (code 719) and written consent was received from all participants. Genomic DNA from 5 ml blood in EDTA-containing glass collected and extracted through the standard method of saturated salt was according to the instructions of Miller and colleagues (34). IL-20 gene rs1400986 polymorphism were selected from information contained in GenBank and genotyped by PCR-RFLP method.

In summary, by using software Gene Runner version 4 primers were designed for amplification of a region of the gene polymorphisms. PCR reaction mixture with a final volume of 25 ml, containing 2.5 ml of 10X PCR Buffer with Mg²⁺, 0.5 mM dNTP, 10 pmol of each specific primers and 1.5 unit Taq polymerase enzyme was ready. Forward primer sequences 5'CTGGCAGTAGGCTTGTATGAAATC3' and reverse primer 5'CCACGACCTGTGCCACCAA3' region of the genome that contains the position of the

polymorphism (C/T) 1807 to replicate-out. PCR reaction by adding 100 ng of DNA template to the reaction mixture and heat cycles were applied as follows:

Denaturation at 94 °C for 10 min, with 35 cycles of 94 °C for 45 seconds, connecting at a temperature of 61 °C for 40 seconds and elongation at 72 °C for 45 seconds, followed by a long final at 72 °C for 10 minutes.

PCR product by restriction enzyme Eco130 I (StyI) was digested with an enzyme recognition sites for 5'C ▼ CWWGG3 ' and TT genotype is not recognized by the enzyme and were not cut, and thus made piece of 437 nucleotides, but CC cutting genotype and caused two pieces of 278 and 179 bp and the CT heterozygous genotype created 3 bands in sizes 437, 278 and 159 bp on 3% agarose gel. Significant differences obtained in the genotype and allele frequencies between the two groups by chi-square test (2χ), respectively.

For data analysis, SPSS version 22 was used. P value less than 0.05 was considered significant. Odds Ratio was calculated at 95%.

Results

C to T nucleotide substitution in the 2 groups of patients and controls were observed with low frequency (in patients (32%) 48 people CT and (4%) 6 people TT, (28.1%) 41 people CT and (2.7%) 4 people TT in the control group).

However, no significant differences in allele and genotype distribution between the 2 groups (respectively p=0.549 and p=0.599). TT homozygous genotype with less frequently in patients (4% vs. 2.7%) and thus a higher frequency of CC homozygous genotype was observed in the control group (69.2% vs. 64%).

96% of patients were C allele carriers while in the control group the 97.3% were C allele carriers which are provided in table 1. CT heterozygote genotype in patients with chronic hepatitis B had the dominant genotype (32% vs 28.1%, p=0.416).

Table 1. Genotype and allele frequency of rs1400986 polymorphism in the IL-20 gene of patients with chronic hepatitis B compared with healthy control

Genotype	Chronic Hepatitis B N=150 (%)	Healthy control N=146 (%)	P-value	OR (CI-95%)
CC	96(64)	101(69.2)	-	-
CT	48(32)	41(28.1)	0.416	0.812(0.492-1.341)
TT	6(4)	4(2.7)	0.490	0.634(0.173-2.315)
Allele				
C	144(96)	142(97.3)	-	-
T	6(4)	4(2.7)	0.551	0.676(0.187-2.447)

Discussion

In this study rs1400986 genotype and allele polymorphism frequency of IL-20 gene in patients with chronic hepatitis B compared with healthy control group and no significant differences were observed. IL-20 is known as an adjustment factor of the inflammatory response. During infection, this interleukin acted on epithelial cells and improves cohesion of tissues and heals the wounds; hence it can be used as a potential marker (9).

Chiu et al (2014) to prove the therapeutic role of IL-20 in the treatment of liver fibrosis, tested its effect on liver injury induced by carbon tetrachloride in a mouse model. Based on the results, the IL-20 in mice with liver injury were significantly higher (16). Although few studies of the IL-20 gene single nucleotide polymorphism has been released however, numerous articles have been reported statistically significant relationship between polymorphisms of this gene and some inflammatory diseases (35-39).

Fife et al (2006) showed an association between rs1400986 single nucleotide polymorphism in IL-20 gene with juvenile idiopathic and also found a significant association between the disease and the haplotype IL10 rs1800896 A / IL20 rs1400986 T (40). In another study, rs1400986 polymorphisms of interleukin-20 gene by clearing hepatitis B infection in populations of European Americans was read. Based on the results of that study people who have cleared the virus from the body without treatment, are more likely to have CC genotype at polymorphism position (17). On the Iranian population Arbabi and co-workers

(2013), reported another single nucleotide polymorphisms of interleukin-20 gene (rs1518108) associated with hepatitis C infection (35).

Traks et al study (2008) showed of IL-20 and IL-24 haplotypes associated with major depressive disorder and said they are involved in the pathogenesis of the disease (41). Another study worked on the relationship between IL-20 gene Polymorphisms and psoriasis and excessive proliferation of keratinocytes (13 and 42-47). The effect of IL-20 gene polymorphism and chronic hepatitis B, IL-20 gene single nucleotide polymorphism rs1400986 in this case-control study studied.

The results showed that this single nucleotide polymorphisms statistically is not associated with chronic Hepatitis B in the study population. Despite significant differences in frequencies of genotypes and alleles of the patient and control groups was not observed. A possible explanation for this result could be probed in the difference in IL-20 gene variations and heterogeneity population genetics. Limited data population, luck factor and the difference in analysis parameters can be considered as other reasons to justify such ambivalence.

The importance of studies such as these, is the practical implications of their. It is suggested that these studies, which are examined as variations of the genes of the immune system for communicating with infectious diseases or inflammatory diseases or are considered as potential biomarkers for prognosis of the disease, are completed with other similar studies on gene expression or proteomics. To investigate the

relationship between IL-20 gene single nucleotide polymorphism with multifactorial diseases such as hepatitis B haplotype analysis is also recommended. Prediction of Chronic hepatitis B potential can lead new therapeutic strategies or "personal care", which can provide a specific treatment for each person. Our results indicate that there is no association between rs1400986 polymorphisms of interleukin-20 gene with chronic hepatitis B and this polymorphism cannot be

considered as a determining factor in the chronic disease in the study population.

Acknowledgments

Hereby, we would like to thank the personnel of the Gastroenterology and Hepatology Research Center, specially Mrs F Jabarian and Mr. M Tloui Moghadam for support of this research.

References

1. Liver eafstot. Easl clinical practice guidelines: management of chronic hepatitis B virus infection. *J Hepatol*. 2012;57(1):167-85.
2. Conde SR, Feitosa RN, Freitas FB, Hermes RB, Demachki S, Araújo MT, et al. Association of cytokine gene polymorphisms and serum concentrations with the outcome of chronic hepatitis B. *Cytokine*. 2013;61(3):940-4.
3. Dandri M, Locarnini S. New insight in the pathobiology of hepatitis B virus infection. *Gut*. 2012;61(1): 6-17.
4. Koziel MJ. Cytokines in viral hepatitis. *Semin Liver Dis*. 1999;19(2):157-69.
5. Sabat R. IL-10 family of cytokines. *Cytokine Growth Factor Rev*. 2010;21(5):315-24.
6. Blumberg H, Conklin D, Xu W, Grossmann A, Brender T, Carollo S, et al. Interleukin 20: discovery, receptor identification, and role in epidermal function. *Cell*. 2001;104(1):9-19.
7. Oral HB, Kotenko SV, Yılmaz M, Mani O, Zumkehr J, Blaser K, et al. Regulation of T cells and cytokines by the interleukin-10 (IL-10)-family cytokines IL-19, IL-20, IL-22, IL-24 and IL-26. *Eur J Immunol*. 2006;36(2):380-8.
8. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. *J Allerg Clin Immunol*. 2008;121(5):1108-11.
9. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol*. 2011;29:71-109.
10. Wegenka UM. IL-20: biological functions mediated through two types of receptor complexes. *Cytokine Growth Factor Rev*. 2010;21(5):353-63.
11. Fickenscher H, Hör S, Küpers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. *Trends Immunol*. 2002;23(2):89-96.
12. Hsu YH, Li HH, Hsieh MY, Liu MF, Huang KY, Chin LS, et al. Function of interleukin-20 as a proinflammatory molecule in rheumatoid and experimental arthritis. *Arthritis Rheum*. 2006;54(9):2722-33.
13. Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, et al. The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol*. 2007;178(4):2229-40.
14. Hsieh M-Y, Chen W-Y, Jiang M-J, Cheng B-C, Huang T-Y, Chang M-S. Interleukin-20 promotes angiogenesis in a direct and indirect manner. *Gen Immun*. 2006;7(3):234-42.
15. Wu J, Wang G, Hao J, Gong W, Wang J, Zhao J, et al. The correlation between IL-20 and the Th2 immune response in human asthma. *Asia Pacific J Allergy Immunol*. 2014;32(4):DOI 10.12932/AP0447. 32.4. 2014.
16. Chiu YS, Wei CC, Lin YJ, Hsu YH, Chang MS. IL-20 and IL-20R1 antibodies protect against liver fibrosis. *Hepatol*. 2014;60(3):1003-14.
17. Truelove AL, Oleksyk TK, Shrestha S, Thio CL, Goedert JJ, Donfield SM, et al. Evaluation of IL10, IL19 and IL20 gene polymorphisms and chronic hepatitis B infection outcome. *Int J Immunogenet*. 2008;35(3):255-64.
18. Azimzadeh P, Romani S, Mirtalebi H, Reza Fatemi S, Kazemian S, Khanyaghma M, et al. Association of co-stimulatory human B-lymphocyte antigen B7-2 (CD86) gene polymorphism with colorectal cancer risk. *Gastroenterol Hepatol Bed Bench*. 2013;6(2):86-91.
19. Wei C-C, Hsu Y-H, Li H-H, Wang Y-C, Hsieh M-Y, Chen W-Y, et al. IL-20: biological functions and clinical implications. *J Biomed Sci*. 2006;13(5):601-12.
20. Wang S, Huang D, Sun S, Ma W, Zhen Q. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis B to interferon alfa. *Virol J*. 2011;8(28):1-6.
21. Najjar Sadeghi R, Sahba N, Vahedi M, Mohebbi SR, Zali MR. Association of intron and exon polymorphisms of p53 gene in Iranian patients with gastritis. *Gastroenterol Hepatol Bed Bench* 2013;6(1):545-51.

- 22.Savabkar S, Azimzadeh P, Chaleshi V, Nazemalhosseini Mojarad E, Asadzadeh Aghdaei H. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with gastric cancer. *Gastroenterol Hepatol Bed Bench*. 2013;6(4):176-82.
- 23.Habibi M, Naderi N, Farnood A, Balaii H, Dadaei T, Almasi S, et al. Association between two single base polymorphisms of intercellular adhesion molecule 1 gene and inflammatory bowel disease. *Gastroenterol Hepatol Bed Bench*. 2016;6(2):87-93.
- 24.Arkani M, Karimi K, Safaei A, Vahedi M, Mohebi S, Fatemi S, et al. Association of IGFBP3 Gene (rs2854744) Polymorphism and Colorectal Cancer. *J Babol Univ Med Sci*. 2012;14(3):46-51.[InPersian].
- 25.Azimzadeh P, Mohebbi S, Romani S, Kazemian S, irtalebi H, Vahedi M, et al. Role of TGF- β 1 Codon 10 Polymorphism in Chronic Hepatitis C Patients. *J Babol Univ Med Sci*. 2011;13(4):26-33.[In Persian].
- 26.Haghighi, M, Mohebbi S, Pour Hoseingholi, MA, Taleghani, MY, Fatemi S, Zali M. Association of Allele 61888G>T Polymorphism VDR Gene and Colorectal Cancer. *J Babol Univ Med Sci*. 2010;12(1):24-8.[In Persian].
- 27.Thompson P, Blumberg H, Chandrasekher YA, Novak JE. Methods of treatment using anti-IL-20 antibodies. Google Patents; 2013.
- 28.Khanizadeh S, Ravanshad M, Mohebbi SR, Naghoosi H, Tahaei ME, Mousavi Nasab SD, et al. Polymorphisms within the Promoter Region of the Gamma Interferon (IFN- γ) Receptor1 Gene are Associated with the Susceptibility to Chronic HBV Infection in an Iranian Population. *Hepat Mon*. 2012;12(11):e7283.
- 29.Behelgard A, Hosseini SM, Mohebbi SR, Azimzadeh P, Derakhshani S, Karimi K, et al. A Study on Genetic Association of Interleukin-16 Single Nucleotide Polymorphism (rs1131445) With Chronic Hepatitis B Virus Infection in Iranian Patients. *Jundishapur J Microbiol*. 2015;8(11):e23411.
- 30.Kazemi Arababadi M, Pourfathollah A, Jafarzadeh A, Hassanshahi GH, Rezvani M. Exon 9 of Vitamin D Receptor Association with Occult Hepatitis B Virus Infection. *Journal of Babol University Of Medical Sciences*. 2009;11(4):19-24.
- 31.Jiang X, Su K, Tao J, Fan R, Xu Y, Han H, et al. Association of STAT4 polymorphisms with hepatitis B virus infection and clearance in Chinese Han population. *Amino Acids*. 2016;48(11):2589-98.
- 32.Komatsu H, Murakami J, Inui A, Tsunoda T, Sogo T, Fujisawa T. Association between single-nucleotide polymorphisms and early spontaneous hepatitis B virus e antigen seroconversion in children. *BMC Research Notes*. 2014;7(1):789.
- 33.Song JE, Kim DY. Diagnosis of hepatitis B. *Annals of Translational Medicine*. 2016;4(18).
- 34.Miller SA ea. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
- 35.Arbabi-Aval E, Mohebbi SR, Rostami F, Habibi M, Vahedi M, Almasi S, et al. Study of Association between Interleukin 20 Polymorphism (Rs1518108) and Chronic Hepatitis C Infection. *Zahedan Journal of Research in Medical Sciences*. 2013;15(7):35-8.
- 36.Chang M-s. Use of IL-20 Antagonists for Treating Liver Diseases. US Patent 20,140,120,094; 2014.
- 37.Chang M-S. Treatment of Osteoarthritis Using IL-20 Antagonists. Google Patents; 2013.
- 38.Fonseca-Camarillo G, Furuzawa-Carballeda J, Llorente L, Yamamoto-Furusho JK. IL-10—and IL-20—Expressing Epithelial and Inflammatory Cells are Increased in Patients with Ulcerative Colitis. *Journal of clinical immunology*. 2013;33(3):640-8.
- 39.Kragstrup TW, Otkjaer K, Holm C, Jørgensen A, Hokland M, Iversen L, et al. The expression of IL-20 and IL-24 and their shared receptors are increased in rheumatoid arthritis and spondyloarthropathy. *Cytokine*. 2008;41(1):16-23.
- 40.Fife MS, Gutierrez A, Ogilvie EM, Stock CJ, Samuel JM, Thomson W, et al. Novel IL10 gene family associations with systemic juvenile idiopathic arthritis. *Arthritis research & therapy*. 2006;8(5):R148.

- 41.Traks T, Koido K, Eller T, Maron E, Kingo K, Vasar V, et al. Polymorphisms in the interleukin-10 gene cluster are possibly involved in the increased risk for major depressive disorder. *BMC medical genetics*. 2008;9(1):111.
- 42.Kunz S, Wolk K, Witte E, Witte K, Doecke WD, Volk HD, et al. Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs. *Experimental dermatology*. 2006;15(12):991-1004.
- 43.Wolk K, Haugen HS, Xu W, Witte E, Waggle K, Anderson M, et al. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN- γ are not. *Journal of molecular medicine*. 2009;87(5):523-36.
- 44.Kingo K, Koks S, Nikopensius T, Silm H, Vasar E. Polymorphisms in the interleukin-20 gene: relationships to plaque-type psoriasis. *Genes and immunity*. 2004;5(2):117-21.
- 45.Koks S, Kingo K, Vabrit K, Rätsep R, Karelson M, Silm H, et al. Possible relations between the polymorphisms of the cytokines IL-19, IL-20 and IL-24 and plaque-type psoriasis. *Genes and immunity*. 2005;6(5):407-15.
- 46.Otkjaer K, Kragballe K, Johansen C, Funding AT, Just H, Jensen UB, et al. IL-20 gene expression is induced by IL-1 β through mitogen-activated protein kinase and NF- κ B-dependent mechanisms. *Journal of Investigative Dermatology*. 2007;127(6):1326-36.
- 47.Rich BE, Kupper TS. Cytokines: IL-20—a new effector in skin inflammation. *Current Biology*. 2001;11(13):R531-R4.