Interleukin-10 family cytokines pathway: genetic variants and psoriasis

E. Galimova[1,2,*], R. Rätsep[2], T. Traks[3], K. Kingo[3], V. Escott-Price[4] and S. Kõks[2,5]

[1]Department of Physiology, University of Tartu, Tartu, Estonia

[2]*Institute of Biochemistry and Genetics, Ufa Scientific Center of Russian Academy of Sciences, Ufa, Russia*

[3] *Department of Dermatology, University of Tartu, Tartu, Estonia*

[4] *MRC Centre for Neuropsychiatric Genetics & Genomics, Cardiff University, Cardiff, UK*

[5] *Department of Pathophysiology, University of Tartu, Tartu, Estonia*

Correspondence

Elvira Galimova

E-mail: elya-4@yandex.ru

Summary

Background Interleukin (IL)-10 family cytokines IL-10, IL-19, IL-20, and IL-24 have been implicated in autoimmune diseases and we have previously reported that genetic variants in *IL10* gene cluster were associated with psoriasis.

Objective To analyze the relationship of genetic polymorphisms in the *IL10* gene cluster with psoriasis. This study also explores whether there are gene–gene interactions among these genetic polymorphisms.

Methods A total of 377 patients with psoriasis and 403 matched healthy controls were enrolled to carry out a case-control study for 48 SNPs of *IL10* gene cluster. Genotyping for the SNPs was conducted on the Applied Biosystems 3730 DNA Analyzer using SNPlex™ technology. Generalized multifactor dimensionality reduction (GMDR) analysis was applied to discover likely gene–gene interaction model among the SNPs.

Results The results showed that the alleles distributions of *IL10* gene cluster SNPs are significantly different between case and control groups. Carriers of *IL10* T allele (rs1554286) and of *IL20* T allele (rs1400986) conferred protection to psoriasis (OR = 0.63, *Pc* = 0.007; OR = 0.62, *Pc* = 0.038, respectively). GMDR analysis displayed a significant gene-gene interaction between *IL10* (rs1554286) and *IL20* (rs1518108) variants. The strongest protective effect was found with the block 1 haplotype ACATA in the *IL10* gene (*Pc* = 0.004).

Conclusions The novel finding of the present study is gene-gene interaction of the IL-10 pathway on the reduced risk of psoriais. Our results indicate that genetic variants of the immunomodulatory *IL10* and *IL20* genes may protective effect in the Europeans from Russia. Future studies are needed to confirm the results and find the possible functional explanation.

Keywords: cytokine; gene; psoriasis; single-nucleotide polymorphism

What's already known about this topic?

- Psoriasis is one of the most prevalent chronic inflammatory disorders caused by an interplay of genetic factors and the environment on the background of dysregulated immune system
- One unifying hypothesis of psoriasis pathophysiology is the cytokine network model. In this model either an endogenous stimulus such as HIV-1, neuropeptides, and medications, or an exogenous stimulus such as trauma, are represented as triggering a plexus of cellular events by inciting a cascade of cytokines

What does this study add?

- Our preliminary data suggest that two polymorphisms rs1554286 and rs1400986 located in *IL10* gene cluster related to inflammatory and immunity processes showed an association with protection to psoriasis.
- Our finding suggest evidence for a two-locus interaction between the *IL10* (rs1554286) and *IL20* (rs1518108) variants in the risk of psoriasis, and highlight further the importance of multilocus effects in the genetic component of psoriasis.
- Haplotype analysis revealed an association of *IL10* haplotype ACATA with a reduced risk for psoriasis.

The molecular basis of the pathogenesis of psoriasis, the chronic inflammatory skin disease, remains unclear, but principal clinical features of psoriasis - proliferation and

abnormal differentiation of epidermal keratinocytes, the growth and dilation of blood vessels and the infiltration of leukocytes into the dermis and epidermis, appear to be driven mainly by various cytokines and chemokines released by the activated T-cell population. [1]

Each inflammatory pathway IL-12/Th1, IL-23/Th17 and IL22/Th22 has its impact on psoriasis pathogenesis. Aberrant cytokine expression has been proposed as an underlying cause of the disease. During the past few years, the IL-10 family cytokines have been shown as the key cytokines involved in psoriasis – these cytokines are enhanced in psoriatic skin, induce many important pathological features in keratinocytes, and, also, IL-10 family members are essential for the development of psoriasis in preclinical models. IL-10 family members possess different biological functions, including immune suppression, elevated antiviral and antibacterial immunity, antitumor activity, and promotion of self-tolerance in autoimmune diseases, but the main functions of IL-10 family cytokine converge on protection of several tissues and organs from damage caused by inflammatory responses and by infections.

It has been reported that expression of IL-19, IL-20, IL-22, and IL-24 cytokines was up-regulated in psoriasis skin.[2-6] Additionally, it was found that primary human keratinocytes express IL-20R1, IL-20R2 and IL-22R1.[6] IL-20 and IL-22 promoted hyperproliferation and abnormal differentiation of keratinocytes *in vitro* and *in vivo*.[2,7,8] Li et al. observed that IL-19 upregulated keratinocyte growth factor transcripts on CD8+ T cells.[9] IL-19, IL-20, IL-22, and IL-24 activate STAT3 either in keratinocyte cell lines [6] or in primary keratinocytes.[2,7,10] Some studies reported that IL-10 family members, but not IL-26, induce the expression of various antimicrobial peptides including β-defensin family genes and S100 family genes.

[7,10,11] These cytokines also activate proinflammatory responses through the induction of chemokines and cytokines from keratinocytes.[7,10] Moreover, the IL-10 family cytokines regulate proteins involved in tissue remodeling, including kallikreins KLKs, marapsin MPN, platelet-derived growth factor PDGF, and Matrix metalloproteinase-1, -3 MMP-1, MMP-3.[10]

Studies with transgenic mice overexpressing IL-10 family cytokines also support their important pathogenic role in psoriasis. Mice overexpressing IL-20, IL-22, and IL-24 under the control of various promoters have been generated. [12-14] Aberrant cytokine expression in transgenic mice causes neonatal lethality with skin abnormalities, including a thickened epidermis, hyperkeratosis and compact stratum corneum. Stenderup et al. investigated the role of IL-20 in the aetiology of psoriasis by using a human skin xenograft transplantation model. These results demonstrated that blocking IL-20 signaling with anti-IL-20 antibodies resolved the psoriasis condition.[15] They also found that continuous IL-20 infusion, together with injection of additional nonactivated leucocytes, promotes induction of psoriasis in nonlesional skin from patients with psoriasis. Stenderup et al. suggested that IL-20 may play a critical role in the induction and maintenance of psoriasis.[15]

Numerous of described cytokines and their receptors are involved in several human diseases and health conditions, including psoriasis, rheumatoid arthritis, lupus nephritis, and asthma.[16-19] In accordance with the proposed role of these cytokine in various inflammatory diseases, the polymorphisms in respective genes have also been associated with many immune-related conditions, especially psoriasis.[20-28] All these findings suggest that these cytokines may contribute to the pathogenesis of psoriasis.

In present study, we investigated the effects of 48 single nucleotide polymorphisms (SNPs) from *IL10* gene cluster on the risk of psoriasis. In addition, a generalized multifactor dimensionality reduction (GMDR) analysis was performed to explore whether there are gene–gene interactions among the 48 SNPs.

Materials and methods

Study subjects

Unrelated psoriasis patients (n=377) of European descent from the Volga-Ural region were studied (Table 1). All patients were hospitalized for diagnosis and treatment at the Republic Dermatological Hospital (Ufa, Bashkortostan). Each patient was evaluated according to the standard protocol including a complete history and physical examination. All patients had the classical pattern of skin lesions (chronic plaque lesions, psoriasis vulgaris), confirmed by a dermatologist. The age distribution of the psoriasis cases ranged from 3 to 93 years. The control cohort comprised 403 healthy individuals. The normal control subjects were matched by sex and age with patients with psoriasis. Informed consent was obtained from all the healthy donors and patients by explaining the details of this study prior to collection of peripheral blood. The study was approved by the research Ethics Committee of our hospital and conducted according to the Declaration of Helsinki Principles.

DNA extraction, marker selection and genotyping

DNA was obtained from peripheral blood leukocytes by standard phenol extraction method.[29] All SNPs were genotyped at the Department of Physiology, University of Tartu by the ligation-based SNPlexTM genotyping system 48-plex (Applied Biosystems, Foster City,CA,USA) following the manufacturer's recommended protocol(http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042019.pdf). Genotype assignments were manually confirmed by visual inspection with the Genemapper 4.0 (Applied Biosystems, Carlsbad, CA, USA) software. For the SNPs selection and SNPlex assay pool design the SNPbrowser v3.5 was used.[30] Using the database from Applied Biosystems, SNP selection was based on density of 10 kb, minor allele frequency >5% and inclusion of all non-synonymous SNPs.

Statistical analysis

The demographic data calculations were made by SPSS 13.0 for Windows. For case-control association studies, χ^2 tests with Pearson 2×2 and 2×3 contingency tables as implemented in PLINK version 1.06 (http://pngu.mgh.harvard.edu) were used to compute the *P* values and corresponding odds ratios (OR) with 95% confidential intervals for allelic association. Hardy-Weinberg equilibrium (HWE) test was performed using PLINK. To adjust for multiple comparisons, corrected *P*-value (*Pc*) for a number of comparisons (Bonferroni correction) was applied.

Pairwise linkage disequilibrium (LD) between SNPs was quantified using the absolute value of Lewontin's D' and r^2 and LD plots were generated using Haploview version 4.1.[31,32] For determining haplotype-based associations, an accelerated expectation-maximization (EM) algorithm was used. To correct for multiple testing in comparing haplotype frequencies between the group of patients with psoriasis and the control group, the *P* values were adjusted by means of permutation testing.

The generalised multifactor dimensionality reduction (GMDR) software (http://www.ssg.uab.edu/gmdr/) was applied to assess gene–gene interactions. In this study, we used 10-fold cross-validation and 1000-fold permutation testing. The null hypothesis was rejected when the P-value derived from the permutation test was 0.05 or lower. The GMDR software provides a number of output parameters including CV consistency, the testing balanced accuracy, and empirical P-values to assess each selected interaction. The CV consistency score is a measure of the degree of consistency with which the selected interaction is identified as the best model among all possibilities considered.

Results

Association of single markers and gene–gene interaction

Table 1 shows the clinical characteristics of all cases and controls. From 48 SNPs (Table 2), the genotyping assay for three SNPs did not work, eleven SNPs were monomorphic, and genotypes distribution of SNP rs1890866 deviates from HWE in the control population ($p<0.05$). Therefore, we analyzed 33 SNPs, all of which fulfilled the inclusion criteria of the minimum allele frequency (MAF)>0.05 for all samples and were in HWE in the control group.

Allele frequencies and allelic P-value of SNPs in the psoriasis patients compared to the control group are presented in the Table 3. There were no significant differences in allele frequencies for any of the *IL10* family SNPs between patients with psoriasis and controls. Only the T allele of rs1554286 and T allele of rs1400986 were significantly increased in healthy controls compared with psoriaisis patients when adjusted for multiple comparisions. Thus, carriers of *IL10* T allele (rs1554286) and of *IL20* T allele (1400986) conferred protection to psoriasis (OR = 0.63, *Pc* = 0.007; OR = 0.62, *Pc* = 0.038, respectively).

Table 4 presents the results of cross-validation consistency (CVC) and testing accuracy obtained from GMDR analysis of the data. GMDR analysis revealed an interaction between the *IL10* and *IL20* polymorphisms (*Pc* = 0.001). The model including two SNPs *IL20* rs1518108/*IL10* rs1554286 had the testing balanced accuracy of 56.17%, the maximum CV consistency of 9/10, and a sign test P-value 0.001, and the result remained statistically significant after correction (Figure1).

Association of haplotypes

Haplotype analysis of the *IL10* gene cluster was performed according to the pairwise linkage disequilibrium pattern observed within each of these genes (cases and

controls: N=780). The results of the haplotype specific analyses are shown in Table 5. The haplotypes with a frequency below 1% were excluded from analyses, improving statistical power. The linkage disequilibrium (LD) analysis indicated the existence of six haplotypes blocks in the chromosome 1 region in Russians (Figure2). The first haplotype block (3 kb) includes five SNPs across the *IL10* gene (rs3024498, rs1878672, rs3024492, rs1554286, rs1518111) and the second haplotype block (4 kb) includes five SNPs from the *IL10* gene (rs3021094, rs3024490, rs1800872, rs1800893, rs1800890). The third haplotype block (1 kb) includes two SNPs across the *IL19* gene (rs2243156, rs2243158) and the fourth haplotype block (5 kb) includes five SNPs from the *IL19* gene (rs2243168, rs2073186, rs2073185, rs2243176, rs2243188). The fifth haplotype block (24 kb) contains one SNP from the *IL19* gene (rs2243191) and five SNPs across the *IL20* gene (rs1713239, rs1400986, rs3024517, rs2981573, rs2232360). The sixth haplotype block (1 kb) contains three SNPs from the *IL20* gene (rs2232363, rs3024523, rs1518108) and six SNPs across the *IL24* gene (rs291111, rs1150254, 1150255, rs1150258, rs291107, rs3748669).

Additionally, the haplotype analysis provided six haplotypes significantly associated with decreased and increased disease susceptibility in the psoriasis patients (Table 5). Namely, the block 2 haplotype AGCAA, the block 5 haplotype CGCAAA, the block 6 haplotype ATCAAGAAC frequencies were significantly higher in patients with psoriasis compared to the control group (35.1% vs. 28.9%, 57.3% vs. 51.5%, 3.3% vs. 1.6%, respectively; Table 5 and Figure 2). While the block 5 haplotype CGTGAA and haplotype CGTAAA frequencies were significantly higher in control group compared to the patients with psoriasis (10.0% vs. 14.0%, 1.7% vs. 3.4%, respectively; Table 5 and Figure2). However, the associations did not survive multiple correction test. Only the block 1 haplotype ACATA in the *IL10* gene displayed a statistically significant

association with psoriasis when adjusted for multiple comparisons (*Pc* =0.004) (Table 5). This association was caused by effect of rs1554286 and rs1518111 SNPs in the *IL10* gene.

Discussion

IL-19, IL-20 and IL-24 have been identified as IL-10-like cytokines, based on their analogous cellular sources, protein structure, receptors, target cell, genomic

localization and exon-intron structures. *IL10* gene cluster locates in a 200 kb region on chromosome 1q31-32, and the three cytokine share a common receptor IL-20R1/IL-20R2 heterodimer. [33, 34] These cytokines display many overlapping functions due to the similarity and shared receptor usage. Significant commonality exist also through conserved signaling cascades: the binding of IL-10 related cytokine to their receptors activates the JAK (Junus kinase), STAT (signal trasducers and activator of transcription), and the MAPK (mitogen-activated protein kinase) pathways.[35-43] Though, the IL-10 family members mediate diverse activities, including enhanced antibacterial and antiviral immunity, immune and antitumor activities, and promotion of self-tolerance in autoimmune diseases. [44-46]

In the present study we analyzed the association of SNPs in the *IL10* gene cluster with psoriasis. We have applied three different statistical to this data set – single SNP analysis, haplotype analysis and gene-gene analysis, each having different strengths with the aim of optimizing our ability to define the genetic architecture of psoriasis in the *IL10* region. In the allelic tests, the strongest associations were seen with two SNPs in *IL10* gene cluster, rs1554286 and rs1400986 (*Pc* = 0.007, *Pc* = 0.038, respectively). We identified a 2-locus interaction on psoriasis in GMDR analyses (*Pc* = 0.001), involving two genetic variants of *IL10* and *IL20*. Our finding suggest evidence for a two-locus interaction between the *IL10* (rs1554286) and *IL20* (1518108) variants in the risk of psoriasis, and highlight further the importance of multilocus effects in the genetic component of psoriasis.

The importance of examining haplotype of gene clusters has clearly been demonstrated in several studies. [47,48,49] The haplotype analysis provided one haplotype significantly associated with decreased disease susceptibility. Namely, the block 1 haplotype ACATA in the *IL10* gene frequency was significantly higher in

patients with psoriasis compared to the control group (*Pc* = 0.004). Therefore, our study supports a role of *IL10* and the *IL20* polymorphisms in the development of psoriasis.

Genetic association studies and preclinical data in psoriatic models support the functions of IL-10 subfamily cytokines in psoriasis. Candidate SNP approaches suggest that *IL10*, *IL19*, *IL20*, and *IL24* are associated with susceptibility to psoriasis [19-27]. These data have not been independently confirmed in genome wide association studies (GWAS). IL-23 plays an important role in the development of psoriasis. The IL-23 p40 subunit and the IL-23 receptor have been associated with psoriasis in genome-wide association studies. [50-53] IL-23 does not directly target keratinocytes. Its pathogenic functions on keratinocytes are mediated through the IL-10 subfamily cytokines, especially IL-22.

The positive association of the *IL10*.G13 allele with familial psoriasis has been reported, suggesting that the *IL10* locus contributes to the heritability of psoriasis susceptibility.[19,20] Another study found that the -1082 (rs1800896) heterozygoys G/A genotype,[21] -2763 (rs6693899) A allele and extended AAGC (rs1800890, rs6693899, rs1800896, rs1800872) haplotype [22] are associated with late-onset psoriasis. Kingo et al. analyzed three SNPs at the *IL10* 5'flanking region (−1082 (rs1800896), −592 (rs1800872), −819C/T (rs1800871)) and identified that the *IL10* ACC haplotype is associated with lower activity of the disease, and ATA haplotype with persistent eruption.[23] A meta-analysis involving Asian psoriasis patients (N = 1018) and controls (N = 1186) found significant association between psoriasis and the *IL10*-1082G allele (P = 0.011).[54]

Associations between *IL19*, *IL20* and *IL24* polymorphisms and psoriasis have also been assessed. In the individual evaluation of SNPs of *IL19*, *IL20* and *IL24* genes in a

sample of unrelated Caucasian psoriasis patients *IL19* SNP rs2243188, *IL20* SNPs rs2981572 and rs1518108 had significant association with psoriasis.[24,25] Kõks et al. showed block-like structure of LD formed by the genes *IL19*, *IL20*, and *IL24*. They found extended haplotype (CACCGGAA) formed by eight SNPs in *IL19* (rs2073186, rs2243174, rs2243188, rs2243191, rs2243193) and *IL20* (rs2981572, rs2981573, rs2232360) genes to be a significant susceptibility factor for psoriasis, while the *IL20*/*IL24* extended haplotypes (SNPs rs1518108, rs3762344, rs1150253, rs1150256, rs1150258) CAAAC, TGGGT, and CGAGT have been demonstrated to be protective against psoriasis. These data indicate that different loci within the chromosome 1q32 possess different effects in susceptibility to psoriasis. [25] Similar haplotype block structure encompassing the *IL10*, *IL19* and *IL20* genes was also established in African Americans and European Americans. Oleksyk et al. found that the *IL10* rs6703630, rs6693899, and rs3024498, and *IL10* haplotype AAGCG, as well as the SNPs in *IL19*, rs22443191, and *IL20*, rs1400986, rs3024517, rs2232360 and also two haplotypes CTGAAC and TCAGGC in the *IL19*/*IL20* region had an effect on HCV clearance in Africans but not European-American patients with HCV infection. [55] Whereas part of the SNPs investigated in our study are distinct from those examined in the above studies, all studies suggest that carriage of *IL10* and *IL19*/*IL20* haplotypes may influence inflammatory response. Our study had a few limitations: firstly, we did not have a replication cohort in the present study, which would have validated our results, and secondly, the limited sample size. Further studies of different populations are required to examine the combined influence of these variants of the IL-10 signaling pathway on the pathogenesis of psoriasis. Two SNPs were associated with psoriasis in this study: rs1554286, which is located at the intronic boundary of intron 3, creating a putative location of an alternate splicing, and rs1400986 at promoter. Polymorphisms

in the promoters are likely to deeply affect RNA amount and consequently protein synthesis as these regions harbour several motifs binding to transcription regulatory factors.

Several authors have demonstrated the role of *IL10* rs1554286 in the pathogenesis Benign Prostate Hyperplasia,[56] Behçet's Disease,[57] Invasive Haemophilus Influenzae Serotype b Infection,[58] Leprosy,[59] and Ischemic Stroke.[60] The SNPs in *IL10*, *IL10RA* and *IL10RB* genes have been studied in Korean population, the TT genotype of rs1554286 were associated with small prostate volume.[56] Nobuhisa M et al. conducted a genome-wide association study in a Japanese cohort including 612 individuals with Behçet's disease and 740 unaffected individuals. Authors identified two suggestive associations on chromosomes 1q32.1 (*IL10*, rs1554286, $P = 8.0\text{-}10^{-8}$) and 1p31.3 (*IL23R-IL12RB2*, rs12119179, $P = 2.7\text{-}10^{-8}$).[57] In a case-control study performed in UK children with Hib vaccine failure, the recessive homozygous genotype for SNP rs1554286 in strong linkage disequilibrium with both the C-819T and C-592A promoter polymorphisms in the *IL10* gene was associated with epiglottitis only (OR = 5.8; $P = 1.1\text{-}10^{-5}$).[58] Aggarwal et al. analyzed the SNP rs1554286 of *IL10* in 807 Indian patients and found a strong association between rs1554286 and leprosy.[59] The rs1554286 (TT vs. CT+CC genotype, OR=1.59; 1.06-2.39) was significantly associated with ischemic stroke even after controlling for age, sex, smoking, systolic blood pressure, total cholesterol, glucose, body mass index and serum IL-10 in a case-control study.[60]

Genetic polymorphism rs1400986 in *IL20* gene has been shown to be associated with Juvenile Idiopathic Arthritis, chronic hepatitis B virus and HCV clearence.[61-64] A study performed in 219 Juvenile Idiopathic Arthritis (JIA) from UK detected that rs1400986 of *IL20* gene conferred a risk of developing the diseases (OR = 1.53, *P*

= 0.0004).[61] Association at this SNP rs1400986 was previously identified by Fife et al. in 172 UK patients with JIA.[41 62] Truelove et al. analyzed rs1400986 of *IL20* gene in patients with chronic hepatitis B virus and found a strong association between rs1400986 and chronic hepatitis B infection outcome.[63] SNP rs1400986 was associated with HCV clearence in Africans Americans (91 clearence cases and 183 chronically infected matched controls; P = 0.005 – 0.002), however, no significant associations were detected in European Americans (108 clearence and 245 chronic).[64] Many associations seen at *IL20*, with is important in the inflammatory response, suggest that this gene may be significant in psoriasis.

In conclusion, the novel finding of the present study is gene-gene interaction of the IL-10 pathway on the reduced risk of psoriais. Our results indicate that genetic variants of the immunomodulatory *IL10* and *IL20* genes may protective effect in the Europeans from Russia. In addition, this observation needs to be confirmed in other population to exclude the possibility of a type I error due to limited sample size.

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Table 1

Demographic and clinical information

Subphenotype	Cases N=377	Controls N=403
Male:female ratio of affected	240:137	226:177
Age affected at entry to the study		
Median	41	43
Range	7-93	8-90
Number of affected with age of onset		
<40 years (type I psoriasis)	280	
>40 years (type II psoriasis)	97	
Number of affected with family history	127	

Table 2

Characteristics of studied SNPs

№	rs SNP	Chr	Base positions	Major/minor alleles	Gene	Function
1	rs10877	1	205258803	C/T	*C1orf116*	3' UTR
2	rs1150254	1	205139138	A/G	*IL24*	intron
3	rs1150255	1	205139582	G/A	*IL24*	intron
4	rs1150258	1	205141528	A/G	*IL24*	exon Tyr125His
5	rs11589	1	204828561	A/G	*RASSF5*	3'UTR
6	rs13208	1	204894785	G/A	*DYRK3*	intron
7	rs1400986	1	205105309	C/T	*IL20*	promoter
8	rs1518108	1	205109797	C/T	*IL20*	3' of a gene
9	rs1518111	1	205011268	G/A	*IL10*	intron
10	rs1539243	1	204714410	C/T	*IKBKE*	exon IIe67IIe
11	rs1554286	1	205010856	C/T	*IL10*	intron
12	rs1713239	1	205104098	C/G	*IL20*	5' of a gene
13	rs1800872	1	205013030	C/A	*IL10*	promoter
14	rs1800890	1	205015988	A/T	*IL10*	promoter
15	rs1800893	1	205013790	G/A	*IL10*	promoter
16	rs1878672	1	205010336	G/C	*IL10*	intron
17	rs188334	1	205146238	T/C	*FAIM3*	exon non-coding
18	rs1890865	1	205219379	A/G	*FCAMR*	promoter
19	rs1890866	1	205229519	G/A	*NA*	intergenic
20	rs2073185	1	205077351	C/T	*IL19*	intron
21	rs2073186	1	205077249	G/A	*IL19*	intron
22	rs2232360	1	205107282	A/G	*IL20*	intron
23	rs2232361	1	205108466	G/A	*IL20*	exon Gln155Gln
24	rs2232362	1	205108564	G/A	*IL20*	3' UTR
24	rs2232363	1	205108656	A/G	*IL20*	3' UTR
26	rs2243156	1	205072837	G/C	*IL19*	intron
27	rs2243158	1	205074264	G/C	*IL19*	5'UTR
28	rs2243164	1	205075189	T/C	*IL19*	intron
29	rs2243168	1	205076011	A/T	*IL19*	intron
30	rs2243176	1	205079067	C/T	*IL19*	intron
31	rs2243188	1	205081095	C/A	*IL19*	intron
32	rs2243191	1	205082580	C/T	*IL19*	missense Phe213Ser
33	rs2275531	1	205175739	G/A	*PIGR*	missense Gly365Ser
34	rs291102	1	205173101	C/T	*PIGR*	missense Ala580Val
35	rs291107	1	205141794	A/G	*IL24*	intron
36	rs291111	1	205136149	A/G	*IL24*	5' of a gene
37	rs2981573	1	205107200	A/G	*IL20*	intron
38	rs3021094	1	205011575	A/C	*IL10*	intron
39	rs3024490	1	205011934	G/T	*IL10*	intron
40	rs3024492	1	205010735	T/A	*IL10*	intron
41	rs3024498	1	205008152	A/G	*IL10*	3' UTR
42	rs3024517	1	205106876	A/G	*IL20*	intron
43	rs3024523	1	205109307	T/C	*IL20*	3' of a gene
44	rs3093426	1	205140097	G/A	*IL24*	intron
45	rs3093438	1	205143833	A/T	*IL24*	3' UTR
46	rs3748669	1	205143646	C/G	*IL24*	3' UTR
47	rs9242	1	204704018	T/C	*SRGAP2*	3' UTR
48	rs944769	1	204758700	C/T	*RASSF5*	intron

Table 3

Association analysis of SNPs from *IL10* gene cluster with psoriasis

Gene	SNP ID	Alleles 1/2	MAF[a](%) Cases	MAF (%) Controls	P-value	Pc-value	OR (95%CI)[b]
IL10	rs1878672	G/C	41.02	37.79	0.145		1.14 (0.93-1.40)
	rs1554286	**T/C**	**19.64**	**27.72**	**0.00023**	**0.007**[c]	**0.63 (0.50-0.81)**
	rs1518111	**A/G**	**23.60**	**29.90**	**0.005**		**0.72 (0.57-0.91)**
	rs3021094	C/A	9.16	11.11	0.211		0.80 (0.57-1.13)
	rs3024490	**T/G**	**25.76**	**30.41**	**0.044**		**0.79 (0.63-0.99)**
	rs3024492	T/A	23.55	22.22	0.537		1.07 (0.84-1.37)
	rs3024498	G/A	23.55	22.72	0.699		1.04 (0.82-1.33)
	rs1800872	**A/C**	**25.62**	**30.54**	**0.034**		**0.78 (0.62-0.98)**
	rs1800893	A/G	41.37	37.60	0.133		1.17 (0.95-1.44)
	rs1800890	**A/T**	**35.22**	**28.92**	**0.008**		**1.33 (1.07-1.66)**
IL19	rs2243156	C/G	11.58	12.50	0.581		0.91 (0.67-1.24)
	rs2243158	C/G	12.36	13.13	0.653		0.93 (0.68-1.26)
	rs2243168	T/A	11.40	12.21	0.624		0.92 (0.67-1.26)
	rs2073186	A/G	30.36	31.65	0.587		0.94 (0.75-1.17)
	rs2073185	T/C	17.95	18.86	0.645		0.94 (0.72-1.22)
	rs2243176	T/C	18.49	19.04	0.786		0.96 (0.74-1.24)
	rs2243188	A/C	29.58	31.35	0.457		0.92 (0.73-1.14)
	rs2243191	T/C	29.78	29.85	0.976		0.99 (0.79-1.24)
IL20	rs1713239	C/G	17.09	16.92	0.932		1.01 (0.77-1.32)
	rs1400986	**T/C**	**11.64**	**17.55**	**0.0011**	**0.038**[c]	**0.61 (0.46-0.82)**
	rs3024517	**G/A**	**10.30**	**14.02**	**0.027**		**0.70 (0.51-0.96)**
	rs2981573	G/A	28.73	29.62	0.703		0.95 (0.76-1.19)
	rs2232360	G/A	28.03	28.83	0.732		0.96 (0.76-1.20)
	rs2232363	**A/G**	**3.56**	**1.89**	**0.045**		**1.90 (1.00-3.63)**
	rs3024523	C/T	2.86	4.43	0.105		0.63 (0.36-1.10)
	rs1518108	T/C	45.47	40.55	0.053		1.22 (0.99-1.49)
IL24	rs291111	G/A	1.36	1.51	0.812		0.90 (0.38-2.10)
	rs1150254	G/A	46.23	41.88	0.089		1.19 (0.97-1.46)
	rs1150255	A/G	45.83	41.84	0.118		1,17 (0.95-1.44)
	rs1150258	G/A	46.27	41.98	0.093		1.19 (0.97-1.45)
	rs291107	G/A	46.98	43.58	0.187		1.14 (0.93-1.40)
	rs3748669	G/C	2.74	2.38	0.662		1.15 (0.60-2.17)
	rs3093438	T/A	2.86	2.13	0.357		1.35 (0.70-2.58)

Significant results are shown in bold face.
[a] MAF – minor allele frequency.
[b] OR: odds ratio; CI: confidence interval.
[c] – statistically significant association after Bonferroni correction

Table 4

Best gene–gene interaction models identified by the generalised multifactor dimensionality reduction method

Models	Training balanced accuracy (%)	Testing balanced accuracy (%)	Cross-validation consistency	Sign test
IL20 rs1518108/ *IL10* rs1554286	58.8	56.1	9/10	**0.001**[a]

[a] - statistically significant association after 1000 permutations

Table 5

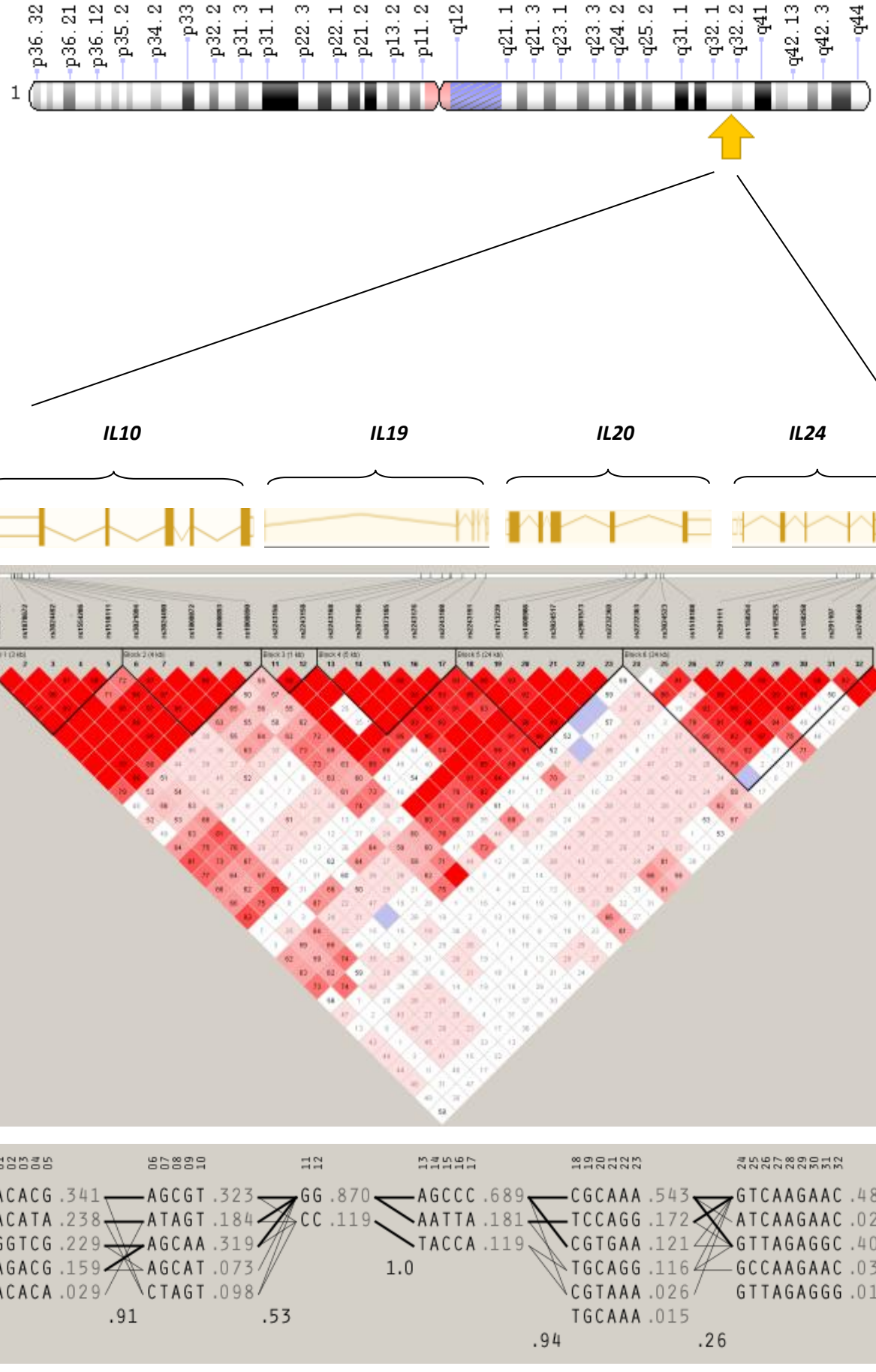
Haplotype analysis of SNPs from *IL10* gene cluster with psoriasis.

Block	Psoriasis (%)	Control sample (%)	χ^2 Statistic	P-value	Pc-value
Block 1 [a] *IL10* (rs3024498, rs1878672, rs3024492, rs1554286, rs1518111)					
ACACG	97(35.5)	98(32.9)	1.14	0.285	
ACATA	**54(19.7)**	**82(27.6)**	**13.21**	**0.0003**	**0.004**[b]
GGTCG	64(23.6)	66(22.2)	0.48	0.488	
AGACG	48(17.5)	44(14.6)	2.28	0.118	
ACACA	10(3.70)	7(2.30)	2.56	0.109	
Block 2 *IL10* (rs3021094, rs3024490, rs1800872, rs1800893, rs1800890)					
AGCGT	90(32.8)	95(31.9)	0.12	0.724	
AGCAA	**96(35.1)**	**86(28.9)**	**6.91**	**0.008**	
ATAGT	46(16.8)	60(20.0)	2.63	0.104	
CTAGT	23(8.60)	33(11.0)	2.39	0.122	
AGCAT	17(6.3)	24(8.2)	1.99	0.158	
Block 3 *IL19* (rs2243156, rs2243158)					
GG	239(87.4)	258(86.7)	0.16	0.681	
CC	32(11.6)	36(12.2)	0.16	0.686	
Block 4 *IL19* (rs2243168, rs2073186, rs2073185, rs2243176, rs2243188)					
AGCCC	190(69.5)	203(68.2)	0.30	0.584	
AATTA	48(17.5)	55(18.6)	0.32	0.570	
TACCA	31(11.3)	37(12.4)	0.40	0.525	
Block 5 *IL19/ IL20* (rs2243191, rs1713239, rs1400986, rs3024517, rs2981573, rs2232360)					
CGCAAA	**156(57.3)**	**153(51.5)**	**5.27**	**0.021**	
TCCAGG	47(17.1)	52(17.3)	0.01	0.898	
CGTGAA	**27(10.0)**	**42(14.0)**	**5.81**	**0.015**	
TGCAGG	31(11.3)	35(11.9)	0.10	0.749	
CGTAAA	**5(1.7)**	**10(3.4)**	**4.74**	**0.029**	
TGCAAA	5(1.8)	4(1.2)	1.09	0.296	
Block 6 *IL20/ IL24* (rs2232363, rs3024523, rs1518108, rs291111, rs1150254, 1150255, rs1150258, rs291107, rs3748669)					
GTCAAGAAC	125(45.9)	151(50.8)	3.63	0.056	
GTTAGAGGC	117(42.9)	115(38.5)	3.10	0.078	
GCCAAGAAC	7(2.60)	11(3.80)	1.80	0.179	
ATCAAGAAC	**9(3.30)**	**5(1.60)**	**4.72**	**0.029**	
GTTAGAGGG	5(1.70)	4(1.40)	0.246	0.619	

Significant results are shown in bold face.

[a] - haplotype combinations with less than 1% frequency are not displayed

[b] - statistically significant association after 1000 permutations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ACACA .029
AGCGT .323
ATAGT .184
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AGCAT .073
CTAGT .098
GG .870
CC .119
AGCCC .689
AATTA .181
TACCA .119
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TGCAGG .116
CGTAAA .026
TGCAAA .015
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