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Association analysis of IL20RA and IL20RB genes in psoriasis

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The interleukin-20-receptor I complex (IL-20-RI) is composed of two chains, IL20RA and IL20RB. Its ligands are the three members of the IL19 subfamily of cytokines, IL-19, IL-20 and IL-24. These cytokines are important in the manifestation of psoriatic lesions and, recently, an association of polymorphisms of IL20 with psoriasis has been described. In the present study we tested the hypotheses that genetic variations of the IL-20-RI influence susceptibility to psoriasis and investigated single nucleotide polymorphisms (SNPs) in the IL20RA and IL20RB genes in psoriasis patients ($n = 254$) and healthy controls ($n = 224$). We found no association of any of the investigated SNPs with the disease. Analysis of pairwise linkage disequilibrium (LD) across studied markers revealed a strong level of LD between SNPs within the IL20RA gene and SNPs within the IL20RB gene, and, for both genes six common haplotypes were identified with an estimated frequency $\geqslant 1\%$. Haplotype analyses suggested that the IL20RA haplotype CCG (rs1184860, rs1167846, rs1167849) is significantly associated with psoriasis (OR 3.14, 95% CI 1.61–6.14), whereas the TTG haplotype had a protective effect (OR 0.20, 95% CI 0.07–0.55). The risk haplotype defining SNPs 1167846 and 1184860 were found to modify paired box 5 and homeobox A9 sites, respectively, two transcription factors related to the differentiation of immune cells. Further studies are needed to confirm the genetic association and to investigate the functional relevance of IL20RA haplotypes in psoriasis.
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Keywords: *psoriasis; interleukin-20 receptor; single nucleotide polymorphisms; haplotypes*

Introduction

Interleukin (IL)-19, IL-20 and IL-24 have recently been identified as a group of IL-10-related cytokines, which are now referred to as the IL-19 subfamily of cytokines based on their analogous genomic localization, protein structure, cellular sources, receptors and target cells.[1–3] It has been verified that IL-19 subfamily cytokines are regulators of epidermal keratinocyte biology and are important in the immunopathology of psoriasis.[4–6]

The expression levels of IL-19, IL-20 and IL-24 in lesional skin of psoriasis patients are increased compared to healthy skin.[2,7–11] Two independent studies have demonstrated that overexpression of IL-20 in transgenic mice induces a skin phenotype similar to psoriasis.[2,5] Both *in vitro* and *in vivo*, IL-20 promotes hyperproliferation and abnormal differentiation of keratinocytes that are characteristic features of psoriasis.[2,12,13] IL-19 has been shown to induce expression of keratinocyte growth factor, which acts as a potent mitogen for human keratinocytes.[14] and is considered to contribute to the epidermal hyperplasia in psoriasis.

We have previously studied the possible impact of single nucleotide polymorphisms (SNPs) in the genes encoding the IL-19 subfamily of cytokines on the risk to develop psoriasis. Evidence was obtained that the *IL19* gene cluster represents a susceptibility region shared by plaque-type psoriasis and palmoplantar pustulosis (PPP), offering a possible explanation at the genetic level for the frequent co-existence of PPP and plaque-type psoriasis.[15–18]

The biological activities of the IL-19 subfamily cytokines are mediated through binding to two distinct cell-surface receptors, the IL-20 receptor type I (IL-20-RI) and type II (IL-20-RII). Both receptors are heterodimers and share the IL-20RB protein as common subunit. IL-20RB associates with IL-20RA to form IL-20RI that binds IL-19, IL20 and IL-24, and IL-20RB associates with IL-22RA1 to form IL-20-RII that binds IL-20 and IL-24.[2,13,19–21] After binding of the different ligands to the receptor complexes, signals are predominantly transducted *via* Janus kinase-signal transducer and activation of transcription pathways.[22] IL-20-RI is mainly expressed in the skin, lung and reproductive organs as well as in various glands. The expression of IL-20-RII seems to be more restricted with high levels detectable only in the skin.[2,13,19–21]

There are conflicting findings with regard to the expression of IL-20R subunits in skin lesions of psoriasis.

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Increased expression of IL-20RA, IL-20RB and IL-22RA1 subunits has been demonstrated in psoriatic lesions compared to healthy skin both at the mRNA and protein level.[10] However, when lesional skin was compared to nonlesional skin of patients with psoriasis, the expression levels of IL-20RA and IL-20RB were higher in the latter,[8,23] whereas expression of IL-22RA1 levels was comparable.[23]

The factors that regulate the expression of IL-20R subunits are unclear. It is possible that polymorphisms of the genes encoding the subunits are involved; however, the exact functional consequence of the different polymorphisms detected in these genes has not yet been thoroughly investigated. In addition, to the best of our knowledge, no studies have so far analyzed possible associations of these polymorphisms with inflammatory and other diseases. The aim of the present study was to explore whether the genes encoding the two chains of IL-20-RI, *IL20RA* and *IL20RB*, located on chromosomes 6q22.33-23.1 and 3q22.3, respectively, are associated with psoriasis.

Results

We performed a dbSNP database search (www.ncbi.nlm.nih.gov/SNP/) to identify SNPs of the *IL20A* and *IL20B* genes suitable for association studies. The selection criteria for SNP inclusion were the following: a frequency of the minor allele >10% and a distance between SNPs of at least 2000 bp. The selected SNPs were rs1184860, rs1167846, rs1167849 and rs276504 of the *IL20RA* gene and rs835634, rs10935213, rs747842 and rs108858 of the *IL20RB* gene (Figures 1 and 2).

Genotype distributions of the analyzed polymorphisms of *IL20RA* and *IL20RB* genes were in Hardy–Weinberg equilibrium both in the group of patients with psoriasis and the control group. Allele frequencies and allelic *P*-values of SNPs in the psoriasis group compared to the control group are presented in Table 1. There was no statistically significant association of any of the investigated SNPs with susceptibility to plaque-type psoriasis. However, in an exploratory analysis of subgroups defined by presence or absence of a family history of psoriasis, the SNPs rs1167846 and rs1167849 of the *IL20RA* gene showed an association with familial psoriasis (Table 2). The frequencies of the minor alleles of *IL20RA* SNPs rs1167846 and rs1167849 were lower in the familial psoriasis group compared to the control group (18.8 versus 25.9%; *P* 0.05, OR 0.66, 95% CI 0.44–1.00; 15.8

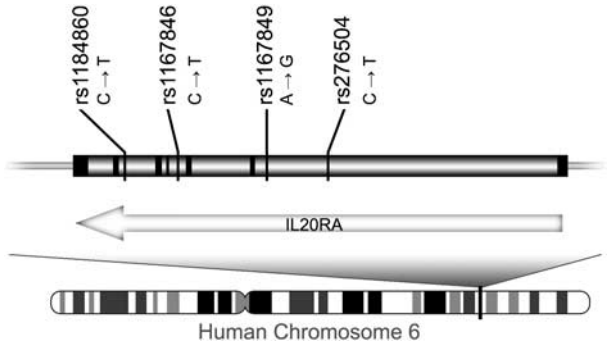

Figure 1 Genomic localization of the single nucleotide polymorphisms (SNPs) in the *IL20RA* gene used in the current study. Relative positions of selected SNPs are represented on the illustration by their cluster ID numbers in Public Polymorphism database (http://www.ncbi.nlm.nih.gov/SNP/) with allelic substitutions included. Arrow indicates the direction of transcription of respective gene, whereas coding region of a gene is represented as box on a narrow bar with dark bands representing exons.

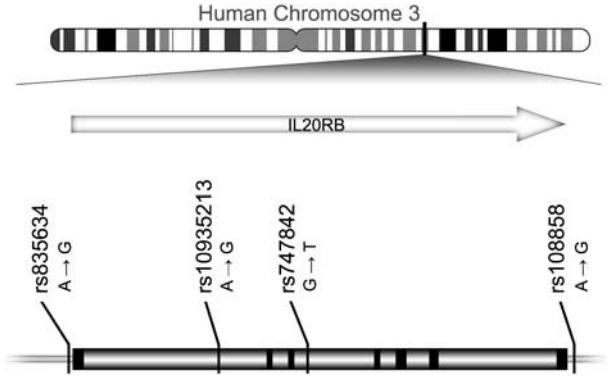

Figure 2 Genomic localization of the single nucleotide polymorphisms (SNPs) in the *IL20RB* gene used in the current study. Relative positions of selected SNPs are represented on the illustration by their cluster ID numbers in Public Polymorphism database (http://www.ncbi.nlm.nih.gov/SNP/) with allelic substitutions included. Arrow indicates the direction of transcription of respective gene, whereas coding region of a gene is represented as box on a narrow bar with dark bands representing exons.

Table 1 Results of association analysis of the genes *IL20RA* and *IL20RB* in psoriasis patients are indicated

Gene	*SNP ID*	*Chr Pos NCBI*	*Alleles*	*Minor allele frequency in controls (%)*	*Minor allele frequency in cases (%)*	*Allelic P-value*	*OR (95% CI)*
IL20RA	rs1184860	137382297	T/C	28.3	32.4	0.19	1.20 (0.91–1.59)
	rs1167846	137385413	C/T	25.9	23.1	0.32	0.86 (0.64–1.16)
	rs1167849	137390718	G/A	23.0	21.8	0.65	0.93 (0.69–1.26)
	rs276504	137394212	T/C	35.0	36.7	0.60	1.07 (0.82–1.40)
IL20RB	rs835634	138159001	G/A	46.6	46.7	0.99	1.00 (0.78–1.29)
	rs10935213	138176157	A/G	41.6	43.1	0.64	1.06 (0.82–1.38)
	rs747842	138185819	T/G	40.8	44.9	0.20	1.18 (0.91–1.54)
	rs108858	138212583	A/G	33.0	32.3	0.82	0.97 (0.73–1.28)

Abbreviations: IL, interleukin; SNP, single nucleotide polymorphism.
Allelic *P*-values were calculated using χ^2-test.

Table 2 Results of association analysis of *IL20RA* gene SNPs in plaque-type psoriasis

	rs1184860			*rs1167846*			*rs1167849*			*rs276504*		
	Alleles (%)		P-*value*	*Alleles (%)*		P-*value*	*Alleles (%)*		P-*value*	*Alleles (%)*		P-*value*
	T	*C*		*C*	*T*		*G*	*A*		*T*	*C*	
Controls ($n=224$)	71.5	28.3	*	74.1	25.9	*	77.0	23.0	*	65.0	35.0	*
Early-onset psoriasis ($n=179$)	68.2	31.8	0.3177	77.9	22.1	0.2076	79.3	20.7	0.4291	63.7	36.3	0.7087
Late-onset psoriasis ($n=75$)	66.2	33.8	0.2300	74.7	25.3	0.8921	76.0	24.0	0.8001	63.3	36.7	0.7193
Familial psoriasis ($n=101$)	72.2	27.8	0.8625	81.2	18.8	0.0494*	84.2	15.8	0.0376*	66.8	33.2	0.6413
Sporadic psoriasis ($n=153$)	64.6	35.4	0.0538	74.2	25.8	0.9529	74.5	25.5	0.4502	61.4	38.6	0.3157

Allele frequencies of the four studied polymorphisms of the *IL20RA* gene were compared between the psoriasis patients and the controls, *P*-values for allelic association were calculated using a χ^2-test.
*P-value <0.05 comparing allelic frequencies between patients and controls.

Table 3 Common haplotypes (estimated frequency $\geqslant 1\%$) formed by SNPs in the *IL20RA* and *IL20RB* genes

IL20RA				*IL20RB*				
rs1184860	*rs1167846*	*rs1167849*	*Frequencies of haplotype (%)*	*rs835634*	*rs10935213*	*rs747842*	*rs108858*	*Frequencies of haplotype (%)*
T	C	G	66.4	A	G	G	A	38.1
C	T	A	17.9	G	A	T	G	30.3
C	C	G	5.3	G	A	T	A	20.4
C	T	G	3.4	A	A	G	A	3.3
C	C	A	3.4	A	G	T	A	2.9
T	T	G	2.5	A	A	T	A	1.8
Haplotype block of the IL20RA—8 kb				Haplotype block of the IL20RB—36 kb				

versus 23.0%; P 0.04, OR 0.63, 95% CI 0.41–0.98; respectively).

Haplotype analysis of the *IL20RA* and *IL20RB* genes was performed according to the pairwise linkage disequilibrium (LD) pattern in the pooled data set. Nearly complete LD ($|D'|$ 0.77–0.93) existed among the polymorphisms rs1184860, rs1167846 and rs1167849 within the *IL20RA* gene. The *IL20RA* rs276504 polymorphism, located in the first intron of the *IL20RA* gene, was not in strong LD with the other investigated polymorphisms of the *IL20RA* gene. This polymorphism was, therefore, excluded from further haplotype analysis. There was nearly complete LD ($|D'|$ 0.83–0.95) among all studied polymorphisms within the *IL20RB* gene. Six common *IL20RA* haplotypes and six common *IL20RB* haplotypes with an estimated frequency of $\geqslant 1\%$ were identified in the pooled group of study participants (Table 3). These common haplotypes consisted of 98.9% of all *IL20RA* haplotypes and 96.8% of all *IL20RB* haplotypes among study participants, respectively.

Haplotype analysis revealed a significantly higher frequency of the *IL20RA* haplotype CGG in patients with plaque-type psoriasis than in healthy individuals (7.6 versus 2.6%; P_{nom} 0.0004, P_{adj} 0.007, OR 3.14, 95% CI 1.61–6.14), whereas the *IL20RA* haplotype TTG was less frequent in patients with psoriasis compared to the control group (0.9 versus 4.4%; P_{nom} 0.0006, P_{adj} 0.007, OR 0.20, 95% CI 0.07–0.55; Table 4). A lower frequency of the *IL20RB* haplotype AGTA was observed in patients with plaque-type psoriasis compared to control subjects (1.9 versus 4.1%), but the difference was only of borderline significance and not significant after correction for multiple testing (P_{nom} 0.04, P_{adj} 0.45 OR 0.44, 95% CI 0.20–0.97; Table 4).

Comparison of controls and different psoriasis subgroups stratified according to the age of onset and family history of psoriasis showed that the strength of association of the *IL20R*A haplotype CGG was similar in the investigated subgroups (OR between 3.02 and 3.49; Table 5). There were also no significant associations between the different *IL20RB* AGTA haplotypes and psoriasis subgroups (data not shown).

In order to obtain some information on whether the SNPs defining the haplotypes associated with psoriasis modify transcription factor (TF)-binding sites, the effect of the base exchanges were modeled using MatInspector and SNPInspector. It was found that two SNPs in *IL20RA* gene modify potential TF-binding sites. In the case of SNP rs1167846, the C to T exchange leads to the loss of a PAX5 (paired box 5) site and generates a new TF-binding site for POU class 2 homeobox 1. The T to C exchange of rs1184860 leads to the loss of homeobox A9 TF site and generates a new site for nuclear factor (erythroid-derived-2)-like-1, TF. The SNP rs1167849 does not appear to modify any TF-binding site.

Table 4 Results of haplotype analysis of the *IL20RA* and *IL20RB* genes

IL20RA haplotypes	*Controls* (n = 224)	*Plaque psoriasis patients* (n = 254)	P_{nom}-*value*	P_{adj}-*value*	*OR (95% CI)*
TCG	66.8	66.1	0.81	1.00	0.97 (0.74–1.26)
CTA	18.2	17.6	0.81	1.00	0.96 (0.69–1.34)
CCG	2.6	7.6	0.0004*	0.007*	3.14 (1.61–6.14)
CTG	3.2	3.6	0.76	1.00	1.12 (0.56–2.2)
CCA	4.2	2.7	0.20	0.96	0.63 (0.31–1.28)
TTG	4.4	0.9	0.0006*	0.007*	0.20 (0.07–0.55)
IL20RB haplotypes					
AGGA	35.9	40.0	0.20	0.96	1.19 (0.91–1.55)
GATG	30.5	30.1	0.89	1.00	0.98 (0.74–1.29)
GATA	20.2	20.5	0.91	1.00	1.02 (0.74–1.39)
AAGA	4.1	2.6	0.18	0.95	0.62 (0.30–1.26)
AGTA	4.1	1.9	0.04	0.45	0.44 (0.20–0.97)
AATA	1.8	1.7	0.91	1.00	0.94 (0.36–2.47)

The haplotype frequencies (%) and haplotypic ORs with their 95% CIs and *P*-values in plaque psoriasis patients compared to controls are indicated. Haplotype–phenotype associations were estimated using an expectation-maximization algorithm. Multiple testing was performed using permutation testing.
*P-value <0.05 haplotypic differences compared to the controls.

Table 5 The *IL20RA* CCG and TTG haplotype frequencies (%) and haplotypic ORs with their 95% CIs and P-values in the subgroups of patients according to the age of onset, family history and severity of disease compared to controls are indicated

Subgroups of psoriasis	*CCG (%)*	P-*value*	*OR (95% CI)*	*TTG (%)*	P-*value*	*OR (95% CI)*
Controls ($n = 224$)	2.6			4.4		
Patients with early-onset psoriasis ($n = 179$)	7.6	0.0008	3.18 (1.57–6 .45)	1.1	0.0052	0.24 (0.08–0.71)
Patients with late-onset disease ($n = 75$)	7.6	0.004	3.27 (1.40–7 .65)	0.7	0.0295	0.15 (0.02–1.09)
Patients with familial psoriasis ($n = 101$)	8.2	0.0009	3.49 (1.61–7 .60)	0.7	0.0132	0.15 (0.03–0.84)
Patients with sporadic psoriasis ($n = 153$)	7.2	0.0021	3.02 (1.45–6 .30)	1.1	0.0097	0.24 (0.08–0.77)

Discussion

In the present study we analyzed the association of four SNPs in the *IL20RA* gene and four SNPs in the *IL20RB* gene with plaque-type psoriasis. We found no significant association of any of the investigated SNPs with the disease. In an exploratory subgroup analysis there was a weak association of two SNPs (rs1167846 and rs1167849) of the *IL20RA* gene with familial psoriasis in that the minor alleles of these SNPs were underrepresented in cases compared to healthy controls, possibly suggesting a mild protective effect of the more frequent allele. However, it cannot be excluded that this finding is a false-positive result due to multiple testing.

LD analysis revealed strong LD over the 8421 bp and 53582 bp region of the *IL20RA* and *IL20RB* gene, respectively, and haplotype blocks for these genes could be established. For *IL20RA*, the block consists of rs1184860, rs1167846 and rs1167849 but not rs276504. For *IL20RB*, all four SNPs are in almost complete LD. Interestingly, association analysis of haplotypes revealed a significant association of the *IL20RA* CCG haplotype with psoriasis ($P_{adj} < 0.01$), whereas carriage of the *IL20RA* TTG haplotype seemed to have a protective effect ($P_{adj} < 0.01$). One *IL20RB* haplotype (AGTA) was associated with a decreased risk for psoriasis, but this effect did not remain significant after correction for multiple testing. Therefore, our study suggests a role of polymorphisms in the *IL20RA* gene and possibly also the *IL20RB* gene in the development of psoriasis.

Previous studies have indicated the importance of IL-19, IL-20 and IL-24 in the manifestation of psoriasis.[7,8,10,16] Psoriatic lesions are characterized by increased expression of IL-19 and IL-20, which is normalized after treatment with cyclosporine or calcipotriol parallel to the clinical improvement of psoriatic lesions.[7,8] Although the data on the expression of IL-20R types I and II in psoriasis are somewhat controversial it seems possible that changes in the expression and/or function of IL-20-RI contribute to the changes of the IL-19 cytokine system that are part of psoriasis pathophysiology.[8,10,23] Therefore, the present association study focused on the *IL-20RA* and *IL-20RB* genes.

The functional consequence of the observed association of the *IL20RA* CCG haplotype with psoriasis (OR 3.14) is currently under investigation. Bioinformatic modeling suggests that the C to T exchange in the case of SNP rs1167846 in the *IL20RA* gene causes the loss of a PAX5 site. PAX5 is a member of the paired box (PAX) family of TFs that are important in the regulation of cell differentiation. Alterations in the expression of these TFs are thought to contribute to abnormal cell proliferation and malignant transformation.[24,25] Recently it was shown that PAX5 deletion induces the conversion of B lymphocytes to functional T lymphocytes,[26] indicating an additional role in the immune system that might be relevant in psoriasis. In the *IL20RB* gene SNP rs747842 leading to a loss of an IRF4 (interferon regulatory factor 4)-binding site could be of particular interest because the function of IRF4 is very closely related to the

differentiation of T lymphocytes. For instance, IRF4 is important during IL-12-induced Th1 cell differentiation and is also necessary for IL-4-induced differentiation of Th2 cells and B cells.[27–29] Recent findings suggest that IRF4 is also involved in the development of Th17 cells[30] that are believed to be centrally involved in psoriasis.

The polymorphisms rs1167846 in the *IL20RA* gene and rs747842 in the *IL20RB* gene appear as interesting candidates for further studies, however, these hypotheses are currently speculative and require functional support. In addition, as there was a strong LD in the investigated genes, it is possible that other polymorphisms of these genes or of other genes in LD with the investigated haplotype might account for the observed association.

In summary, to the best of our knowledge, this is the first study investigating the association of *IL-20RA* and *IL-20RB* polymorphisms with a chronic inflammatory disease. Although none of the investigated SNPs of the *IL-20RA* and *IL-20RB* genes were individually associated with psoriasis, we identified an *IL-20RA* haplotype that seems to increase psoriasis susceptibility, and a second *IL-20RA* haplotype that was associated with a protective effect. There is initial evidence that the identified *IL20RA* risk haplotype may be functionally relevant because it carries altered binding sites for TFs involved in cell differentiation and immunological functions, but the exact effects of the *IL-20RA* polymorphisms on transcriptional activation and function warrant further studies.

Materials and methods

Ethical considerations

The study was approved by the Ethics Review Committee on Human Research of the University of Tartu and written informed consent was obtained from all participants.

Characteristics of study participants

Unrelated Caucasian patients living in Estonia with a clear clinical diagnosis of plaque psoriasis ($n=254$, 119 women, 135 men, age range 18–89 years, mean age of onset of psoriasis 29.7 years) were enrolled at the Department of Dermatology, University of Tartu, Estonia as described.[17]

Patients with plaque psoriasis were divided into subgroups according to the age of disease onset and family history of psoriasis. In total, 179 patients had early-onset disease (onset before the age of 40), and 75 patients had late-onset disease (onset at the age of 40 or later). Patients were considered to have familial psoriasis if they had at least one first- or second-degree relative with psoriasis ($n=101$), otherwise they were considered to have sporadic disease ($n=153$).

The control group consisted of 224 healthy unrelated Caucasians (110 women, 114 men, age range 18–71 years) without a personal or family history of psoriasis. These individuals were recruited at the University of Tartu from among medical students, health-care personnel and patients presenting at the dermatological outpatient clinic with mild expression of either facial teleangiectasis or skin tags as described earlier.

Preparation of genomic DNA and analysis of IL19, IL20 *and* IL24 *polymorphisms*

Genomic DNA was extracted from whole blood and the SNPs of the *IL20RA* and *IL20RB* genes were analyzed by the tetra-primer Amplification Refractory Mutation System (ARMS)-PCR method as previously described.[11,15–17] For each SNP, a set of four primers was designed using the online program available at http://cedar.genetics.soton.ac.uk/public_html/primer1.html. Each PCR reaction was carried out in a total volume of 10 μl, containing 100 ng of template DNA, 20 pmol of each inner primer, 2 pmol of each outer primer, 0.2 mM deoxyribonucleotide triphosphate, 1 × reaction buffer (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 0.1 mg ml^{-1} Tartrazine, 1% Sucrose (Naxo, Tartu, Estonia), 0.5 U Smart-Taq DNA polymerase (Naxo). Sequences and melting temperatures of primers, annealing temperatures for specific PCR reactions and optimized concentrations of Mg^{2+} used for genotyping of SNPs are shown in Table 6. The direct sequencing of incidental DNA samples was performed using ABI Genetic Analyzer 310 (Applied Biosystems, Foster City, CA, USA) for verification of the tetra-primer ARMS-PCR results and validation of the polymorphisms. The results of the tetra-primer ARMS-PCR were completely consistent with the results of direct sequencing.

Statistical and bioinformatic analysis

Single-marker association analysis and multimarker haplotype association tests of individuals were performed using the Haploview program.[31] Allele frequencies were investigated using the χ^2-test. To evaluate deviation from the Hardy–Weinberg equilibrium, observed and expected genotype frequencies were compared by Fisher's exact test in the examined groups (cases and controls). For determining haplotype-based associations, an accelerated expectation-maximization (EM) algorithm similar to the partition/ligation method described by Qin *et al*.[32] was used. The haplotype association test was performed on the set of blocks selected based on the LD. Pairwise LD was estimated by a log-linear model and standardized D′ characteristics were used to demonstrate the extent of disequilibrium. Haplotype analysis was performed only for the haplotypes with a frequency of $\geqslant 1\%$.

The significance level of the tests for declaring a probability value as significant was set to 0.05. To correct for multiple testing in comparing allele frequencies between the group of patients with psoriasis and the control group, the *P*-values (P_{nom}-value) were adjusted by permutation testing (P_{adj}-value). Altogether 1000 permutations were performed for correction of multiple comparisons. In addition, in an exploratory analysis, we compared allele frequencies and haplotype frequencies of subgroups of psoriasis stratified according to age of onset and presence of a positive family history for psoriasis with healthy controls.

The sequence of *IL20RA* gene was analyzed with MatInspector version 7.4.8 (http://www.genomatix.de) to find potential binding sites for the TFs in the close proximity of SNPs. The SNPInspector (http://www.genomatix.de) program was used to identify TF-binding sites affected by SNPs investigated in our study.

Table 6 Sequences and other details of primers used for genotyping of SNPs of *IL20RA* and *IL20RB* genes

Genetic polymorphism	*Primers (5′–3′)*	*Temperature*	*Annealing temperature*	*Mg^{2+}*	*Amplicon size*
rs1184860 T/C	Forward inner primer (C allele):		60 °C	2.5 mm	289 bp–control
	TTTTAATGTGAGTAAAGAAATGACAGCGC	65 °C			
	Reverse inner primer (T allele):				193 bp—T allele
	TTTTTGGGTATGTTTTAGGCATCTTGATAA	65 °C			
	Forward outer primer:				155 bp—C allele
	TTTATAGTAGAGATGGGGTTTTGCCATG	65 °C			
	Reverse outer primer:				
	AAAATTGCTTTGTTTCCTTATGACAGCA	65 °C			
rs1167846 C/T	Forward inner primer (C allele):		60 °C	2.5 mm	376 bp—control
	CAGTCATTCAACTCATATTTATTGGGGGC	64 °C			
	Reverse inner primer (T allele):				244 bp—T allele
	AGAGGAACACAATTCAACCCATAATCA	64 °C			
	Forward outer primer:				188 bp—C allele
	TACTCTGGTTTATGTTTAGTTGCCGAGA	64 °C			
	Reverse outer primer:				
	CCACCTGACTTCAGTATGATCTCATGTT	64 °C			
rs1167849 G/A	Forward inner primer (A allele):		60 °C	2.5 mm	356 bp—control
	CATTTAGGTAAGTGGGAAATGCTCCAAA	66 °C			
	Reverse inner primer (G allele):				236 bp—G allele
	TATAATCTTTTCTTCCCACAACACTGTCCC	66 °C			
	Forward outer primer:				178 bp—A allele
	AGAAAGAGCTCAGGAATTATTCGCTCAG	66 °C			
	Reverse outer primer:				
	AACTATGAACAGTTCCACCAGGAAAAGC	66 °C			
rs276504 T/C	Forward inner primer (C allele):		65 °C	2.6 mm	408 bp—control
	AACCACTGGTGCACACTTCAGAAATCAC	69 °C			
	Reverse inner primer (T allele):				275 bp—T allele
	ACTGAACGTTGTTTTGCAGCACGTGCCA	75 °C			
	Forward outer primer:				189 bp—C allele
	GGCCAATGGCAGTGGACATCAAAAGACT	72 °C			
	Reverse outer primer:				
	CCC CTA GGC TAC CAA CCT GTA CTG CAT GC	72 °C			
rs835634 A/G	Forward inner primer (A allele):		55 °C	2.5 mM	285 bp—control
	GAAGAGAAAAATGGGAACAAGACTTGTA	63 °C			
	Reverse inner primer (G allele):				200 bp—A allele
	TCAAACGATATACAAAGATATTCATTC	57 °C			
	Forward outer primer:				140 bp—G allele
	TTTGCAAAAAGAAAAACTAAAAGAAGAT	60 °C			
	Reverse outer primer:				
	TATTCCACTATTGTATTTGCACATTAGG	60 °C			
rs10935213 A/G	Forward inner primer (G allele):		55 °C	2.5 mM	354 bp—control
	TCTAACTATAATGACCATTTTACATTTTG	57 °C			
	Reverse inner primer (A allele):				235 bp—A allele
	AGGAGGCTCATGATGTCCAGAGATAT	64 °C			
	Forward outer primer:				174 bp—G allele
	TGTAATAGTCTTGGCTAGCAGGTTTT	60 °C			
	Reverse outer primer:				
	TTTATGTATTATCAGTATCCCTAAGGGG	60 °C			
rs747842 T/G	Forward inner primer (G allele):		65 °C	2.5 mM	415 bp—control
	CTCAGGAGCTGTTCTATTCTCCTTTCCGTG	70 °C			270 bp—T allele
	Reverse inner primer (T allele):	71 °C			
	AGGGAAGGGCACCAGGGTAGAGGGTA				201 bp—G allele
	Forward outer primer:	70 °C			
	TTTCACAACAGAAGCTCTTCTGCCACATC				
	Reverse outer primer:	70 °C			
	GATTATAGGAATGAGCCATCGCACAGGG				
rs108858 A/G	Forward inner primer (G allele):		60 °C	2.5 mM	426 bp—control
	GCCAGGGTGGCCCTTTTTATTTGATG	71 °C			
	Reverse inner primer (T allele):				162 bp—G allele
	CCTTTTTTCTGTTGGTAAAGTACAGAAGTT	62 °C			120 bp—A allele
	Forward outer primer:	66 °C			
	TATCCTTTGGAGCCTCTCTGGACTTG				
	Reverse outer primer:	66 °C			
	CTGTAAAGTGTTGACAGTGTGTGCACAC				

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