Association analysis of class II cytokine and receptor genes in vitiligo patients

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Abstract

The loss of melanocytes in vitiligo is mainly attributed to defective autoimmune mechanisms and lately autoinflammatory mediators have become more emphasized. Among these, a number of class II cytokines and their receptors have displayed altered expression patterns in vitiligo. Thus, we selected 30 SNPs from the regions of respective genes to be genotyped in Estonian case-control sample (109 and 238 individuals, respectively). For more precise analyses, patients were divided into subgroups based on vitiligo progression activity, age of onset, sex, occurrence of vitiligo among relatives, extent of depigmented areas, appearance of Köbner's phenomenon, existence of halo nevi, occurrence of spontaneous repigmentation, and amount of thyroid peroxidase antibodies. No associations appeared in whole vitiligo group. In subgroups, several allelic and haplotype associations were found. The strongest involved SNPs rs12301088 (near *IL26* gene), that was associated with familial vitiligo and existence of halo nevi, and rs2257167 (*IFNAR1* gene), that was associated with female vitiligo. Additionally, haplotypes consisting of rs12301088 and rs12321603 alleles (*IL26-IL22* genes), that were associated with familial vitiligo and existence of halo nevi. In conclusion, several genetic associations with vitiligo subphenotypes were revealed and functional explanations to these remain to be determined in respective studies.

Keywords: vitiligo, class II cytokine, genetic association study, single-nucleotide polymorphism, haplotype

1. Introduction

Vitiligo is a complex disease characterised by depigmented areas on the skin and hair which occur as a result of melanocyte destruction. The definite causes are yet to be established and may involve multiple genetic and environmental factors [1]. Still, among several hypotheses, the autoimmune theory has received the most support, with the autoinflammatory component of the disease gaining more attention recently [2-4]. For instance, increased levels of proinflammatory cytokines and receptors such as interleukin 6 (IL-6), IL-8, tumor necrosis factor α (TNF-α), and IL-2R have been measured in vitligo patients [5]. As to the genetic factors, estimates of vitiligo heritability range from 16% to 46% in different populations. Overwhelming majority of nearly 90% of candidate genes encode immunoregulatory proteins and only 10% encode melanocyte proteins [6].

Our study focused on seven genetic regions of class II cytokine and their receptor genes. Specifically, interferon gamma (IFN-γ) that is primarily produced by natural killer (NK) cells, CD4+ and CD8+ T cells, and deviations in its expression have been associated with autoinflammatory and autoimmune diseases [7]. IL-26 is secreted by NK cells and T cells, and induces the production of IL-10, IL-8, and intercellular adhesion molecule 1 (ICAM-1) in epithelial cells [8, 9]. IL-22 is produced by variety of immune cells including different types of T cells, innate lymphoid cells (ILCs), NK cells, and dendritic cells [10]. Epithelial cells are the main targets of IL-22 and binding of this cytokine leads to induction of anti-microbial proteins, and also proliferative and anti-apoptotic pathways [11]. The corresponding genes *IFNG*, *L26*, and *IL22* of these cytokines form a cluster at 12q15 chromosomal region. The functions of adjacently placed *MDM1* gene are yet to be established, but the mRNA levels were increased in vitiligo skin and peripheral blood mononuclear cells (PBMCs) in our recent report [12]. Another group of cytokines are IL-28B, IL-28A and IL-29 (alternatively labeled IFN-λ3, IFN-λ2 and IFN-λ1, respectively) encoded by gene cluster of *IL28B*, *IL28A* and *IL29* in the 19q13.2 chromosomal region. They can be expressed by almost any nucleated cell, especially dendritic cells, and induce anti-viral and anti-tumor pathways in a restricted population of target cells mainly comprised of epithelial cells and hepatocytes [13].

The receptors of class II cytokines are composed of two subunits which can be coupled alternatively. One cytokine can associate with more than one complex and a certain complex can bind more than one cytokine. IL-20R2 can be coupled with IL-20R1 to form a receptor for IL-19, IL-20 and IL-24, or with IL-22R1 to form a receptor for IL-19 and IL-20. IL-10R2 can be paired with IL-10R1 (IL-10 receptor), IL-20R1 (IL-26 receptor), IL-22R1 (IL-22 receptor), and IL-28R1 (receptor for IL-28A, IL-28B and IL-29). IFN-αR1 and IFN-αR2c complex binds IFN-α, -β, -ε, -κ, -ω, -δ, and –τ. IFN-γR1 and IFN-γR2 complex binds IFN-γ. In addition to these, IL-22R2 is a soluble protein that binds IL-22, serving as an antagonist of this cytokine. Single-nucleotide polymorphisms (SNPs) from the gene regions of all described receptor subunits except for IL-20R2 were selected for genotyping. Finally, tissue factor gene *F3* was also included due to large degree of homology to these receptors [14].

The preceding gene expression studies had revealed substantial changes in many class II cytokine and receptor genes in vitiligo. Notably, *IL22* expression was elevated in vitiligo patients along with one of its receptor subunits *IL10RB* [15]. A following study demonstrated changes in mRNA expression pattern of *IL20RB*, *IL22RA2*, *IL28A*, *IL28B*, *IL28RA*,*MDM1*, *IFNA1*, *IFNB1*, *IFNG*, and *ICAM1* in skin and/or PBMCs of vitiligo patients [12]. Additionally, genetic associations were found with SNPs of *IL19*, *IL20*, and *IL20RB* genes [16]. Considering those findings, a genetic association study was designed with SNPs from the region of class II cytokine (*IFNG*, *IL26*, *IL22*, *IL28B*, *IL28A*, and *IL29*), their receptor (*IL20RA*, *IL22RA1*, *IL10RA*, *IL10RB*, *IL28RA*, *IL22RA2*, *IFNAR1*, *IFNAR2*, *IFNGR1*, and *IFNGR2*), receptor homolog (*F3*), and adjacently placed (*MDM1*) genes to be genotyped in Estonian case-control sample.

2. Materials and Methods

2. 1. Study sample

Vitiligo patients (n = 109) and healthy control individuals (n = 328) were enrolled at the Department of Dermatology, University of Tartu, Estonia. All were unrelated, of Caucasian origin, and living in Estonia. Vitiligo diagnoses were based on characteristic skin depigmentation at typical locations and

whiteness of skin lesions under Wood's lamp. The patients were further divided into subgroups according to several characteristics. The patients were classified to have active vitiligo (n = 79) in case new areas of depigmentation had appeared during the previous 3 months and stable vitiligo (n = 30) if new areas or enlargement of previously existing depigmentation had not occurred. Early onset vitiligo (n = 33) was assigned in case the symptoms appeared before the age of 20 and late onset (n = 76) in case 20 or after. Female (n = 76) and male (n = 33) patients were analyzed separately with their respective controls (n = 182; n = 146) and also comparatively against each other. Familial vitiligo (n = 26) was defined by occurrence of vitiligo in patients' relatives and absence indicated the sporadic cases (n = 83). Two groups were formed according to the extent of affected areas: extent < 10% (n = 54) and extent ≥ 10% (n = 55). Patients expressing the Köbner's phenomenon, manifested by the development of new vitiligo patches at sites of skin injury comprised the Köbner+ group (n = 11) and those without it belonged to Köbner– (n = 98). The existence (n = 17) or absence (n = 92) of halo nevi was another differentiating factor. Patients displaying spontaneous repigmentation (n = 30) were analysed apart from the rest (n = 79). Finally, the amount of thyroid peroxidase antibodies (TPO-Ab) was determined by chemiluminescence immunoassay and used as a criterion for TPO-Ab<10 (n = 49) and TPO-Ab≥10 (n = 51) groups.

The Ethics Committee of the University of Tartu approved the study and informed consent was obtained from all participants.

2.2. SNP selection and genotyping

SNPbrowser version 3.5 was used for SNP selection and SNPlexTM assay pool design. The inclusion of SNPs was based on density with spacing criterion around 10 kb, minor allele frequency cut-off 5%, and non-synonymous SNPs always preferred. Genomic DNA was extracted from 9 ml blood samples and Applied Biosystems SNPlexTM method was used for genotyping [17]. SNPlexTM platform utilises allele-specific hybridization probes and ensuing PCR to amplify the resulting products. Each amplicon contains a unique sequence that binds a specific fluorescent-labeled ZipChute probe. The

latter are eventually separated and detected by capillary electrophoresis, enabling automatic allele calling by manufacturer provided software.

2.3. Data analysis

The Haploview v4.2 program was used for Hardy-Weinberg equilibrium (HWE) calculations in control group and also for allelic association and haplotype association tests between groups of patients and controls [18]. The Solid spine of LD algorithm integrated in Haploview v4.2 was applied to define the haplotype blocks and the resulting blocks were used in the haplotype association test. SNPs of each chromosome were analysed separately and rs3849301 was further separated from the rest of chromosome 1 SNPs due to the distance between them. Differences in allele or haplotype frequencies between cases and controls were assessed by chi square test. Null hypothesis was rejected when significant differences in allele or haplotype frequencies occurred and corresponding p values are presented in the text and tables. The statistical significance threshold was set to 0.05 for all tests. Ten thousand permutations were performed to correct p values for errors of multiple testing.

3. Results

A total of 30 SNPs selected from Class II cytokine and their receptor loci were genotyped (Table 1). All these SNPs met the inclusion criteria for minor allele frequency and Hardy-Weinberg equilibrium.

3.1. Allelic association analysis

A number of SNPs displayed significantly different allele frequencies among vitiligo patients compared to healthy control individuals. The results from whole vitiligo, early onset, late onset, familial, sporadic, extent<10%, extent≥10%, active, and stable vitiligo groups are shown in Table 2 and significant associations from the remaining groups are described below in the following section.

No associations were found when analyzing the whole vitiligo group, but several appeared in the subgroups. The most substantial results concerned the SNP rs12301088 in early onset (p = 0.0154, OR 1.95, CI: 1.13 – 3.39), familial (p = 0.0048, OR 2.41, CI: 1.29 – 4.51), extent≥10% (p = 0.0138, OR 1.7, CI: 1.11 – 2.61), and halo+ (p = 0.0033, OR 3.49, CI: 1.44 – 8.42) subgroups. Notably, the associations in familial and halo+ subgroups remained significant after correction for multiple testing (respective p values 0.0245 and 0.021). Also, rs2257167 produced a more convincing association in female vitiligo subgroup (p = 0.0020, OR 0.27, CI: 0.11 – 0.66), that withstood the correction for multiple testing (p = 0.0102), and additionally in familial vitiligo subgroup (p = 0.033, OR 0.15, CI: 0.02 – 1.12). The rs1342642 association in stable vitiligo subgroup was one of the strongest revealed in this study (p = 0.0058, OR 0.26, CI: 0.09 – 0.72), but narrowly missed the statistical significance threshold after correction for multiple testing (p = 0.0777). The remaining SNP associations came from various subgroups and were weaker compared to those already described. Rs276586 was associated with sporadic (p = 0.0469, OR 1.44, CI: 1 – 2.1) and stable (p = 0.0325, OR 1.89, CI: 1.05 – 3.42) vitiligo. Rs30461 was associated with early onset (p = 0.0278, OR 0.14, CI: 0.02 – 1.06) and female (p = 0.0384, OR 0.43, CI: 0.19 – 0.98) vitiligo. Rs2834117 was associated with sporadic (p = 0.0354, OR 0.56, CI: 0.33 – 0.97), spontaneous repigmentation (p = 0.0221, OR 0.27, CI: 0.08 – 0.89), and TPO-Ab<10 (p = 0.0216, OR 0.4, CI: 0.18 – 0.9) vitiligo. Additionally, rs3795299 and rs276571 were associated in Köbner+ subgroup (p = 0.0228, OR 2.76, CI: 1.11 – 6.87 and p = 0.0331 OR 0.34, CI: 0.12 – 0.96, respectively), rs7749054 and rs955155 in halo+ subgroup (p = 0.0484, OR 2.3, CI: 0.98 – 5.38 and p = 0.0251, OR 2.41, CI: 1.09 – 5.32, respectively), rs2834167 and rs7282780 in familial vitiligo subgroup (p = 0.0378, OR 0.41, CI: 0.17 – 0.98 and p = 0.0175, OR 2.08, CI: 1.12 – 3.85, respectively).

When comparing allele frequencies among female patients against male patients, rs2257167 minor allele C was significantly more frequent among males (p = 0.0136, OR 3.65, CI: 1.23 – 10.77).

3.2. Haplotype analysis

Haplotype blocks were formed by SNPs of chromosome 1, 6, 12, 19, and 21. According to haplotype association analysis, the most significant differences in haplotype frequencies between patients and healthy controls occurred with Block 2 haplotypes CC and TC of chromosome 12 (harboring *IL26-IL22* genes). The results from this region in early onset, late onset, familial, sporadic, extent<10%, and extent≥10% groups are shown in Table 3 and significant associations from the remaining groups are described in the following section. These haplotypes were formed by alleles of rs12301088 and rs12321603, and the haplotype associations were evident in early onset (CC, p = 0.0249, OR 0.54, CI: 0.31 –0.93; TC, p = 0.0123, OR 2, CI: 1.15 – 3.45), familial (CC, p = 0.008, OR 0.44, CI: 0.23 – 0.82; TC, p = 0.0038, OR 2.46, CI: 1.31 – 4.6), extent≥10% (CC, p = 0.0256, OR 0.62, CI: 0.4 – 0.95; TC, p = 0.0104, OR 1.74, CI: 1.14 – 2.66), and halo+ (CC, p = 0.0049, OR 0.3, CI: 0.12 – 0.73; TC, p = 0.0027, OR 3.56, CI: 1.48 – 8.59) subgroups. The associations concerning TC in the last three subgroups and CC in halo+ subgroup remained significant after correction for multiple testing (p values 0.019, 0.0433, 0.0207, and 0.0251). The remaining haplotype associations involved various subgroups and none of those stayed significant when corrections for multiple testing were applied. Block 1 haplotype GC of chromosome 1 (*IL22RA1*) and Block 2 haplotype ACA of chromosome 6 (*IL22RA2*) were associated in Köbner+ subgroup (p = 0.0175, OR 2.9, CI: 1.17 – 7.19 and p = 0.0243, OR 0.29, CI: 0.09 – 0.9, respectively). Block 1 haplotype CA of chromosome 6 (near *IL20RA*) was associated with sporadic (p = 0.0388, OR 1.45, CI: 1.02 – 2.07) and stable (p = 0.0213, OR 1.93, CI: 1.09 – 3.41) vitiligo. Block 1 haplotype GG of chromosome 12 (near *IFNG*) was associated with halo+ (p = 0.0408, OR 2.61, CI: 1.01 – 6.78). Block 1 haplotype TT of chromosome 19 (*IL28B*) was associated with spontaneous repigmentation (p = 0.0319, OR 2, CI: 1.06 – 3.79) and TPO-Ab<10 (p = 0.0176, OR 1.87, CI: 1.11 – 3.17) vitiligo. Additionally, Block 1 haplotype CT of chromosome 19 was associated in halo+ (p = 0.0433, OR 0.45, CI: 0.2 – 0.99) and Block 2 haplotype TC (*IL29*) in early onset (p = 0.201, OR 7.49, CI: 1.02 – 54.87) subgroup.

When comparing haplotype frequencies among female patients against male patients, significant differences were found in three instances (Table 4). Block 1 haplotype CC of chromosome 1

(*IL22RA1*) was more frequent among females (p = 0.0298, OR 2.99, CI: 1.07 – 8.38), whereas Block 1 haplotype GGC of chromosome 12 (*IFNG*) and Block 1 haplotype TC of chromosome 21 (*IFNAR1*) were more frequent among males (p = 0.0297, OR 2.79, CI: 1.07 – 7.25; p = 0.0173, OR 3.51, CI: 1.18 – 10.47). None of those three associations withstood the correction for multiple comparisons.

4. Discussion

In order to conduct genetic association analyses, 30 SNPs from class II cytokine and their receptor gene regions were genotyped in Estonian case-control sample. There were no associations in whole vitiligo group, but altogether 21 allelic and 20 haplotype associations were found in several subgroups. These occurred with SNPs from five out of seven studied genetic regions. The 1p23.3 locus that contained *F3* gene and 11q23.3 locus that contained *IL10RA* gene did not contribute any statistical results.

The first region under investigation was within 1p36.11 and contained cytokine receptor genes *IL22RA1* and *IL28RA*. Genetic associations appeared in Köbner+ subgroup where G allele of rs3795299 and haplotype GC (composed of rs3795299 and rs16829204 alleles) were significantly more frequent among patients compared to controls. Secondly, alternative haplotype block composed of rs3934861 and rs3795299 was formed exclusively in male-female vitiligo comparative analysis and haplotype CC was significanlty more frequent in females. Two of these SNPs are situated in *IL22RA1* gene and are non-synonymous by function: rs3795299 C/G alleles cause arginine/glycine substitution at position 518 and rs16829204 C/T alleles cause valine/isoleucine substitution at position 205 in the IL-22R1 peptide sequence. The IL-22R1 protein can be coupled with IL-10R2 to form a receptor for IL-22 or with IL-20R2 to form a receptor for IL-20 and IL-24. It is possible that the described genetic mutations cause changes in the formation of these receptors and/or alter their interactions with cytokines, but this cannot be further speculated. In addition, it has been demostrated, that IL-22R1 is a driver of inflammation in mice [19] and variants of *IL22RA1* gene have been linked to common

inflammatory disease chronic rhinosinusitis [20], which enhance the plausibility of its contribution to vitiligo pathogenesis.

The SNPs of 6q23.3 region surrounded the genes *IL20RA*, *IL22RA2* and *IFNGR1*. As a general remark, the 6q23.3 region has been indentified as a susceptibility region for rheumatoid arthritis [21, 22], systemic lupus erythematosus [23] and multiple sclerosis [24]. Several allelic and haplotype associations were found. Non-synonymous SNP rs1342642C/T that causes leucine/phenylalanine substitution at position 382 in the IL-20R1 peptide sequence was associated with stable vitiligo and the given result withstood the correction for multiple testing. The IL-20R1 protein can be coupled with IL-20R2 to form a receptor for IL-19, IL-20 and IL-24 or with IL-10R2 to form a receptor for IL-26. Interestingly, *IL20RA* mRNA expression was decreased in the skin of vitiligo patients in our previous study and especially among those with stable vitiligo [15]. This suggests that the C allele of rs1342642 that was more frequent in stable vitligo group may be related to lower *IL20RA* mRNA levels in those patients. Moreover, the same allele was also significantly more frequent in psoriasis patients in our previous work [25]. The second associated SNP of this region was rs276586 in stable and sporadic vitiligo groups. It also formed a haplotype block with rs276526 and CA haplotypes of this block were significantly more frequent in those groups compared to controls. It was evident in our psoriasis genetic association study that both of these SNPs were in strong linkage disequilibrium with rs276504 in the first intron of *IL20RA* gene [25] and thus these results may reflect changes in IL-20R1 functioning. Our former vitiligo genetic association study involved SNPs rs1184860, rs1167846, and rs1167849 that are postioned between the two associated SNPs discussed above [16]. Nominal associations were found for rs1167849 and haplotypes CTA and CTG, although these did not remain significant after correction for multiple testing. The third significant SNP was rs276571 in Köbner+ group. Although it is an intergenic SNP, it belongs to the same haplotype block with rs719640 and rs276466, the last one being situated in *IL22RA2* gene. Therefore, this association may be related to IL-22BP encoded by *IL22RA2*. Additionally, haplotype ACA of this block was significantly less frequent in Köbner+ vitiligo patients. IL-22BP is a soluble monomeric protein that binds IL-22, functioning as its antagonist. In our previous work, *IL22RA2* mRNA was upregulated in uninvolved

skin of vitiligo patients compared to involved and control skin [12]. Further analysis indicated a downregulation of *IL22RA2* mRNA in involved skin of active vitiligio patients [12]. We have also demonstrated an upregulated state of proinflammatory IL-22 in vitiligo patients [15]. Taken together, these findings suggested substantial changes in IL-22 signaling in vitiligo and the presently described mutations may have an effect of this. The fourth associated SNP was rs7749054 in patients manifesting halo nevi. This SNP in positioned between *IL22RA2* and *IFNGR1*. The latter gene encodes IFN-γR1, a ligand-binding chain of the receptor for inflammation promoting cytokine IFN-γ. Notably, polymorphisms in *IFNGR1* have been associated with allergic diseases [26] and systemic lupus erythematosus [27].

The SNPs of 12q15 region were near the genes *IFNG*, *IL26*, *IL22*, and *MDM1*. This gene cluster has been associated with chronic inflammatory diseases including rheumatoid arthritis, type I diabetes, ulcerative colitis, and asthma [28-31]. The single associated SNP stood out from the rest, namely rs12301088 that produced significantly differing allelic frequencies in early onset, familial, extent ≥ 10%, and halo+ groups. Secondly, haplotypes CC and TC of Block 2 that involved alleles of this SNP and rs12321603 were associated in the same groups. Results in familial and halo+ groups also withstood the correction for multiple testing. None of the four studied SNPs were in the gene coding regions. Since all the above described statistical results came from Block 2, they can be mainly related to *IL26*, *IL22*, and *MDM1* genes that fall in the range of this haplotype block. Only one result involved Block 1 (rs10784680 and rs10878763), when haplotype GG was associated in halo+ group. Exceptional to this was male-female vitiligo comparative analysis, where rs1078468, rs10878763, and rs12301088 were grouped into a haplotype block. The revealed association of haplotype GGC points to *IFNG* gene enclosed in this block. Still, in some of the other groups all four SNPs were included in a single haplotype block, but no haplotype associations occurred in those cases. The expression of *MDM1* and all three cytokine genes of this cluster has been measured in vitiligo patients in the course of our previous studies. *IFNG* mRNA expression was increased in vitiligo involved skin and in PBMCs of the patients [12]. *IL26* levels in skin did not differ between patients and controls [12]. *IL22* was upregulated on mRNA level in PBMCs and on protein level in the sera of the patients [15].

MDM1 mRNA expression was increased in vitiligo skin and in PBMCs [12]. Together, those findings suggested an active role for IFN-γ, IL-22, and MDM1 in vitiligo pathogenesis. The significant SNPs of this study do not directly affect the amino acid composition of these proteins but may be in linkage disequilibrium with the polymorphisms that do or with the ones that are involved in gene regulation.

The SNPs of 19q13.2 region were in the area of *IL28B*, *IL28A*, and *IL29* genes. Together they constitute interferon type III family and signal through a common receptor that is composed of IL-10R2 and IL-28R1. In our analysis, allelic associations were revealed for two SNPs: rs955155 was associated in patients manifesting halo nevi and rs30461 was associated with early onset and female vitiligo. In addition, Block 1 (alleles of rs955155 and rs10853727) halplotype CT was associated with halo nevi and haplotype TT was associated with spontaneous repigmentation and TPO-Ab<10. Block 2 (alleles of rs30461 and rs12972575) haplotype TC was associated with early onset vitiligo. The results concerning rs955155 and Block 1 haplotypes could be primarily related to *IL28B* that is situated within that haplotype block. Remarkably, polymorphisms near *IL28B* gene have been repeatedly associated with hepatitis C virus treatment response and clearance [32] and lately also with allergic disease [33]. The results concerning rs30461 and Block 2 haplotype are inclined towards *IL29*, since rs30461T/C is a non-synonymous SNP that causes asparagine/aspartate substitution at position 188 in IL-29 peptide. The same SNP has been associated with psoriasis in Russian [34] and Estonian (unpublished data) populations. Similarly to this study, the C allele was more prevalent among controls in both instances. With regard to expression, *IL28B* mRNA was found to be elevated and *IL28A* mRNA downregulated in vitligo patients PBMCs [12].

The SNPs of 21q22.11 region were in the area of *IFNAR2*, *IL10RB*, *IFNAR1*, and *IFNGR2* genes. Earlier investigations have yielded genetic associations with various immune-related diseases from this region: multiple sclerosis [35], systemic sclerosis [36], graft-versus-host disease [37], systemic lupus erythematosus [38], and asthma [39]. Several allelic associations were found. Rs2834117 was associated with vitligo sporadic form, spontaneous repigmentation, and TPO-Ab<10. Interestingly, three associations appeared in familial vitiligo group with SNPs rs2834167, rs7282780, and rs2257167. The latter SNP was also associated with female vitiligo and displayed significantly

different allele frequencies when comparing female patients to male patients. The result in female vitiligo group remained significant when correcting for multiple testing. Lastly, in male-female vitiligo comparative analysis, haplotype TC comprising rs7282780 and rs2257167 alleles was significantly more frequent among males. Rs2834167A/G is a non-synonymous SNP that causes lysine/glutamate substitution at postition 47 in IL-10R2 peptide. The A allele was more common in familial vitiligo group, which was also apparent in patients of systemic sclerosis [36] and systemic lupus erythematosus [38] studies. Further, the respective *IL10RB* mRNA had unchanged expression in vitiligo skin, but was upregulated in patients PBMCs [15]. Rs2257167G/C is a non-synonymous SNP that causes valine/leucine substitution at position 168 in *IFNAR1* peptide. Again, the definite functional roles of associated SNPs can not be asserted. Changes in *IFNAR1* and *IFNAR2* functions could affect type I interferon signaling i.e. IFN-α, IFN-β and, IFN-ω. Changes in *IL10RB* could affect IL-10, IL-22, IFN-λ, and IL-26 signaling. Finally, changes in *IFNGR2* could affect proinflammatory action of IFN-γ.

To conclude, the present genetic association study did not find any SNPs to be associated with general vitiligo diagnosis. However, many SNP allele and haplotype frequencies differed significantly from controls when analyzing specific vitiligo subgroups that were defined according to additional characteristics of their phenotypes. The strongest associations were evident for SNPs rs12301088 (near *IL26*) and rs2257167 (*IFNAR1*), and haplotypes that consisted of rs12301088 and rs12321603 alleles (*IL26-IL22*). These results support the suggested role of class II cytokines in vitiligo pathogenesis and remain to be verified in replication genetic studies and functional studies.

Acknowledgements

This study was financially supported by the grant PUT177 from the Estonian Research Council and by the funding from the Estonian Ministry of Education and Research targeted financing SF0180043s07.

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Tables

Table 1. Characteristics of studied SNPs

SNP	Chr region	Base Position	Gene	Major/minor alleles	Function	MAF
rs3934861	1p36.11	24304543	MYOM3	T/C	intron	0.448
rs3795299	1p36.11	24320055	IL22RA1	C/G	R518G	0.349
rs16829204	1p36.11	24327275	IL22RA1	C/T	V205I	0.184
rs1416834	1p36.11	24359247	IL28RA	A/G	intron	0.359
rs3849301	1p21.3	94750787	ABCD3	G/A	intron	0.127
rs4896227	6q23.3	137341703		C/T	intergenic	0.274
rs1342642	6q23.3	137364906	IL20RA	C/T	L382F	0.251
rs276586	6q23.3	137417095		A/C	intergenic	0.48
rs276526	6q23.3	137432224		G/A	intergenic	0.489
rs276571	6q23.3	137468286		G/A	intergenic	0.479
rs719640	6q23.3	137484439		T/C	intergenic	0.479
rs276466	6q23.3	137508307	IL22RA2	A/G	3'UTR	0.197
rs7749054	6q23.3	137542479		T/G	intergenic	0.151
rs1343677	6q23.3	137625487		T/C	intergenic	0.422
rs12197182	6q23.3	137675944		G/A	intergenic	0.129
rs3135932	11q23.3	117369273	IL10RA	A/G	S159G	0.181
rs10784680	12q15	66814383		G/A	intergenic	0.049
rs10878763	12q15	66829965		G/T	intergenic	0.37
rs12301088	12q15	66876215		C/T	intergenic	0.449
rs12321603	12q15	66973117	MDM1	C/T	3' near gene	0.013
rs955155	19q13.2	44421319		C/T	intergenic	0.263
rs10853727	19q13.2	44432303		T/C	intergenic	0.105
rs30461	19q13.2	44480955	IL29	T/C	N188D	0.098
rs12972575	19q13.2	44548086	SAMD4B	C/T	intron	0.045
rs2834117	21q22.11	33464020	C21orf54	G/A	intron	0.198
rs2834133	21q22.11	33485037		T/C	intergenic	0.471
rs2300370	21q22.11	33526427	IFNAR2	G/A	intron	0.319
rs2834167	21q22.11	33562658	IL10RB	A/G	K47E	0.261
rs7282780	21q22.11	33609224		T/A	intergenic	0.311
rs2257167	21q22.11	33637569	IFNAR1	G/C	V168L	0.116

MAF – minor allele frequency among vitiligo patients and healthy controls.

Table 2. Results of allelic association analysis

SNP	Gene	Control MAF	Vitiligo		Early onset		Late onset		Familial		Sporadic		Extent<10%		Extent≥10%		Active		Stable	
			MAF	P value	MAF	P value	MAF	P value	MAF	P value	MAF	P value	MAF	P value	MAF	P value	MAF	P value	MAF	P value
rs1342642	IL20RA	0.262	0.213	0.1714	0.268	0.9258	0.189	0.0795	0.19	0.3041	0.219	0.2835	0.229	0.492	0.196	0.1714	0.257	0.9033	0.083	**0.0058**
rs276586		0.463	0.536	0.0754	0.552	0.1955	0.529	0.1602	0.477	0.8547	0.553	**0.0469**	0.562	0.0693	0.51	0.3844	0.507	0.3414	0.62	**0.0325**
rs12301088		0.438	0.485	0.2499	0.603	**0.0154**	0.435	0.9502	0.652	**0.0048***	0.433	0.923	0.396	0.4414	0.57	**0.0138**	0.486	0.2933	0.481	0.5483
rs30461	IL29	0.108	0.063	0.0683	0.017	**0.0278**	0.083	0.3991	0.023	0.0714	0.075	0.2406	0.076	0.3496	0.051	0.081	0.077	0.2791	0.021	0.0544
rs2834117	C21orf54	0.212	0.149	0.0703	0.12	0.1227	0.161	0.2113	0.211	0.9882	0.131	**0.0354**	0.143	0.1421	0.155	0.2261	0.167	0.2542	0.095	0.0703
rs2834167	IL10RB	0.27	0.232	0.2975	0.217	0.3742	0.239	0.4615	0.13	**0.0378**	0.263	0.87	0.23	0.4036	0.235	0.4657	0.236	0.41	0.222	0.4488
rs7282780		0.305	0.33	0.5173	0.397	0.1496	0.3	0.9132	0.477	**0.0175**	0.285	0.6355	0.298	0.8912	0.362	0.2676	0.321	0.7009	0.354	0.4759
rs2257167	IFNAR1	0.128	0.079	0.0661	0.054	0.1036	0.09	0.2181	0.022	**0.033**	0.097	0.3122	0.096	0.3785	0.062	0.0659	0.077	0.0939	0.083	0.3686

Only SNPs that were associated in at least one of these groups are presented

P value ≤ 0.05 bolded

* P value ≤ 0.05 after 10 000 permutations

Table 3. Results of chromosome 12 haplotype analysis

Haplotype block	rs12301088	rs12321603	Controls	Early onset		Late onset		Familial		Sporadic		Extent<10%		Extent≥10%	
			Freq.	Freq.	P value	Freq.	P value	Freq.	P value	Freq.	P value	Freq.	P value	Freq.	P value
Block 1 (near *IFNG*)	G	G	0.588	0.644	0.407	0.536	0.2714	0.687	0.1858	0.532	0.2145	0.529	0.2762	0.607	0.7181
	G	T	0.360	0.339	0.7544	0.413	0.242	0.269	0.2175	0.428	0.1196	0.430	0.186	0.353	0.9003
	A	G	0.049	0.015	0.242	0.050	0.9966	0.041	0.8064	0.039	0.5825	0.041	0.7048	0.039	0.6329
Block 2 (*IL26-IL22*)	C	C	0.550	0.397	**0.0249**	0.565	0.7502	0.348	**0.0080**	0.567	0.7179	0.604	0.3236	0.430	**0.0256**
	T	C	0.432	0.603	**0.0123**	0.435	0.9563	0.652	**0.0038***	0.433	0.9803	0.396	0.5032	0.570	**0.0104***
	C	T	0.017	0.000	0.3108	0.000	0.1184	0.000	0.3666	0.000	0.1036	0.000	0.1925	0.000	0.1835

P value ≤ 0.05 bolded

* P value ≤ 0.05 after 10 000 permutations

Table 4. Female and male vitiligo comparative haplotype association analysis

Chromosome	Haplotype Block	Haplotype				Female freq.	Male freq.	P value
Chr1	Block 1	rs3934861	rs3795299					
	(*IL22RA1*)	T	C			0.478	0.538	0.4357
		C	G			0.275	0.322	0.5072
		C	C			0.203	0.078	**0.0298**
		T	G			0.044	0.062	0.5902
Chr6	Block 1	rs276586	rs276526					
	(near *IL20RA*)	C	A			0.491	0.566	0.3341
		A	G			0.470	0.399	0.3552
		C	G			0.024	0.018	0.8022
		A	A			0.015	0.018	0.9136
	Block 2	rs276571	rs719640	rs276466	rs7749054			
	(*IL22RA2*)	A	C	A	T	0.398	0.389	0.9048
		G	T	A	T	0.243	0.244	0.9889
		G	T	G	T	0.174	0.113	0.2733
		G	T	A	G	0.141	0.160	0.7261
		A	C	G	T	0.032	0.054	0.4686
		A	C	A	G	0.012	0.024	0.5255
Chr12	Block 1	rs10784680	rs10878763	rs12301088				
	(*IFNG*)	G	G	T		0.499	0.432	0.3934
		G	T	C		0.389	0.350	0.6014
		G	G	C		0.067	0.167	**0.0297**
		A	G	C		0.044	0.033	0.7251
Chr21	Block 1	rs7282780	rs2257167					
	(*IFNAR1*)	T	G			0.601	0.578	0.7641
		A	G			0.352	0.272	0.2742
		T	C			0.045	0.144	**0.0173**

P value ≤ 0.05 bolded