Original paper

Evaluation of polymorphisms and expression of *PTPN22*, *NLRP1* and *TYR* genes in vitiligo patients

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Abstract

Introduction: Vitiligo is a pigmentary disorder associated with a selective loss of melanocytes in the skin, its appendages and mucous membranes.

Aim: The aim of the study was to evaluate the association between the rs2476601 polymorphism of the *PTPN22* gene, the rs2670660 and rs6502867 polymorphisms of the *NLRP1* gene and the rs1847134 and rs1393350 polymorphisms of the *TYR* gene and vitiligo. Another aim was to compare the gene expression in lesional and symmetrically non-lesional skin of vitiligo patients and healthy controls.

Material and methods: The experimental group consisted of 42 patients and the control group consisted of 38 healthy volunteers. The polymorphisms of the genes were assessed with PCR-RFLP technique and gene expression with qRT-PCR technique.

Results: We found that the CT genotype of the *PTPN22* rs2476601 polymorphism is more frequent in vitiligo patients, in the case of the *NLRP1* rs2670660 polymorphism it was the AG genotype, in the *NLRP1* rs6502867 polymorphism they were the CT and CC genotypes and in the *TYR* rs1393350 polymorphism it was the AG genotype. There was no association between vitiligo and the *TYR* rs1847134 polymorphism. We found statistically significant differences in gene expression in the lesional and symmetrical non-lesional skin of vitiligo patients compared to the control group.

Conclusions: Our analysis showed genotypes predisposing to vitiligo. We found that the gene expression is different not only in lesional but also in non-lesional skin of vitiligo patients, what may change the approach to treatment of the disease.

Key words: *PTPN22* gene, *NLRP1* gene, *TYR* gene, vitiligo.

Introduction

Vitiligo is a pigmentary disorder associated with a selective loss of melanocytes leading to depigmentation of the skin, its appendages and mucous membranes. The incidence of the disease is 0.5–4% of the worldwide population [1]. Vitiligo is considered as an autoimmune/autoinflammatory disease developing in genetically predisposed patients [2]. Thanks to genome-wide association studies (GWAS) 50 different loci associated with vitiligo were identified [3–5]. About 85% of associated genes encode proteins involved in the functioning of the immune system and the process of apoptosis [6]. About half of these genes were identified in independent genetic studies of other autoimmune diseases [7].

The *PTPN22* gene is located on chromosome 1 and it encodes the lymphoid-specific tyrosine phosphatase (LYP) [8, 9]. Many studies of the rs2476601 polymorphism showed its association with the higher risk of autoimmune diseases, such as vitiligo, type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus and autoimmune thyroid diseases [10–13].

The *NALP1* gene is located on chromosome 17 and it takes part in controlling of inflammation and apoptosis [14–16]. There is an association between *NALP1* gene polymorphisms and autoimmune diseases such as vitiligo, Addison's disease, type 1 diabetes, celiac disease, systemic lupus erythematosus, rheumatoid arthritis and systemic sclerosis [17–23].

The *TYR* gene is located on chromosome 11, it encodes tyrosinase recognized as the main autoantigen in vitiligo [24, 25].

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Aim

The aim of the study was to evaluate the association between the rs2476601 polymorphism of the *PTPN22* gene, the rs2670660 and rs6502867 polymorphisms of the *NLRP1* gene and the rs1847134 and rs1393350 polymorphisms of the *TYR* gene and vitiligo. Other aims were to analyse the relationship between the polymorphisms of the investigated genes and their expression; to analyse the relationship between polymorphisms of the *PTPN22* and *NLRP1* genes and concomitant autoimmune diseases in vitiligo patients; to compare expression of *PTPN22*, *NLRP1* and *TYR* genes in lesional and symmetrically non-lesional skin of vitiligo patients and in the healthy skin of the control group; and to analyse the relationship between expression of *PTPN22*, *NLRP1* and *TYR* genes and clinical features as age, sex, age of vitiligo onset and the VASI score in vitiligo patients.

Material and methods

Patients

The material consisted of skin biopsies collected from 42 Caucasian vitiligo patients of the Dermatology Clinic and skin biopsies collected from 38 Caucasian healthy volunteers hospitalized in the Plastic Surgery Clinic of the University Hospital in Bydgoszcz, Poland. The study was conducted with the consent of the Bioethics Committee of the Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun (Protocol No. 197/2014).

The inclusion criteria of the experimental group were signing the informed consent and diagnosis of vitiligo at least 6 months before the study. Patients with acute inflammatory skin diseases, pregnant women, patients less than 18 years old and incapacitated persons were excluded from the study. The inclusion criteria of the control group was signing the informed consent. The exclusion criteria were diagnosis of vitiligo, personal history or family history of autoimmune diseases, acute and chronic skin diseases, pregnancy, age less than 18 and incapacitation.

The vitiligo patients were asked about the age of vitiligo onset, possible relationship between the occurrence of the first lesions and environmental factors, previous treatment of vitiligo, concomitant autoimmune diseases, family history of vitiligo or other autoimmune diseases, illnesses, surgical procedures, and medications. The extent of skin lesions was assessed based on the VASI (Vitiligo Area Scoring Index) score. Laboratory tests of the blood included morphology, glucose test, thyroid function tests (TSH, fT3, fT4), antithyroglobulin antibodies test, antithyroid peroxidase antibodies test and antinuclear antibodies test.

Methods

The skin biopsies of the experimental group were collected from the centre of the vitiligo lesions and symmetrically from the healthy skin. The skin was not exposed to sunlight/phototherapy and not treated with topical glucocorticosteroids/calcineurin inhibitors for at least 2 months prior to the study. The skin samples of the control group were a part of the material intended for disposal after the completed surgical procedure. They were collected from the sites unexposed to UV radiation. The skin biopsies were stored at –80°C until further analysis.

The isolation of the genomic DNA was performed using a commercially available isolation kit, Genomic Mini A&A Biotechnology. The spectrophotometric analysis was performed to assess the concentration and purity of obtained DNA material. The analysis of the DNA was performed using polymerase chain reaction (PCR) enabling amplification of the specific DNA fragment in a large number of copies in a relatively short period of time. The amplified fragment was exposed to a specific restriction enzyme recognizing the characteristic site within the DNA (restriction fragment length polymorphism, RFLP) (Table 1).

Table 1. Starters used for the PCR reaction in the study (IBB PAN)

Gene	Polymorphism	Sequence
PTPN22	rs2476601	Forward: 5'-TCACCAGCTTCCTCAACCACA-3' Reverse: 5'-GATAATGTTGCTTCAACGGAATTTA-3'
NLRP1	rs2670660	Forward: 5'-ATACCCAGGTGTTCAGGAGC-3' Reverse: 5'-GCCTGTGTTGTACCTTCAGC-3'
NLRP1	rs6502867	Forward: 5'-TGGGGGTTTGGGTTCTTAG-3' Reverse: 5'-TTTCTCATGATAGTTTGGGTGTG-3'
TYR	rs1393350	Forward: 5'-ACGTTGGATGGGAAGGTGAATGATAACACG-3' Reverse: 5'-ACGTTGGATGATGCGTGCATATCCACCAAC-3'
TYR	rs1847134	Forward: 5'-TGGGGAGCGTTCCAACAG-3' Reverse: 5'-TGTTGGCCACCCATGTATTC-3'
G6PD	Reference gene	Forward: 5'-TGGGACCTAGCCCTCCATA-3' Reverse: 5'-AGGGGAATGAGGGAGAGTG-3'

For the isolation of the total RNA, the commercial kit of A&A Biotechnology was used. After the isolation, every sample was analysed for the concentration and purity with the spectrometer. The obtained RNA material was transcribed on the complementary DNA (cDNA) using reverse transcriptase (RT) with a commercial kit *dATR RT-PCR* EURx and random hexamers as starters. The obtained cDNA was subjected to quantitative analysis qRT-PCR with intercalating dyes *EvaGreen* and relative quantification of the expression level. As the reference gene, *G6PD* (glucose-6-phosphate dehydrogenase) gene was used. The UPL (Universal Probe Library) program was used for selection of the reference gene and the starters for examined genes and the reference gene.

Statistical analysis

The statistical analysis was performed using Statistica 13 software. The non-parametric tests were used in the study because of the non-parametric distribution of the results assessed with Shapiro-Wilk test. The Wilcoxon test was used for dependent parameters (comparison of the results obtained within the experimental group). The Spearman's rank correlation test was used for the assessment of correlation of the analysed parameters. Tests used for assessment of differences between the groups was Mann-Whitney test for two groups and Kruskal-Wallis test for three groups. The test used for the analysis of the variables in the nominal scale was Pearson's χ^2 test and Yates test. At the verification of all analysis the significance factor was $\alpha = 0.05$, what enabled to consider statistically significant variables at $p < 0.05$.

Results

42 patients with non-segmental vitiligo and 38 healthy controls were enrolled in the study. The demographic and clinical characteristics of the study subjects are shown in Table 2.

Table 3 shows differences in the incidence of genotypes between the groups.

In the case of the rs2476601 polymorphism of the *PTPN22* gene, the differences between the groups were statistically significant ($p = 0.001$). The heterozygous CT genotype was more frequent in the experimental group, while homozygous CC and TT genotypes were more frequent in the control group.

In the case of the rs2670660 polymorphism of the *NLRP1* gene, the differences were statistically significant between the investigated groups ($p = 0.014$). Homozygous GG and heterozygous AG genotypes were more frequent in the experimental group while the homozygous

Table 2. Demographic and clinical characteristics of the study subjects

Parameter	Experimental group ($n = 42$)	Control group ($n = 38$)
Demographic details:		
Male, *n* (%)	14 (33.33)	14 (36.84)
Female *n* (%)	28 (66.67)	24 (63.16)
Age, mean ± SD [years]	40.98 ±15.09	58.45 ±20.20
Clinical details:		
VASI score, mean ± SD	5.016 ±2.44	
Age of onset of the disease, mean ± SD [years]	29.69 ±15.78	
Duration of the disease, mean ± SD [years]	11.29 ±10.53	
Family history of vitiligo, *n* (%)	13 (30.95)	
Personal history of autoimmune	18 (42.86%)	
Diseases, *n* (%):		
Autoimmune thyroid diseases	14 (33.33)	
Psoriasis	3 (7.14)	
Triggering factor, *n* (%):	23 (54.76)	
Emotional stress	16 (38.09)	
Sunburn/skin injuries	7 (16.67)	
Antibodies, *n* (%):		
Elevated antithyroid peroxidase antibodies	11 (26.19)	
Elevated antithyroglobulin antibodies	4 (9.52)	
Wlevated antinuclear antibodies	10 (23.81)	

Table 3. Statistical analysis of genotype distribution of the *PTPN22*, *NLRP1* and *TYR* polymorphisms in the experimental and control group

Genotype	Experimental group		Control group		Test result	*P*-value
	N	%	*N*	%		
PTPN22 rs2476601:						
CC	14	33.33	16	42.11	13.656	0.001
CT	22	52.38	6	15.79		
TT	6	14.29	16	42.11		
NLRP1 rs2670660:						
AA	8	19.05	18	47.37	8.482	0.014
AG	19	45.24	8	21.05		
GG	15	35.71	12	31.58		
NLRP1 rs6502867:						
CC	17	40.48	9	23.68	9.532	0.009
CT	20	47.62	13	34.21		
TT	5	11.90	16	42.11		
TYR rs1393350:						
AA	17	40.48	6	15.79	19.946	< 0.001
AG	24	57.14	16	42.11		
GG	1	2.38	16	42.11		
TYR rs1847134:						
AA	8	19.05	13	34.21	2.369	0.306
AC	19	45.24	14	36.84		
CC	15	35.71	11	28.95		

Pearson's c2 test.

AA genotype was more frequent in the control group. In the case of the rs6502867 polymorphism the differences were also statistically significant ($p = 0.009$). Homozygous CC and heterozygous CT genotypes were more frequent in the experimental group while the homozygous TT genotype was more frequent in the control group.

In the case of the rs1393350 polymorphism of the *TYR* gene, the differences between the groups were statistically significant ($p < 0.001$). The analysis showed that especially the homozygous AA genotype and to a lesser degree the AG genotype were more frequent in the experimental group while the homozygous GG genotype was more frequent in the control group. In the case of the rs1847134 polymorphism the differences were not statistically significant ($p = 0.306$).

We performed the analysis of the correlation of the investigated gene polymorphisms and incidence of concomitant autoimmune disorders but the results were not statistically significant ($p > 0.1$).

Statistical analysis of the gene expression

Table 4 shows statistical analysis of the expression of the genes between the healthy skin of the control group and the healthy skin of the experimental group.

There were no statistically significant differences between the expression of the *PTPN22* gene between the healthy skin of the control group and the healthy skin of the experimental group, although there was a statistical trend ($p = 0.069$). The analysis of the expression of the *NLRP1* gene and *TYR* gene showed statistically significant differences ($p < 0.001$). In the healthy skin of the experimental group, the expressions of these genes were significantly lower than in the healthy skin of the control group.

Table 5 shows statistical analysis of the expressions of the genes between the lesional and symmetrical non-lesional skin of the experimental group.

Analysis of the expression of the investigated genes in lesional and non-lesional skin of the experimental group showed statistically significant differences ($p > 0.001$). Expressions of the *PTPN22* and *NLRP1* genes were increased in the lesional skin, and in the case of the *TYR* gene, the expression was lower comparing to symmetrical non-lesional skin.

We investigated the relationship between the expression of *PTPN22*, *NLRP1* and *TYR* genes and age of the patients (non-lesional skin) and healthy volunteers. We found a correlation only in the case of the *TYR* gene

Table 4. Statistical analysis of differences in expression of the genes between the healthy skin of the control group and the healthy skin of the experimental group

Group	*N*	$\bar{x}$	SD	Min.	Q_1	Me	Q_3	Max.	Test result	*P*-value
mRNA expression of the PTPN22 gene:										
Experimental	42	1.90	0.39	1.0	1.8	1.9	2.0	2.9	1.818	0.069
Control	38	1.70	0.56	1.0	1.1	1.8	2.0	3.0		
mRNA expression of the NLRP1 gene:										
Experimental	42	16.6	1.53	13.9	15.3	16.7	17.9	19.2	–6.500	< 0.001
Control	38	18.4	0.90	17.0	17.9	18.3	19.0	21.0		
mRNA expression of the TYR gene:										
Experimental	42	14.2	1.99	10.1	12.5	14.5	15.9	18.2	–7.655	< 0.001
Control	38	20.0	1.05	17.0	19.5	20.0	21.0	22.0		

Mann-Whitney test.

Table 5. Statistical analysis of differences in expression of the genes between the lesional and symmetrical non-lesional skin of the experimental group

Skin sample	*N*	$\bar{x}$	SD	Min.	Q_1	Me	Q_3	Max.	Test result	*P*-value
mRNA PTPN22 expression:										
Lesional skin	42	2.94	0.64	1.8	2.4	2.9	3.5	4.0	5.645	< 0.001
Non-lesional skin	42	1.90	0.39	1.0	1.8	1.9	2.0	2.9		
mRNA NLRP1 expression:										
Lesional skin	42	19.68	1.21	16.3	19.0	19.6	20.9	21.9	5.645	< 0.001
Non-lesional skin	42	16.60	1.53	13.9	15.3	16.7	17.9	19.2		
mRNA TYR expression:										
Lesional skin	42	0.87	0.15	0.6	0.8	0.9	1.0	1.1	5.645	< 0.001
Non-lesional skin	42	14.22	1.99	10.1	12.5	14.5	15.9	18.2		

Wilcoxon test.

and based on Spearman's rank correlation test, the result was $r = –0.375$, with p-value of 0.020. The test showed that expression of the *TYR* gene decreased with age. Conducting an analogous analysis in both groups without any significant difference in age, the correlation was not found (smaller age range excluded the significant relationship).

There was no association between the gene expression and sex in both groups ($p > 0.3$). We also did not find any statistically significant differences between gene expression and VASI index ($p > 0.2$) or age of disease onset ($p > 0.3$) within the experimental group.

The analysis of the association between the genotypes of each investigated polymorphism and gene expression did not show statistically significant results ($p > 0.06$).

Discussion

The pathogenesis of vitiligo is not fully understood. Genetic studies of polymorphisms and gene expressions are necessary to broaden the knowledge about the disease, determine the genetic risk of the disease and improve the effectiveness of therapies as none of them guarantee full repigmentation of vitiligo lesions.

The experimental group consisted of 42 vitiligo patients. Women constituted 66.67%, and men 33.33%. Many studies showed the same prevalence of the disease in women and men [26–29]. But in some studies, as in ours, the disease was more frequent in women, what might be the result of women's greater attention to aesthetic aspects of the disease [30–32].

The average age of first lesions onset in vitiligo patients ranges between 18 and 32 years [28, 29, 33], what is consistent with the experimental group as the average age in the study was 29.69 years.

In the experimental group 54.76% of patients observed a relationship between the lesion occurrence and the environmental factors. Most of them (38.1%) observed the association with emotional stress, others with physical factors (excessive UV exposure/injury). In studies on vitiligo, factors such as malnutrition, stress, injuries, medications, infections and chemical agents were considered to be the main environmental triggering fac-

tors, but it is difficult to determine which of them is the most relevant, because the results are divergent [34, 35].

In the study, 30.95% of the patients had a family history of vitiligo. Depending on the population, publications show values ranging from 9% to 36% [27, 36–41].

The comorbidity of autoimmune diseases in patients with vitiligo varies between populations (13.1–41.5%) [29, 38, 42]. However, in all cases the frequency is higher than in the general population, where the prevalence of autoimmune diseases reaches 3–8% [43]. In the experimental group, 42.86% of patients had at least one autoimmune disease, most frequently autoimmune thyroid diseases (33.3%).

The *PTPN22* gene is located on chromosome 1p 13.3-13.1. It encodes cytoplasmic lymphoid-specific phosphatase (Lyp), which plays an important role in the negative control of T cells activation [8, 9]. The phosphatase is expressed on hematopoietic cells and on cells of the immune system, especially on neutrophils and natural killer cells [44].

Agarwal *et al.* [45] conducted a meta-analysis of seven studies published up to 2016, including 2,094 cases and 3,613 controls, to verify the association of the 1858 C/T polymorphism of the *PTPN22* gene and vitiligo. They concluded that this allele predisposes to vitiligo in Europeans, but not in Asians and Americans. In recent studies, Huraib *et al.* [46] and Rajendiran *et al.* [47] showed an association of this polymorphism in the Saudi and South Indian Tamil populations, respectively. However, Akbas *et al.* [48] did not observe such correlations in the Turkish population.

In our study we showed statistically significant differences ($p = 0.001$) in the frequency of genotypes between the experimental and the control group, what confirms the association of the 1858 C/T polymorphism of the *PTPN22* gene with vitiligo. CT heterozygotes were the most frequent genotype in the experimental group, what is consistent with other studies [46, 47, 49, 50].

Comparing the expression of the *PTPN22* gene in the healthy skin of the experimental and the control group the difference was not statistically significant, there was only a statistical trend. However, there was a significant difference ($p < 0.001$) between lesional and non-lesional skin of the experimental group. Within the vitiligo lesions the expression of the gene was increased.

The results differ from the study conducted by Tang *et al.* [51] where the gene expression between the lesional and non-lesional skin in patients with vitiligo did not show statistically significant differences ($p > 0.05$). The study was conducted on a smaller number of samples (17 samples from lesional and 17 samples of non-lesional skin). In addition, biopsies were taken from the lesional skin and from the healthy skin surrounding the lesions, not from distinct symmetrical areas of healthy skin, as in our study.

The inflammasome is a protein complex found mainly in the cytosol of active cells of the immune system. Activation of NLRP1 (NOD-Like Receptor Family Pyrin Domain Containing 1), which is a part of inflammasome, causes release of active caspase-1, IL-1 and IL-18, what induces pyroptosis [52]. The *NLRP1* gene is located on chromosome 17. The studies show that in cultured cells even a slight increase in its expression can induce apoptosis [53].

The genetic association of the *NLRP1* gene variants with vitiligo was found in studies of the US, UK, Romanian, Jordanian and Indian populations. In the case of rs2670660 and rs6502867 polymorphisms it was shown that they contain high-risk alleles of the disease [18, 19, 54, 55].

In the case of the rs2670660 polymorphism, we concluded that the heterozygous AG genotype predisposes to vitiligo, and the homozygous AA genotype reduces the risk of the disease.

In the case of the rs6502867 polymorphism, we showed that the homozygous CC genotype predisposes to the disease, and the homozygous TT reduces the risk of developing vitiligo.

Results of the Dwivedi *et al.* [55] analysis differ. In the case of the rs2670660 polymorphism, the AG genotype occurred with a comparable frequency in both groups. In the case of the rs6502867 polymorphism, as in our study, the CC genotype was more frequent in the experimental group, but the difference was not statistically significant.

In the study conducted by Alkhateeb and Qarqaz [54] within the Arab population, in the case of the rs2670660 polymorphism, as in ours, the AG genotype was more frequent in the experimental group and the AA genotype was more frequent in the control group. These results are consistent with our observations, but they were not statistically significant. In the case of the rs6502867 polymorphism in the study of Alkhateeb and Qarqaz [54], the CT genotype was more frequent in the experimental group, what is inconsistent with the results of our research.

Li *et al.* [56] performed the meta-analysis to investigate the association between rs12150220, rs2670660 and rs6502867 polymorphisms of the *NLRP1* gene and vitiligo and concomitant autoimmune diseases. In the case of the rs2670660 polymorphism, they analysed 5 studies with negative results. In the case of the rs6502867 polymorphism they showed a possible increased risk in CC vs. TT+TC genotypes, what is consistent with our results as the CC genotype was the most frequent in the experimental group. As in our study, they did not observe any association between rs2670660 and rs6502867 polymorphisms and concomitant autoimmune diseases in vitiligo patients. The limitations of this meta-analysis were sizes of groups, their heterogeneity and small number of studies that met the requirements of the analysis.

The expression of the *NLRP1* gene was significantly higher in the lesional skin comparing to non-lesional skin

of the experimental group and healthy skin of the control group ($p < 0.001$).

Dwivedi *et al.* [55] and Bhardwaj *et al.* [57] showed a significantly higher expression of the *NLRP1* gene in the blood of vitiligo patients than in the control group. In the latter study, the NLRP1 protein expression was increased in skin samples of patients with active disease. In the study 5 skin biopsies from the patients and 3 biopsies from the healthy volunteers were collected.

Marie e*t al.* [58] showed in 14 vitiligo patients that the expression of the NLRP1 protein was increased in both melanocytes and keratinocytes in the periphery of enlarging vitiligo lesions while the concentration of NLRP1 in the affected skin was mostly low.

Three enzymes, tyrosinase (TYR), tyrosinase related protein 1 (TRP1) and tyrosinase related protein 2 (TRP2), are involved in the melanogenesis process, but only TYR is essential [59]. Many studies showed that tyrosinase is the primary antigen that induces synthesis of autoantibodies in vitiligo patients [60–63]. However genetic studies on the *TYR* gene are not that obvious. Jin *et al.* [5] conducted the GWAS study in a Caucasian population comparing the genotypes of 1,514 patients with vitiligo with a control group of 2,813 people. Two SNPs (rs1847134 and rs1393350) related to the *TYR* gene region were identified and haplotype analysis showed a strong association with a block of six SNPs [5].

In our study we showed statistically significant differences in the frequency of genotypes between the experimental and the control group in the case of rs1393350 polymorphism. We showed that the homozygous AA genotype predisposes to vitiligo, while the homozygous GG genotype reduces the risk of developing the disease. These results differ from the results of the study conducted by Jin *et al.* [5], where the G allele was shown as the risk allele of vitiligo.

In the case of the rs1847134 polymorphism, in our study, there were no statistically significant differences between the groups. However, Jin *et al.* [5] showed that allele A is the risk allele of vitiligo.

Expression of the *TYR* gene in the lesional skin was lower comparing to symmetrical non-lesional skin and the skin of the control group. Interestingly, also the expression of the *TYR* gene in the non-lesional skin of vitiligo patients was significantly lower compared to the control group.

Mansuri *et al.* [64] analysed the expression of the *TYR* gene in three groups (lesional and non-lesional skin of the experimental group and healthy skin of the control group) showing results consistent with ours.

Similarly, Yu *et al.* [65] compared the gene expression in three groups showing a lower expression in vitiligo lesions compared to the non-lesional skin of 23 vitiligo patients and the normal skin of 16 healthy volunteers. Contrary to our study, they did not observe statistically significant differences in the expression of the *TYR* gene between non-lesional skin of vitiligo patients and the normal skin of the control group.

Some scientists speculate that the decreased expression of most melanocyte-related genes (including *TYR*) within vitiligo lesions results from the death of melanocytes [65]. However, this does not explain the fact that some studies, including ours, showed that the expression of the *TYR* gene in patients with vitiligo is also decreased within the skin distant from the vitiligo lesions. It might be a sign of a subclinical melanocyte destruction and genetic predisposition in vitiligo patients.

Our analysis showed genotypes predisposing to vitiligo but did not show genotypes increasing the risk of other autoimmune diseases. The limitation of this study was the size of the experimental group. However, the epidemiological analysis clearly showed an increased incidence of these diseases among vitiligo patients compared to the general population.

We did not show any relationship between polymorphisms of investigated genes and genetic expression, what indicates contribution of additional factors influencing the gene expression, e.g. miRNA molecules or oxidative stress.

We showed statistically significant differences of gene expression both in the lesional and non-lesional skin of the experimental group compared to the control group. Based on these observations, it seems that treatment of the entire surface of the skin may increase the chance of obtaining optimal therapeutic effects and may reduce the risk of developing new lesions.

Conflict of interest

The authors declare no conflict of interest.

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