Original paper

Genetic similarities and differences between discoid and systemic lupus erythematosus patients within the Polish population

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Abstract

Introduction: Many studies have shown that some SNPs might be a risk factor for systemic lupus erythematosus (SLE), but little is known about potential susceptibility loci of the skin types of the disease. Discoid lupus erythematosus (DLE) is the most common form of the cutaneous lupus erythematosus. Nevertheless, a genetic contribution to DLE is not fully recognized.
Aim: We aimed to analyze three SNPs located in the *STAT4* (rs7574865), *ITGAM* (rs1143679) and *TNXB* (rs1150754) genes in both DLE and SLE patients from Poland.
Material and methods: SNPs were genotyped using real-time polymerase chain reaction (PCR). Statistical significance of the differences between patient and control groups in both allele and genotype frequencies were calculated using two tailed Fisher's exact test. The correction for multiple testing by the Bonferroni adjustment and odds ratio were also calculated.
Results: For the first time, we have shown that the polymorphisms located in the *STAT4* (rs7574865), but not in the *ITGAM* (rs1143679) nor the *TNXB* (rs1150754) genes, might be associated with the development of DLE within the Polish population. The variation of the three investigated SNPs was found to be associated with SLE in our dataset.
Conclusions: The results of our study suggest differences in the molecular background between DLE and SLE within the Polish population.

Key words: discoid lupus erythematosus, systemic lupus erythematosus, SNP, genotype, allele, polymorphism.

Introduction

Lupus erythematosus (LE) is a complex, heterogeneous autoimmune disease that manifests with a variety of clinical symptoms [1–3]. Some LE types, like discoid lupus erythematosus (DLE), primarily affect the skin, whereas others, like systemic lupus erythematosus (SLE), affect many organs [1, 2]. The SLE susceptibility is influenced by genetic, hormonal and environmental factors [4, 5]. Although previous studies have shown that some SNPs might be a risk factor for SLE, little is known about potential susceptibility loci, which may be associated with the development of the skin types of the disease. The DLE is the most common form of the cutaneous LE, which in about 16.7% of cases progresses to SLE [6]. The genetic background of DLE may be similar to that of SLE [4], but the genetic contribution to DLE has not been fully recognized as yet.

In our current study, we have selected three SNPs found to be strongly associated with SLE within the European origin [5] population and analyzed their variation in DLE and SLE patients from within the Polish population.

Aim

The aim of this study was to investigate the variability of rs7574865 (located in the *STAT4* gene), rs1150754 (located in the *TNXB* gene) and rs1143679 (located in the

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ITGAM gene) SNPs in SLE and DLE patients from the Polish population.

Material and methods

The study was approved by the Bioethics Committee of the Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University in Bydgoszcz, Poland (statements no. KB 223/2013 and KB 562/2013).

Patients and clinical data

All participants (patients and healthy volunteers) gave informed written consent to participate in the study. The DLE was diagnosed based on commonly accepted clinical, histological and immunofluorescence findings [1]. The SLE was diagnosed according to revised American College of Rheumatology criteria [2]. Altogether, 21 DLE and 35 SLE patients from the Polish population were recruited for the study (clinical characteristics are given in Table 1). The control group consisted of 50 unrelated healthy volunteers from the Polish population (60% were women). Buccal swabs or blood samples were collected from DLE, SLE and control groups at the Department of Dermatology, Sexually Transmitted Diseases and Immunodermatology in Bydgoszcz (Poland). Clinical data for the DLE and SLE cases were obtained from the medical records.

Genotyping

DNA was isolated from blood samples or buccal swabs using the *GeneMatrix Bio-Trace DNA Purification Kit* according to the manufacturer's protocols (Eurx, Gdansk, Poland). The rs7574865, rs1150754 and rs1143679 SNPs were genotyped using *TaqMan assays* (Life Technologies) by Real-Time PCR on a *ViiA™7 Real-Time PCR System* (Applied Biosystems). Genotyping was performed according to the manufacturer's instructions. Allele discrimination was achieved by fluorescence detection.

Statistical analysis

The *Arlequin* software v. 3.1 was used to determine the Hardy-Weinberg equilibrium (HWE) [7]. Statistical significance of the differences between patient and control groups in both allele and genotype frequencies were calculated using two tailed Fisher's exact test. Associations between clinical manifestations and allele or genotype distribution in SLE patients versus controls were determined by two tailed Fisher's exact test. Moreover, the odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. The *p*-values < 0.05 were considered as statistically significant. Correction for multiple testing was performed using the Bonferroni adjustment. Statistical calculations were performed using Statistica v. 12.5 (StatSoft Inc.) software.

Table 1. Clinical and demographic characteristics of the Polish discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) patients

Parameter	DLE *N* (%)	SLE *N* (%)
Female	12 (57.1)	31 (88.6)
Malar rash	10 (47.6)	30 (85.7)
Discoid rash	21 (100.0)	5 (14.3)
Photosensitivity	19 (90.5)	32 (91.4)
Oral ulcers	4 (19.0)	12 (32.3)
Arthritis	0 (0.0)	14 (40.0)
Serositis	0 (0.0)	3 (8.6)
Renal disorder	0 (0.0)	12 (32.3)
Neurologic disorder	0 (0.0)	6 (17.1)
Hematologic disorder	0 (0.0)	17 (48.6)
ANA positive	12 (57.1)	34 (97.1)

Results

Three SNPs (rs7574865, rs1150754 and rs1143679) were successfully genotyped in all individuals from the DLE, SLE and control groups. The allelic frequencies of rs7574865 and rs1143679 loci were in Hardy-Weinberg equilibrium in all of the DLE, SLE and control groups. Also, the allelic frequencies of rs1150754 locus showed no deviation from Hardy-Weinberg equilibrium in the DLE and control groups. The only statistically significant deviation from HWE was observed in the SLE cases for rs1150754 locus ($p = 0.002$). Therefore, in SLE patients, the hypothesis that the population is in Hardy-Weinberg frequencies for rs1150754 locus was rejected, which suggests that this SNP might be a susceptibility locus to develop SLE.

The summary of allelic and genotype associations with DLE or SLE are given in Table 2.

STAT4 polymorphism associations with LE

The differences between DLE patients and healthy controls were not statistically significant for any allele. The only statistically significant differences between DLE patients and healthy controls were observed for the TT genotype of rs7574865 ($p = 0.0233$, odds ratio (OR) = ∞ with 95% CI = 0 – infinity). Nevertheless, these differences did not reach statistical significance after the Bonferroni correction ($p_B = 0.0699$). A statistically significant prevalence of the TT genotype of rs7574865 was also found in SLE patients ($p_B = 0.0297$, OR = ∞ with 95% CI = 0 – infinity). Moreover, we found an association of this genotype with the presence of ANA (Bonferroni corrected $p_B = 0.027$, OR = ∞ with 95% CI = 0 – infinity) in SLE individuals.

Table 2. SNPs association results in Polish discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) patients

SNP (gene)	Allele/ genotype	*N* (%)		Fisher's *p*-value	Bonferroni corrected *p*-value (p_B)	*N* (%)	Fisher's *p*-value	Bonferroni corrected *p*-value (p_B)
		Controls	SLE			DLE		
rs7574865 (STAT4)	G	79 (79.0)	47 (67.1)	0.1190	0.3570	28 (66.7)	0.1379	0.4137
	T	21 (21.0)	23 (32.9)			14 (33.3)		
	GG	29 (58.0)	17 (48.6)	0.5075	1.0000	10 (47.6)	0.4459	1.0000
	GT	21 (42.0)	13 (37.1)	0.8222	1.0000	8 (38.1)	0.7977	1.0000
	TT	0 (0.0)	5 (14.3)	**0.0099**	**0.0297**	3 (14.3)	**0.0233**	0.0699
rs1150754 (TNXB)	A	17 (17.0)	24 (34.3)	**0.0112**	**0.0336**	5 (11.9)	0.4669	1.0000
	G	83 (83.0)	46 (65.7)			37 (88.1)		
	AA	0 (0.0)	0 (0.0)	1.0000	1.0000	0 (0.0)	1.0000	1.0000
	GA	17 (34.0)	24 (68.6)	**0.0021**	**0.0063**	5 (23.8)	0.5748	1.0000
	GG	33 (66.0)	11 (31.4)	**0.0021**	**0.0063**	16 (76.2)	0.5748	1.0000
rs1143679 (ITGAM)	A	11 (11.0)	17 (24.3)	**0.0343**	0.1029	9 (21.4)	0.1175	0.3525
	G	89 (89.0)	53 (75.7)			33 (78.6)		
	AA	0 (0.0)	1 (2.8)	0.4118	1.0000	0 (0.0)	1.0000	1.0000
	GA	11 (22.0)	15 (42.9)	0.0558	0.1674	9 (42.9)	0.0894	0.2682
	GG	39 (78.0)	19 (54.3)	**0.0324**	0.0972	12 (57.1)	0.0894	0.2682

TNXB polymorphism associations with LE

We have not found any statistically significant differences in the allele or genotype frequencies of rs1150754 between DLE and normal controls. However, the frequency of the *TNXB* allele A was about twice as high in the SLE patients compared to that of the healthy individuals (p_B = 0.0336, *OR* = 2.54 with 95% CI: 1.24–5.22). Moreover, the GA genotype of rs1150754 was about two times more frequent in the SLE group compared to that of the healthy volunteers (p_B = 0.0063, *OR* = 4.24 with 95% CI: 1.68–10.66). The A allele of rs1150754 was observed statistically more frequently in the SLE patients with photosensitivity (p_B = 0.0261, *OR* = 2.74 with 95% CI: 1.32–5.68), renal disorder (p_B = 0.0156, *OR* = 4.13 with 95% CI: 1.58–10.76) or the presence of ANA (p_B = 0.0486, *OR* = 2.49 with 95% CI: 1.21–5.15). We have also found statistically significant associations between the *TNXB* GA genotype and malar rash (p_B = 0.0174, *OR* = 3.88 with 95% CI: 1.49–10.12), photosensitivity (p_B = 0.0042, *OR* = 4.96 with 95% CI: 1.89–13.05), arthritis (p_B = 0.0486, *OR* = 4.85 with 95% CI: 1.32–17.79), renal disorder (p_B = 0.0018, *OR* = 21.35 with 95% CI: 2.54–179.53) and the presence of ANA (p_B = 0.0108, *OR* = 4.06 with 95% CI: 1.61–10.25).

ITGAM polymorphism associations with LE

Although the A allele and GA genotype of rs1143679 in DLE patients were found at a frequency of almost twice as high as that of the normal controls, these differences did not reach statistical significance (p_B = 0.3525 and p_B = 0.2682, respectively). However, a statistically significant higher frequency of the ITGAM allele A was observed in SLE patients in comparison to healthy volunteers (*p* = 0.0343, *OR* = 2.59 with 95% CI: 1.13–5.96). Despite an approximately two times higher frequency of the *ITGAM* allele A in SLE patients in comparison to healthy volunteers, the result was not significant after the Bonferroni correction (p_B = 0.1029). Also, GA + AA genotype frequencies of rs1143679 were significantly higher in the SLE group than in the control group (*p* = 0.0324, *OR* = 0.33 with 95% CI: 0.13–0.86). Nevertheless, the results were not significant after the Bonferroni correction (p_B = 0.0972).

Discussion

Increased interferon α signalling was shown to be a pathogenic factor in both DLE and SLE [8–10]. Kariuki *et al.* [11] have shown that the T allele of rs7574865, located in intron 3 of the *STAT4* gene [12], is associated with dysregulation of the interferon α pathway and upregulation of the interferon α induced gene expression. Previous studies have also revealed that the T allele of rs7574865 locus has been associated with an increased risk of SLE in individuals of European ancestry [12]. In our current study we have shown that the TT genotype of rs7574865 (*STAT4*) increases the risk for SLE. We have also found strong associations between the TT genotype of the *STAT4* gene and the presence of ANA in SLE patients, which coincides with previous observations concerning European populations of SLE patients [12].

However, in contrast to previous findings [12], we have not detected any statistically significant associations between renal disorder and the frequency of T allele or the TT genotype in Polish SLE patients. Moreover, we have found differences in TT genotype frequencies between DLE patients and healthy controls for rs7574865 in the Polish population (p = 0.0233), but the result was not significant after the Bonferroni correction (p_B = 0.0699). Taking into account also the fact that about 17% of DLE patients progress to SLE [6], the contribution of *STAT4* variation to DLE pathogenesis should be further verified. The results of our study suggest an association between *STAT4* polymorphism and DLE. This may further indicate that the dysregulation of interferon α signalling pathway, which is observed in both DLE [9] and SLE patients [11] may have a common molecular background.

The rs1150754 is located in the intronic region of the *TNXB* gene [13], which encodes a glycoprotein of the extracellular matrix. Little is known about *TNXB* involvement in LE pathogenesis, but the rs1150754 was found to be the locus most significantly associated with SLE in patients with European origin so far [5]. In this study we have confirmed the strong association between A allele as well as the GA genotype of rs1150754 and the occurrence of SLE. We have also shown that the A allele or GA genotype of the *TNXB* gene was associated with malar rash, photosensitivity, arthritis, renal disorder and the presence of ANA in SLE patients. Nevertheless, the allele and genotype frequencies of the rs1150754 were not different in DLE patients in comparison with healthy individuals. Although the particular molecular mechanism of *TNXB* involvement in LE pathogenesis is not well understood, the results presented in this paper reveal the differences in the molecular background between DLE and SLE.

The rs1143679 is located in exon 3 of the *ITGAM* gene. A minor A allele causes amino acid change from R to H at position 77 of the ITGAM polypeptide chain [14]. The rs1143679 was shown to be strongly associated with SLE and DLE patients of European origin [4, 15, 16]. While our results suggest the association between a polymorphism in rs1143679 and SLE within the Polish population (this association for the Polish population has previously been shown by Warchoł *et al*. [16]), the differences between allele and genotype frequencies from our study have not reached statistical significance after Bonferroni correction. Although the results of our study for rs1143679 in SLE and DLE patients were not significant after Bonferroni correction, one should take into account that the A allele and GA + AA genotypes of rs1143679 in SLE and DLE patients were found at a frequency of almost twice as high as that of normal controls. Therefore, one should be careful with suggesting the exclusion of the *ITGAM* involvement in DLE molecular pathogenesis within the Polish population, due to the small sample size of the DLE group. Thus, further studies are necessary to verify the contribution of *ITGAM* variation to DLE pathogenesis.

Conclusions

The results of our study suggest that *ITGAM*, *TNXB* as well as *STAT4* nucleotide sequence variation may play a role in the molecular pathogenesis of SLE in Polish patients. Moreover, we have shown for the first time that the variation of SNPs located in the *STAT4* (rs7574865), but not in the *ITGAM* (rs1143679) nor the *TNXB* (rs1150754) genes might be associated with the development of DLE within the Polish population. Nevertheless, further studies employing more samples from different ethnicities (geographical origins) are still needed to elucidate the significance of the *ITGAM*, *TNXB* and *STAT4* polymorphisms in the susceptibility to DLE.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Gilliam JN, Sontheimer RD. Distinctive cutaneous subsets in the spectrum of lupus erythematosus. J Am Acad Dermatol 1981; 4: 471-5.
2. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40: 1725.
3. Szczęch J, Rutka M, Samotij D, et al. Clinical characteristics of cutaneous lupus erythematosus. Adv Dermatol Allergol 2016; 33: 13-7.
4. Järvinen TM, Hellquist A, Koskenmies S, et al. Polymorphisms of the ITGAM gene confer higher risk of discoid cutaneous than of systemic lupus erythematosus. PLoS One 2010; 5: e14212.
5. Chung SA, Taylor KE, Graham RR, et al. Differential genetic associations for systemic lupus erythematosus based on anti-dsDNA autoantibody production. PLoS Genet 2011; 7: e1001323.
6. Grönhagen CM, Fored CM, Granath F, Nyberg F. Cutaneous lupus erythematosus and the association with systemic lupus erythematosus: a population-based cohort of 1088 patients in Sweden. Br J Dermatol 2011; 164: 1335-41.
7. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online 2005; 1: 47-50.
8. Crow MK. Type I interferon in the pathogenesis of lupus. J Immunol 2014; 192: 5459-68.
9. Jabbari A, Suárez-Fariñas M, Fuentes-Duculan J, et al. Dominant Th1 and minimal Th17 skewing in discoid lupus revealed by transcriptomic comparison with psoriasis. J Invest Dermatol 2014; 134: 87-95.
10. Braunstein I, Klein R, Okawa J, Werth VP. The interferon-regulated gene signature is elevated in subacute cutaneous

lupus erythematosus and discoid lupus erythematosus and correlates with the cutaneous lupus area and severity index score. Br J Dermatol 2012; 166: 971-5.
11. Kariuki SN, Kirou KA, MacDermott EJ, et al. Cutting edge: autoimmune disease risk variant of STAT4 confers increased sensitivity to IFN-alpha in lupus patients in vivo. J Immunol 2009; 182: 34-8.
12. Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. Nat Rev Rheumatol 2010; 6: 683-92.
13. Bristow J, Tee MK, Gitelman SE, et al. Tenascin-X: a novel extracellular matrix protein encoded by the human XB gene overlapping P450c21B. J Cell Biol 1993; 122: 265-78.
14. Nath SK, Han S, Kim-Howard X, et al. A nonsynonymous functional variant in integrin-alpha(M) (encoded by ITGAM) is associated with systemic lupus erythematosus. Nat Genet 2008; 40: 152-4.
15. Lee YH, Bae SC. Association between the functional ITGAM rs1143679 G/A polymorphism and systemic lupus erythematosus/lupus nephritis or rheumatoid arthritis: an update meta-analysis. Rheumatol Int 2015; 35: 815-23.
16. Warchoł T, Lianeri M, Łącki JK, et al. ITGAM Arg77His is associated with disease susceptibility, arthritis, and renal symptoms in systemic lupus erythematosus patients from a sample of the Polish population. DNA Cell Biol 2011; 30: 33-8.