Original Article

Feasibility of establishing deletion of the late cornified envelope genes *LCE3B* and *LCE3C* as a susceptibility factor for psoriasis

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Abstract

Background: Psoriasis is a chronic hyperproliferative inflammatory disease of the skin, genetic predisposition to which is well-established. The late cornified envelope genes *LCE3B* and *LCE3C* are involved in maintaining the integrity of skin barrier especially following skin barrier disruption. The deletion of these genes would lead to an impaired epidermal response following damage to the skin barrier thus predisposing to psoriatic lesions. This study aimed to evaluate the common deletion of late cornified envelope genes (*LCE 3B/3C*) in psoriasis patients of Kashmiri ethnic population of North India.

Materials and Methods: It was a hospital-based, case-control study which included 100 psoriasis cases and an equal number of controls. Blood samples were obtained, and DNA was extracted from all the samples by a kit-based method. To determine the *LCE3C_LCE3B-del* genotype, a three-primer polymerase chain reaction assay was performed.

Results: The genotype for the common *LCE3C_LCE3B* deletion in 100 psoriasis patients and 100 controls was determined. Among the cases, 17 cases were homozygous for insertion genotype (I/I), 40 cases were heterozygous for insertion/deletion genotype (I/D) and 43 cases were homozygous for deletion genotype (D/D), compared to controls where 20 cases were homozygous for insertion genotype (I/I), 45 cases were heterozygous for insertion/deletion genotype (I/D), and 35 cases were homozygous for deletion genotype (D/D). The del/del frequency was higher among psoriatic patients compared to controls (43% vs. 35%) although the difference was not statistically significant ($P = 0.507$).

Conclusion: We hereby infer that *LCE3C_LCE3B* deletion does not appear to be associated with the risk of psoriasis in our population.

Key Words: Keratinocyte differentiation, late cornified envelope genes, psoriasis

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INTRODUCTION

Psoriasis is a chronic hyperproliferative inflammatory disease of the skin, scalp, nails, and joints that present in several clinical forms. The prevalence of psoriasis varies widely among different groups and in different parts of the world (0.1–3%).[1,2] The prevalence of

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psoriasis in Kashmir has been estimated to be about 3.4%.[3]

Psoriasis has a multifactorial etiology, involving environmental (infections, drugs, stress, smoking, and climate) and genetic factors. Currently, 41 genetic susceptibility loci for psoriasis have been established at genome-wide level of significance ($P < 5 \times 10^{-8}$).[4-13] Among these, the psoriasis susceptibility (*PSORS*) 1 locus on chromosome 6p21 is the one most strongly associated with psoriasis. Recent studies have demonstrated the association of a common deletion of late cornified envelope genes *LCE3B* and *LCE3C* (in the region that contains the *PSORS4* locus on chromosome 1q21), with psoriasis in several populations.[14-17] These genes form a part of the epidermal differentiation complex, a cluster of genes that encodes proteins found in the stratum corneum. These proteins are of great importance for keratinocyte differentiation and skin barrier maintenance. The late cornified envelope proteins are incorporated into the cornified envelope late in the process of maturation during epidermal differentiation; hence the name. The *LCE* gene cluster consists of 18 members which are divided into 6 families *LCE1-6*.[18] The expression of late cornified envelope genes is induced following skin barrier disruption even in individuals who normally lack the mRNA for these genes, thus helping in the restoration of the skin barrier.[19] Individuals homozygous for *LCE3C_LCE3B-del* fail to mount this response thus conferring a higher risk for the development of psoriasis. This study was conducted to establish a correlation between *LCE3C_LCE3B* deletion and psoriasis susceptibility in ethnic Kashmiri population of North India.

MATERIALS AND METHODS

This was a hospital based, case-controlled study conducted on patients attending the out-patient unit of our department over a period of 1 year (from April 01, 2012 to March 31, 2013). The recruitment process was started only after ethical clearance from the Institutional Ethical Committee as per norms and all the individuals gave their informed consent to participate. A total of 100 clinically typical cases of psoriasis from the ethnic Kashmiri population and an equal number of age and gender matched controls comprising of patients presenting to our department for some other unrelated insignificant complaint, the hospital staff, and healthy volunteers were included in the study. Patients diagnosed with arthritis prior to psoriasis and non-Kashmiri population was excluded from the study. A detailed history was taken from all patients, and a complete clinical examination was carried out. The severity of disease was assessed on the basis of psoriasis area and severity index (PASI). PASI ranges from 0 to 72 with PASI > 10 recognized as indicative of severe disease. In addition, routine investigations, wherever deemed necessary, were carried out. The blood samples from the psoriasis patients and controls were obtained and were collected in 5 ml collection vials containing ethylenediaminetetraacetic acid and immediately stored at –80°C. Genomic DNA from the blood samples was isolated using a kit based method [Figure 1]. The kit used was quick-g DNA™ MiniPrep supplied by Zymo Research (India). The integrity of the genomic DNA was examined by gel electrophoresis using 1% agarose gel. The quantity of the DNA was determined by measuring optical density at 260 and 280 nm by double beam spectrophotometer. To determine the *LCE3C_LCE3B-del* genotype, we performed a three-primer polymerase chain reaction (PCR) assay in 100 psoriasis cases and 100 controls. The *LCE3B* fragment was 422bp long and was amplified with primers GGGCTTCATAAAACCATTTGTAGAG (forward) and TTTCCTCTAAAGTCGCTTGTCTCA (reverse). The *LCE3C* was a 448bp fragment amplified with primers GGTCTGAGGGTTCTGTGCTCA (forward) and TCTGGAAAAGCATGCATCAGG (reverse). Genomic DNA from patients and controls was PCR-amplified in a single tube containing each of *LCE3F* (forward), *LCE3CR* (reverse), and *LCE3CR2D* (reverse) primers. The PCR approach was described previously by de Cid *et al*.[19] and was used to amplify nondeletion allele and deletion allele. The principle of this PCR method lies in the amplification of the nondeletion allele visualized as a fragment of 240 bp (*LCE3CF-LCE3CR* product), and the deletion as a fragment of 199 bp (*LCE3CF-LCE3CR2D* product). PCR amplification was achieved using a thermal cycler (gradient thermal cycler from Eppendorf Mastercycler Pro). Reactions were hot-started at 95°C for 5 min, followed by addition of Taq polymerase, followed by 35 cycles of melting (95°C for 30 s), annealing (60°C for 30 s) and extension (72°C

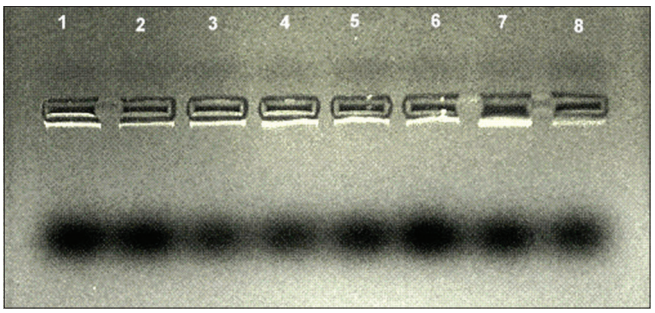

Figure 1: Representative gel picture showing the integrity of the isolated genomic DNA run on 1.0% agarose gel. Lane 1–8 contains the genomic DNA isolated from blood samples of eight psoriasis cases

for 30 s) and by final extension step at 72°C for 4 min. After amplification, 10 μl of each reaction were electrophoresed on 2% agarose gel, stained with ethidium bromide, in which the deletion and nondeletion alleles were visualized as a fragment of 199 and 240 bp, respectively.

Categorical variables were analyzed using Chi-square test and quantitative variables presented as a mean ± standard deviation. $P < 0.05$ was considered to be statistically significant.

RESULTS

The study comprised 100 psoriasis patients, 69 (69%) being males and 31 (31%) being females with a male: female ratio of 2.2:1. The age of the cases ranged from 9 to 65 years with a mean age of 35.67 ± 14.21 years. Of the 100 controls, 64 were males and 36 were females with a mean age of 31.23 ± 12.32 years (range: 12–58 years). The age of onset of psoriasis was ≤40 years in 75 (75%) patients, and 25 (25%) patients developed the disease for the 1st time at an age of more than 40 years. The duration of disease in the cases ranged from 6 days to 27 years and the mean duration of disease was 6.3261 ± 6.06 years. A family history of psoriasis was reported by a total of 14 (14%) patients, of which 8 (57.2%) were males and 6 (42.8%) were females. First-degree relatives were affected in 9 (64.3%) of 14 patients. Various types of psoriasis seen in our study included chronic plaque psoriasis which was seen in 87 (87%) patients, guttate psoriasis is seen in 6 (6%) patients, pustular psoriasis in 3 (3%) patients, and erythrodermic psoriasis in 4 (4%) patients. Nail changes were observed in 38 (38%) patients. The PASI score was more than 12 in 23 (23%) patients and <12 in 77 (77%) patients. Genomic DNA was successfully isolated from all 200 samples (100 cases and 100 controls).

The genotype for the common *LCE3C_LCE3B* deletion in 100 psoriasis patients and 100 controls was determined [Figure 2].

Genotype and allele frequencies for the *LCE3C_LCE3B-del* in patients and controls are given in Table 1.

Genotype frequencies for the *LCE3C_LCE3B-del* were compared between sporadic and familial cases, between patients with early onset and those with late onset psoriasis and between patients with severe and those with nonsevere psoriasis. Comparison of genotype frequencies among different groups is given in Table 2.

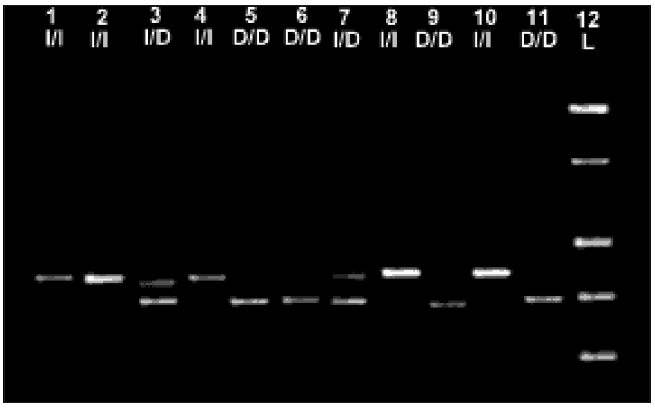

Figure 2: Representative gel picture depicting *LCE3B_3C* ins/del genotypes. The *LCE3B_3C* insertion and deletion alleles were visualized as polymerase chain reaction fragments of 240 and 199 bp. Lane 1, 2, 4, 8 and10: Cases homozygous for insertion. Lane 3 and 7: Cases heterozygous for insertion and deletion. Lane 5, 6, 9 and 11: Cases homozygous for deletion. Lane 12: DNA ladder (molecular weight marker)

The difference in genotype frequencies was analyzed using the Chi-square test.

Hardy–Weinberg test for equilibrium: The observed values for genotype frequencies were as follows - D/D (0.43), I/D (0.4), I/I (0.17) against the expected genotype frequencies of 0.4,0.5 and 0.1, respectively, with a χ^2 test $P = 0.88$. Hence, the observed genotype frequencies were consistent with Hardy–Weinberg equilibrium.

DISCUSSION

Psoriasis is a chronic hyperproliferative inflammatory disease of the skin with a prevalence of 0.1–3% in different parts of the world.[1,2] Psoriasis has a multifactorial etiology with a polygenic mode of inheritance.[20] The *LCE3C_LCE3B-del* leads to a compromised skin barrier function which may play a significant role in the development of psoriasis,[21] as demonstrated in several Caucasian populations. The results of these studies, however, cannot be generalized to non-Caucasians. The present study was undertaken to establish a correlation between *LCE3C_LCE3B* deletion and psoriasis susceptibility in ethnic Kashmiri population of North India.

The age of the patients in this study ranged from 9 to 65 years with a mean age of 35.67 ± 14.21 years. Males constituted 69% of the patients in our study with a male: female ratio of 2.2:1. It is generally believed that psoriasis is equally common in males and females.[22] In a study where comprehensive data was collected from various medical colleges in India, the ratio of male: female in psoriatic patients was very high (2.46:1) which could not be clearly accounted for.[23]

Table 1: Genotype frequencies for the *LCE3C_LCE3B-del* among cases and controls

Study group	*LCE3C_LCE3B-del* genotypes			*P*
	I/I genotype	I/D genotype	D/D genotype	
Cases (*n*=100)	17	40	43	0.507
Controls (*n*=100)	20	45	35	

I/D: Insertion/deletion, I/I: Insertion, D/D: Deletion

Table 2: Comparison of genotype frequencies for *LCE3C_LCE3B-del* among different groups

Disease characteristics	*LCE3C_LCE3B-del* genotype (%)			*P*
	I/I genotype	I/D genotype	D/D genotype	
Early onset psoriasis (*n*=74)	13 (17.6)	30 (40.5)	31 (41.9)	
Late onset psoriasis (*n*=26)	4 (15.4)	10 (38.4)	12 (46.2)	0.939
Sporadic cases (*n*=86)	14 (16.3)	34 (39.5)	38 (44.2)	
Familial cases (*n*=14)	3 (21.4)	4 (28.6)	7 (50)	0.719
Nonsevere psoriasis (*n*=77)	13 (16.9)	33 (42.8)	31 (40.3)	
Severe psoriasis (*n*=23)	4 (17.4)	7 (30.4)	12 (52.8)	0.530

I/D: Insertion/deletion, I/I: Insertion, D/D: Deletion

In another prevalence study of psoriasis from North India, male: female sex ratio was found to be 2.5:1.[24] Similar results were replicated in other studies.[25,26] Hence, it can be inferred that in India, psoriasis is twice more common in males compared to females.

The majority of patients (47%) belonged to the age group of 20–40 years. The age of onset of psoriasis was <40 years in 74% of patients and more than 40 years in 26% of patients. A bimodal age of onset of psoriasis has been recognized in several large studies. Henseler and Christophers reported two clinical presentations of psoriasis, Type I and II distinguished by a bimodal age at onset where Type 1 begins on or before age 40 years; Type II begins after the age of 40 years and Type I disease accounts for more than 75% of cases.[27,28]

A family history of psoriasis was reported by 14% of patients in our study. First-degree relatives were affected in 9 (64.3%) out of 14 patients. Of patients with psoriasis, 36–71% have one relative who is also affected by psoriasis.[29] Indian studies, however, report a lower familial incidence of the disease. Bedi reported a positive family history of psoriasis in 14% of their patients while Kaur *et al*. reported family history in only 2% of their patients with first degree relatives being affected in 84% of the cases and second-degree relatives in 12% of cases.[25,26]

The most common type of psoriasis encountered in this study was chronic plaque psoriasis seen in 87% of patients followed by guttate psoriasis seen in 6% of patients. Chronic plaque psoriasis is the most common form of psoriasis, occurring in about 80% of all psoriasis patients. Bedi and Kaur *et al*. reported chronic plaque psoriasis in 90% and 93% of their cases, respectively while guttate, pustular, and erythrodermic psoriasis were uncommon accounting for <2% of cases.[25,26]

In our study, the homozygous deletion (D/D) genotype frequency was higher among psoriatic patients compared to controls (43% vs. 35%) although the difference did not reach statistical significance (P = 0.507).

de Cid *et al*. found a significantly higher frequency of D/D homozygotes among the patients (total D/D frequency, 0.45 in patients vs. 0.34 in controls; odds ratio = 2.04, 95% confidence interval = 1.59–2.62, P = 1.71e-08).[19] This was further confirmed by Hüffmeier *et al*. in individuals of German origin.[15] We did not find this difference when the psoriasis patients were compared to controls. However, in agreement with these authors, the D/D genotype was more frequent among patients with psoriasis.

A small study in North African population failed to detect any evidence of an association between *LCE3C_LCE3B-del* and psoriasis.[30]

Wiwanitkit did a summative meta-analysis of the case-control studies of the frequency of *LCE3C_LCE3B-del* genotype among patients with psoriasis and controls and found that there is no relationship between *LCE3C_LCE3B-del* genotype and psoriasis.[31]

A meta-analysis including 12,196 psoriatic patients and 13,092 controls from 19 comparative studies, conducted by Song *et al*. however demonstrated a significant association between psoriasis and *LCE3C_LCE3B-del* polymorphism in Europeans and Asians.[32]

On comparing the genotype frequencies for the *LCE3C_LCE3B-del* between sporadic and familial cases, the D/D frequency was higher (50%) among familial cases compared to sporadic cases (44.2%). The difference, however, was not statistically significant (P = 0.719). Coto *et al*. found a higher D/D frequency of 39% among sporadic cases compared to familial cases where the D/D frequency was only 24%.[14] In a study[33] in Tunisian psoriatic population, frequency of the *LCE3C_LCE3B-del* was similar between patients and healthy controls but subanalyses by family history revealed that the frequency of *LCE3C_LCE3B-del* was significantly higher in

patients with a positive family history than in control individuals, as well as in individuals with a positive family history versus those without family history in the case cohort. Xu *et al*.[16] also found a significant difference in deletion frequency between patients with a positive family history and controls.

The D/D genotype for *LCE3C_LCE3B-del* occurred in a frequency of 41.9% in patients with early onset psoriasis and 46.2% in patients with late onset psoriasis in our study. The *LCE3C_LCE3B-del* does not appear to be related to early onset of disease in our population. Coto *et al*.,[14] Hüffmeier *et al*.[15] and Li *et al*.[17] also found that the deletion was not significantly associated with the age of disease onset. Xu *et al*.[16] however found that *LCE3C_LCE3B* D/D frequency was significantly different between patients with early-onset psoriasis and controls.

Among the 23 patients with severe form of psoriasis, D/D genotype for *LCE3C_LCE3B-del* was seen in 52.8% of cases. This was much higher compared to the D/D frequency in patients with a nonsevere form of psoriasis (40.2%). This implies that cases with a common deletion of *LCE3C_LCE3B* genes may be at risk of developing more severe forms of psoriasis. The difference, however, when compared statistically was not found to be significant (P = 0.530). This finding is supported by a previous study[34] where the del/del genotype frequency between these two groups was compared, and no difference was observed between the two groups.

From our study, no significant association was found between *LCE3C_LCE3B* deletion and the risk of psoriasis in general. However, further studies with larger sample size need to be carried out in our population to find the exact correlation between *LCE3C_LCE3B* deletion and susceptibility to psoriasis.

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Conflicts of interest
There are no conflicts of interest.

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