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Association analysis of GWAS and candidate gene loci in a Pakistani population with psoriasis

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ABSTRACT

Psoriasis is a common inflammatory and hyper proliferative condition of the skin and a serious chronic systemic autoimmune disease. We undertook an association study to investigate the genetic etiology of psoriasis in a Pakistani population by genotyping single-nucleotide polymorphisms (SNPs) previously reported to be associated in genome-wide association (GWAS) or in candidate gene studies of psoriasis. Fifty seven single-nucleotide polymorphisms (SNPs) from 42 loci were genotyped in 533 psoriasis patients and 373 controls. Our results showed genome wide significant association of the MHC region (rs1265181 being the most significant from five SNPs used with overall OR = 3.38; $p = 2.97E{-}18$), as well as nominally significant associations at ten other loci ($p < 0.05$) in the Pakistani population (*LCE3B*, *REL*, *IL13*/*IL4*, *TNIP1*, *IL12B*, *TRAF3IP2*, *ZC3H12C*, *NOS2* and *RNF114* from GWAS and *PRR9* from a previous candidate gene study). Overall, only nine SNPs out of the 42 GWAS loci, displayed an odds ratio in the opposite allelic direction and only three did not reach similar odds ratio within 95% confidence interval as previously reported (*SLC45A1*/*TNFRSF9*, *ELMO1* and *IL28RA*). This indicates similar genetic risk factors and molecular mechanisms behind disease in Pakistani psoriasis patients as in other populations. In addition, we show that the MHC and *TNIP1* regions are significantly different in patients with psoriasis onset before the age of 40 (type I) compared to after 40 years of age (type II). MHC being associated mainly with type I while *TNIP1* with type II patients.

1. Introduction

Psoriasis is a chronic inflammatory skin disease with a worldwide distribution, affecting 1–3% of the general population (Henseler, 1998; Kurd and Gelfand, 2009). Among the characteristic histological features are epidermal hyper-proliferation and infiltration of both dermis and epidermis by inflammatory cells including neutrophils, lymphocytes, macrophages, and mast cells. Like many other immune-mediated diseases, psoriasis results from a combined action of multiple genes and environmental triggers. The age at disease onset is highly variable, however, studies have shown that 75–90% of patients develop psoriasis before the age of 40, with a peak around puberty and a smaller peak around 50–60 years of age (Henseler and Christophers, 1985). Based on the age of onset variability two types of psoriasis can be differentiated: type I, manifesting itself early in life before the age of 40 and type II with a later onset after the age of 40. Among psoriatic patients, up to 40% develop psoriatic arthritis (PsA)—an associated inflammation of the joints (Weger, 2010).

Although, the etiology of psoriasis is still unknown, there is a common consensus that it is a complex disorder and multiple genes are involved in the pathogenesis of psoriasis. The major genetic determinant however remains within the MHC region. MHC is the home of genes encoding human leukocyte antigens (HLA) and HLA associations in psoriasis have been recognized for over 35 years (Russell et al., 1972).

The genetic basis of the disease is well documented by a large number of families and population based studies, and has convincingly demonstrated a complex mode of inheritance for psoriasis (Capon et al., 2012). Currently there are 41 significant ($p < 5 \times 10^{-8}$)

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Table 1
Characteristics of the study group.

Parameters	Psoriatic subjects (n = 533)	Controls (373)
Gender ratio M/F	358/175	195/178
Mean age ± SD (years)	37 ± 16	26 ± 14
Mean onset age ± SD (years)	29.9 ± 15	–
Onset at ≤40/>40 years	417/116	–
Familial occurrence (533 patients)±	227/306	–
Positive familial occurrence for type I/II	227/47	

SD = standard deviation.

genome-wide susceptibility loci established for psoriasis including the HLA region on chromosome 6 (Shaiq et al., 2013). A vast majority of the identified susceptibility loci harbor genes within the active immune and inflammatory pathways, affirming the interplay between genetic susceptibility and immune responses in psoriasis.

In addition, various ethnic groups and geographical locations show variation in genetic susceptibility. One association study published previously has identified 30 markers in 24 known psoriasis susceptibility loci in a Pakistani population (Shaiq et al., 2013). Several other smaller studies from India have reported association of psoriasis with MHC genes (Pitchappan et al., 1989; Rani et al., 1998; Gandhi et al., 2011; Umapathy et al., 2011) whereas little is known about the genetic basis of psoriasis in South Asian populations. In this report we comprehensively tested a population from Pakistan in order to determine association with psoriasis susceptibility loci known from GWAS (Liu et al., 2008; Capon et al., 2008; Nair et al., 2009; Zhang et al., 2009; Strange et al., 2010; Ellinghaus et al., 2010; Stuart et al., 2010; Yang et al., 2013) and autoimmunity chip (Tsoi et al., 2012) as well as some candidate gene studies (Hüffmeier et al., 2005; Chen et al., 2009; Lu et al., 2013a).

2. Materials and methods

Our study group consisted of 533 psoriasis patients and 373 controls, both patients and controls were from a similar ethnic background, and belonged to various castes and tribes from northern Punjab and the Khyber Pakhtunkhwa. Diagnosis of psoriasis was established as part of routine clinical care by dermatologists. In the patient group, mean age was 37 years and mean age at onset of disease was 29.9 years. The patient group consisted of 358 males (mean age = 39 years) and 175 females (mean age = 34 years) and 31% had a family history of psoriasis. Seventy eight percent of the patients had type I psoriasis, with an age at onset ≤40 years, as defined by Henseler and Christophers (Table 1). The control group consisted of unrelated subjects and was randomly recruited from blood and bone marrow donors. The study was conducted following ethical guide lines of the Helsinki-II Declaration and formal approval from the ethical committee of the Institute of Biomedical and Genetic Engineering, Islamabad was obtained. Written informed consent was obtained from all the patient and control subjects enrolled in the study.

Genomic DNA was extracted from blood samples using standard organic extraction protocols (Sambrook et al., 2001). Single-nucleotide polymorphisms (SNPs) that have genome wide significant association with psoriasis were selected from published GWAS reported in the GWAS catalog and from the immunochip data (Tsoi et al., 2012). A few were selected on the basis of association reported in various candidate gene studies (Hüffmeier et al., 2005; Chen et al., 2009; Lu et al., 2013a). PCR was performed according to the manufacturer's protocol (LGC Genomics, UK). All quality control steps and statistical analyses were performed using the PLINK (v1.07) software (Purcell et al., 2007). SNPs with less than 90% success rate were excluded from the study. In total, 57 SNPs in 42 previously reported psoriasis susceptibility loci were investigated using KASP genotyping assay (LGC Genomics, UK). Allele and genotype frequencies were calculated and tested for Hardy–Weinberg (HW) Equilibrium to check the goodness of fit with one degree of freedom. Linkage disequilibrium values for SNPs present in the same locus/gene and for the genes present on the same chromosome were calculated and haplotype analysis was carried out in case of linkage using Arlequin ver 3.11 software (Excoffier et al., 2005).

3. Results

Results of the genetic association analysis for psoriasis susceptibility for 42 loci tested are summarized in Tables 2 and 3. For each locus, one or more of the best known associated markers were tested. Genotypic frequencies for patients and controls were in HW-Equilibrium except for rs2233278 & rs17728388 which showed linkage disequilibrium (r2 = 0.8). One SNP was excluded due to a call rate below 90% (rs2066819). The strongest association was shown to be at the MHC loci in the *HLA-C* region with type I psoriasis group. From the 5 SNPs genotyped from the MHC region, rs1265181 was most significant in the type I group (OR = 4.06, $p = 9.32 \times 10^{-23}$) and overall (OR = 3.38, $p = 2.97 \times 10^{-18}$). Apart from MHC, nominally significant allelic associations were observed at ten loci (*LCE3B*, *PRR9*, *REL*, *IL13/IL4*, *TNIP1*, *IL12B*, *TRAF3IP2*, *ZC3H12C*, *NOS2* and *RNF114*). All of these except *IL12B* (rs3213094), associated in the Chinese population (Zhang et al., 2009) were associated to the same risk variant as in the previous studies. In addition most SNPs which were not significant still had an OR in the same allelic direction, including some *IL12B* SNPs detected previously in the European population (Ellinghaus et al., 2010). A total of nine SNPs out of the 57 SNPs displayed an OR in the opposite allelic direction and only three loci totally lacked any SNP within a 95% confidence interval of the odds ratio in the same allelic direction as previously reported. When comparing the allelic association between type I and type II psoriasis, three loci were significantly different between the two groups (MHC, *ELMO1* and *TNIP1*). The most strongly associated gene in our study with type II psoriasis was the *TNIP1*, which has been reported as a psoriasis susceptibility gene in the Caucasian population (Nair et al., 2009; Tsoi et al., 2012). We genotyped two SNPs in the *TNIP1* gene (rs17728338 and rs2233278) and found association with both SNPs in 'all' sample group ($p = 8.21 \times 10^{-3}$, 7.44×10^{-3} and OR = 1.56 for both). However, when analyzed according to age of onset, the effect observed with type II samples was stronger with an OR of 2.34 and with a significant difference between the two types of psoriasis for rs17728338 (Table 2).

4. Discussion

In compliance with previous studies (Tsoi et al., 2012; Lu et al., 2013b), present data showed MHC as the most strongly associated locus for psoriasis, with rs1265181 being the most significant disease marker and showing even higher significance with type I patients. The second most strongly associated locus in our study was *LCE3B/LCE3C* ($p = 3.01 \times 10^{-4}$, OR = 1.54) also in compliance with previous studies, and showing a possible difference between the two types of patients ($p = 0.08$, Table 2). *LCE3B/LCE3C* belong to the EDC complex, and a previous candidate gene study using high-density SNP genotyping within the 2 Mb EDC region in Singaporean Chinese psoriasis patients provided evidence for type I disease association within *IVL* vicinity. The study also found association at the *PRR9* locus (Chen et al., 2009), one of our three selected SNPs (rs1078886) upstream *PRR9* region showed marginal association in both types of patients in this study. This finding is quite

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