DISSECTING GENES, LOOKING FOR PSORIASIS: AN IL-6 GENE POLYMORPHISM

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Abstract

Background. *Psoriasis is a common chronic inflammatory disorder, affecting mostly the skin. Recent studies associated this disease to a number of genetic variants.*

Aim. *Following their direction, we analyzed the association of a nucleotide sequence variation in the gene encoding for interleukin-6 (IL-6) and psoriasis.*

Methods. *We genotyped a group of 67 psoriasis patients and 69 healthy subjects for IL6 rs1800795. Data was than statistically analyzed.*

Results. *The major allele of IL6 rs1800795 showed significant association to the disease in the case-control analysis.*

Conclusion. *We report a genetic polymorphism related to IL6 as a risk marker in psoriasis.*

Keywords: psoriasis risk loci, IL6 rs1800795, single nucleotide polymorphism.

Introduction and aim

Psoriasis is a chronic inflammatory, hyper-proliferative, immune-mediated disease. With a highly variable prevalence, depending on ethnicity and climate [1], it affects 2-3% of individuals in Europe [2]. Clinically psoriasis affects mostly the skin, the lesions are easy to notice and stigmatizing, with a strong impact on the patients' quality of life [3]. Furthermore, the patients are exposed to risk of the well-known comorbidities [4].

The etiopathophysiological basis of psoriasis have been extensively reviewed by the literature [5,6]. As for the genetics of this multifactorial disease, studies on monozygotic twins and its familial clustering postulate clearly the genetic background of psoriasis. Even so, the triggering role of environmental factors such as stress, trauma or infection, cannot be overlooked. Supporting this, it is worth noticing that the major risk locus, HLA-Cw6 (Human Leukocyte Antigen Cw6), is present only in 60-65% of psoriasis patients and is found in 15% of healthy persons [7].

Psoriasis is mostly known for its cutaneous lesions, the red scaly patches, localized on elbows and knees, but its clinical features vary a lot, allowing the classification into several clinical phenotypes, psoriasis vulgaris being the most common form. Plus, psoriasis is not always limited to the skin, but often involves nails, joints and/or oral mucosa. The pathophysiology of psoriasis is characterized by excessive epidermal growth, altered differentiation, vascularisation, and immunologic abnormalities [5,6].

Regarding the immunopathogenesis of psoriasis, most data supports the idea of psoriasis being mediated by a positive feed-back loop from activated Tcells to APCs (antigen-presenting cells). Recent studies highlight the involvement of Th17, a subset of T helper cells, in psoriasis. Polymorphisms of cytokine genes of the IL23/Th17 axis have also been associated to the disease [2,7]. In the development of Th17 cells from naive T cells, IL-6 plays an important role. This pleiotropic cytokine has a

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wide range of biological activities, including immune regulation and inflammation and the immunopathogenesis of psoriasis it appears to be involved at different key points, like the Th17, or the IL-23-induced skin inflammation, that can not occur in its absence [8,9,10,11].

Besides all research and many studies comprehensively reviewed, a lot of questions still need to be answered for a better understanding of the disease, and development of improved therapies.

Following the direction of previous valuable studies, aiming to find psoriasis risk loci, we conducted a retrospective study, assessing a genetic variant of IL6, in relation to its potential as a risk marker in psoriasis.

Patients and methods
Subjects
A group of 67 patients affected by psoriasis vulgaris and 69 subjects who served as controls, all aged over 18 years, were recruited in the Dermatology unit at the University of Rome "Tor Vergata". Demographic data of patients and of the control group were similar. The control group of subjects did not have any medical history of psoriasis. All subjects were genotyped for IL6 rs1800795.

The informed consent was obtained from all subjects, under protocols in accordance with the Helsinki Declaration and STROBE statement. The study was approved by the Tor Vergata Ethics Committee and the Ethics Committee of Iuliu Hațieganu University of Medicine and Pharmacy.

DNA extraction
Venous blood was collected and stored at -70°C. Than genomic DNA was extracted according to protocol, using the commercially available kit and stored at -20°C, after proper labeling of tubes.

Genotyping
For IL6 rs1800795 PCR with restriction fragment length polymorphism (RFLP) was used to identify each subject as C/C, A/C, or A/A, as previously described [12]. All primers, Taq polymerase, dNTP, and MgCl2 were purchased from QiaGene and the assay was performed in a Techne-Genius thermal-cycler. Negative and positive controls were used to assure accurate subject genotyping. Results of the positive amplification with specific band size were detected by UV light after electrophoresis on 2.5% agarose gel with SYBR® Safe DNA Gel Stain.

Statistical analysis
The statistical analysis was performed useing SPSS (Statistical Package for the Social Sciences) version 20. Data were modeled as two nominal variables, and frequencies were calculated. For the univariate analysis we used the Pearson Chi-Square Test. Odds ratios (OR) and their 95 percent confidence intervals were also calculated. Statistical significance was considered if the value of the parameter P was <0.05.

Results
Allele frequency analysis
The allele frequency for all 67 patients and 69 controls was calculated for the SNP, with results shown in Table no I.

Table I. Frequency Table.

Gene	Alleles	Nr. of subjects	Percentage(%)
IL6 rs1800795	GG	62	45.6
	CG	65	47.8
	CC	9	6.6
Total		136	100

Case-control association analysis
The frequencies of the major allele, heterozygous and minor allele, as well as the asymptotic p-values for IL6 rs1800795 are reported in Table no II. Strong association with the disease was found for IL6 rs1800795 (p=0.001).

Table II. Case-control allelic frequencies.

SNPs	Alleles	Nr. (frequency) of			p
		cases	controls	total	
IL6 rs1800795	GG	40	22	62	0.001
	CG	26	39	65	
	CC	1	8	9	
Total		67	69	136	

Case-control genotyping associations
Trying to determine the risk of IL6 rs1800795 G allele carriers, we conducted a univariate analysis. The results are shown in Table no III. As show, the major allele of IL12B rs6887695, compared to the minor one, has a strong association to psoriasis (p=0.025, OR=14.54).

Table III. Case-control genotyping associations for IL6 rs1800795.

Genotypes	Nr. (frequency) of			p	OR	95% CI	
	cases	controls	total			lower	upper
GG	40	22	62				
CG	26	39	65				
CC	1	8	9				
GG vs. CC				0.014	14.54	1.706	123.9
GC vs. CC				0.125	5.33	0.629	45.2

Conclusions
In this study we assessed a polymorphism in a cytokine gene (IL6 rs1800795) in relation to its potential as a risk marker for psoriasis. We report statistically significant values for the carriers of the major allele compared to those carrying the minor one. The GG carriers have an approximately 14 fold higher risk of developing psoriasis, compared to CC carriers.

Starting from the implication of IL-6 in the immunopathogenesis of psoriasis, previous studies have also looked into the matter of IL6 rs1800795. Some reported an association of this SNP to the disease [11], but there are also studies with opposite results [13]. The discording results are likely to be caused by the small sample size,

week point met in our study as well.

These results, after further confirmation on larger samples, could be integrated into the clinical practice. Such translational research could improve the management of psoriasis, increasing the accuracy of diagnosis, or offer a more appropriate counseling to patients with familiarity for this disease.

Acknowledgements

References
1. Jacobson CC, Kumar S, Kimball AB. Latitude and psoriasis prevalence. J Am Acad Dermatol, 2011; 65:870-873.
2. Cargill M, Schrodi SJ, Chang M, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet, 2007; 80:273-290.
3. Kurd SK, Troxel AB, Crits-Christoph P, Gelfand JM. The risk of depression, anxiety, and suicidality in patients with psoriasis: a population-based cohort study. Arch Dermatol, 2010; 146:891-895.
4. Armstrong AW, Schupp C, Bebo B. Psoriasis Comorbidities: Results from the National Psoriasis Foundation Surveys 2003 to 2011. Dermatol Online J, 2012;225:121-126.
5. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet, 2007; 370:263-271.
6. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. Nature, 2007; 445:866–873.
7. Duffin KC, Krueger GG. Genetic variations in cytokines and cytokine receptors associated with psoriasis found by genome-wide association. J Invest Dermatol, 2009; 129:827-833.
8. Suttle MM, Nilsson G, Snellman E, Harvima IT. Experimentally induced psoriatic lesion associates with interleukin (IL)-6 in mast cells and appearance of dermal cells expressing IL-33 and IL-6 receptor. Clin Exp Immunol, 2012; 169:311-319.
9. Goodman WA, Levine AD, Massari JV, Sugiyama H, McCormick TS, Cooper KD. IL-6 signaling in psoriasis prevents immune suppression by regulatory T cells. J Immunol, 2009; 183:3170-3176.
10. Lindroos J, Svensson L, Norsgaard H, et al. IL-23-mediated epidermal hyperplasia is dependent on IL-6. J Invest Dermatol, 2011; 131:1110-1118.
11. Settin AA, Hassan HA, El-Baz RA, Hassan TA. Association of cytokine gene polymorphisms with psoriasis in cases from the nile delta of egypt. Indian J Dermatol, 2011; 56:272-277.
12. Kristiansen OP, Nolsøe RL, Larsen L, et al. Association of a functional 17beta-estradiol sensitive IL6-174G/C promoter polymorphism with early-onset type 1 diabetes in females. Hum Mol Genet, 2003; 12:1101-1110.
13. Baran W, Szepietowski JC, Mazur G, Baran E. IL-6 and IL-10 Promoter Gene Polymorphisms in Psoriasis Vulgaris. Acta Derm Venereol, 2008; 88:113-116.