Research Article
Gene Variant in the NF-κB Pathway Inhibitor NFKBIA Distinguishes Patients with Psoriatic Arthritis within the Spectrum of Psoriatic Disease

Pablo Coto-Segura,1,2,3 Eliecer Coto,1,3,4 Leire González-Lara,4 Belén Alonso,4 Juan Gómez,4 Elías Cuesta-Llavona,4 and Rubén Queiro2,5

1Dermatology Division, Hospital Álvarez Buylla-Mieres, Mieres, Spain
2Instituto Investigación Sanitaria del Principado de Asturias (IISPA), Oviedo, Spain
3Department of Medicine, University of Oviedo, Oviedo, Spain
4Molecular Genetics Unit, Hospital Universitario Central Asturias, Oviedo, Spain
5Rheumatology Division, Hospital Universitario Central Asturias, Oviedo, Spain

Correspondence should be addressed to Eliecer Coto; eliecer.coto@sespa.es and Rubén Queiro; rubenque7@yahoo.es

Received 14 May 2019; Revised 27 July 2019; Accepted 26 August 2019; Published 11 November 2019

Academic Editor: Elena Orlova

Copyright © 2019 Pablo Coto-Segura et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background and Aims. The NF-κB pathway has been implicated in the genetic aetiology of psoriatic disease. However, since most patients with arthritis have psoriasis, discerning the genetic contributions to both aspects of psoriatic disease is not easy. Our aim was to study the association of common polymorphisms in genes of the NF-κB pathway in patients with psoriatic disease in order to dissect the contribution of this pathway in the appearance of each component (skin and joint) of the disease. Patients and Methods. We investigated the association between three common variants in NFKB1 (rs230526), NFKBIA (rs7152376), and NFKBIZ (rs3217713 indel) and the risk of developing psoriatic disease. We genotyped a total of 690 psoriatic disease patients and 550 controls. Patients with cutaneous psoriasis of at least 10 years of evolution without associated arthritis were defined to have pure cutaneous psoriasis (PCP).

Results. The rare NFKBIA rs7152376 C was significantly more frequent in the PsA group vs. controls (OR = 2.03 (1.3–3.1), \( p < 0.01 \)). The difference was even higher between PsA and PCP patients (OR = 3.2 (2.1–5.1), \( p < 0.001 \)). Neither NFKB1 rs230526 nor NFKBIZ rs3217713 indel was associated with the risk of developing psoriatic disease as a whole compared to controls. Conclusions. Our study supports a significant effect of the NFKBIA gene on the risk of developing PsA, thus contributing to better discerning of the polymorphisms of this pathway that explain this risk within the spectrum of psoriatic disease. Additional studies with larger cohorts and from different populations are necessary to validate these results.

1. Introduction

In recent years, great advances have been made in the knowledge on the genetic basis of psoriasis and psoriatic arthritis (PsA). The nuclear factor-kappa beta (NF-κB) is pivotal in the regulation of several biological processes, and its deregulation would affect immunological pathways that have been implicated in several pathological processes [1–3]. The NF-κB pathway would play an important role in psoriasis [4–8]. Psoriatic skin exhibits increased expression of NF-κB that would promote the expression of cytokines/chemokines that drive the proliferation of several immune cell types [6]. In this way, the NF-κB is regarded as a mediator between immunity and the keratinocyte alteration characteristic of psoriasis [9]. This role is also supported by the fact that the blockade of the NF-κB binding sites would reduce the expression of several proinflammatory cytokines implicated in psoriasis [8].

The transcriptional activity of NF-κB is regulated by several cytoplasmic inhibitors (IκBα) that bind to the complex and prevent its translocation to the nucleus. Also, atypical nuclear proteins bind to NF-κB and regulate its
function. Among these nuclear inhibitors, IxBκ (encoded by NFKBIZ) would play a prominent role in the pathogenesis of psoriasis [7, 10]. IxBκ is induced by several proinflammatory molecules. In particular, its expression is regulated by IL-17A and might contribute to the activation of Th17-mediated pathways [11–13]. Therefore, IxBκ plays an important role in the pathogenesis of psoriatic disease through IL-17-mediated mechanisms [13, 14].

The HLA-Cw*06 is the most recognised genetic risk factor for psoriasis. In addition, other genes that encode components of immune pathways have been linked to the risk for psoriasis and PsA. These include components of the NF-κB pathway [15–18]. The association with NF-κB polymorphisms has been investigated in cancer and several immunological diseases, including psoriasis and arthritis. The best characterised variant is rs28362491, a four-nucleotide biallelic indel in the NFKB1 promoter that was linked to differences in gene expression and seems to be associated with the risk of developing several types of cancer [19, 20]. Recently, genome-wide association studies (GWAS) have found a significant association between psoriasis and single-nucleotide polymorphisms (SNPs) within NFKBI, NFKBIA, and NFKBIZ [15–17, 21]. Recently, we reported the association between psoriasis and an NFKBIZ intronic indel (rs3217713), conditioned by the Cw6 status, with the insertion allele significantly more frequent in Cw6-positive patients compared to Cw6-negative patients [22].

The greatest challenge in the study of the genetic aetiology of psoriatic disease is to dissect the genetic restriction elements of the cutaneous disease from those that would identify joint disease. The aim of our study herein was to characterise the genetic epidemiology of common NF-κB gene polymorphisms and their association with the risk of developing psoriasis and PsA.

2. Patients and Methods

2.1. Study Population. The detailed information of the studied subjects is summarised in Table 1. All the participants were Caucasians from the region of Asturias (Northern Spain; total population: 1 million), older than 18 years. The study involved 690 patients with psoriatic disease (mean age: 48 ± 15 years; 55% men), recruited through the Dermatology and Rheumatology Department of Hospital Universitario Central de Asturias and Hospital Alvarez-Buylla, Mieres. They were diagnosed based on clinical findings and defined to have severe or nonsevere psoriasis according to the Psoriasis Area and Severity Index (PASI; severe when a PASI score ≥10). Early-onset psoriasis was defined as the onset of disease manifestations at age <40 years. The existence of arthritis was assessed by a rheumatologist according to the CASPAR (CLASSification of Psoriatic ARTHRitis) criteria. Accordingly, a total of 187 patients (27%) were diagnosed with PsA. Individuals affected with psoriasis for 10 or more years without developing arthritis were classified as pure cutaneous psoriasis (PCP; n = 309) cases. The 10-year period is the average time between the onset of psoriasis and the onset of arthritis. Thus, patients with psoriasis duration of this magnitude will most likely not develop arthritis [23]. Therefore, the population of PCP is a good comparator in relation to those patients with PsA.

The control group consisted of 550 nonrelated healthy individuals (mean age: 55 ± 16 years; 57% men) recruited through the Primary Healthcare Centres of Asturias, Spain. None of these controls had been diagnosed with psoriasis or arthritis at the time of inclusion in the study. There was no family history of psoriasis/PsA in the controls.

This study was approved by the Ethics Committee of Clinical Investigation of Principado de Asturias, and all the participants gave their written informed consent. The patient’s cohort was registered as a Biobank Collection by Spanish Instituto de Salud Carlos III (reference C.0003441).

2.2. NF-κB Variant Genotyping. We genotyped three variants in the NFKB1 (rs230526), NFKBIA (rs7152376), and NFKBIZ (rs3217713 indel) genes. They were either previously directly associated or in strong linkage disequilibrium (LD) with other variants associated with cancer, psoriasis, arthritis, or coronary artery disease. Information on these variants including the flanking sequence, reported population frequencies, and LD values was obtained from the Ensembl website (http://www.ensembl.org) and LDlink (https://ldlink.ncbi.nlm.gov/).

The DNA was obtained from 5 mL of blood. All the variants were genotyped through polymerase chain reaction (PCR) amplification of genomic DNA with specific primer pairs (Supplementary Table 1) followed by digestion with a restriction enzyme (PCR-RFLP) and electrophoresis on agarose gels to visualise different alleles. In brief, approximately 100 ng of DNA was amplified (32 cycles of 95°C-30 s, annealing at 65°C-60 s, and 72°C-60 s) in a final volume of 30 μl containing 10 pmol of each primer, 1 unit of Taq DNA polymerase, and 1X buffer. The amplifications were digested with 10 units of the appropriated restriction enzyme and electrophoresed on 4% agarose gels to visualise the alleles in a UV transilluminator.

The NFKB1 rs230526 A/G was in complete LD (r2 = 1) with rs28362491 (−94 delATTG), a common insertion/deletion (indel) polymorphism in the promoter region of NFKB1 that has been widely studied in cancer and immune-mediated processes and was associated with differences in gene expression (the deletion would drive less promoter activity) [19]. NFKBIA rs7152376 was in complete LD with rs12883343 (r2 = 1.0), an SNP previously associated with psoriasis and PsA in GWAS [17]. The NFKBIZ rs3217713 is a 23 nt insertion/deletion (indel) polymorphism that was previously associated with the risk of psoriasis [22]. The PCRs were electrophoresed on agarose gels to visualise the two indel alleles.

All patients were genotyped for HLA-Cw6 (PSORS1).

2.3. Statistical Analysis. All patients and controls’ anthropometric, analytical, and genetic data were stored in an Excel file. The genotype frequencies for each polymorphism were tested online for the Hardy–Weinberg equilibrium (http://www.oeg.org/software/hwe-mr-calc.shtml). The statistical analysis was performed with R software (http://www.r-project.org).
We investigated the association between three common variants in *NFKB1* (rs230526), *NFKBIA* (rs7152376), and *NFKBIZ* (rs3217713 indel) and the risk of developing psoriasis/PsA or their main clinical outcomes. A total of 690 psoriatic disease patients, 187 (27%) with arthritis, and 550 controls were genotyped. The main characteristics of the study cohorts are summarised in Table 1.

The genotype frequencies for the three variants are summarised in Table 2. The minor allele frequencies (MAFs) in our population were almost identical to those reported for other populations of European ancestry. Allele and genotype frequencies did not significantly differ (*p* > 0.05) between patients and controls for the three gene variants, and thus, we concluded that none of these polymorphisms contributed significantly to the risk for psoriatic disease in our population. None of the three variants was associated with psoriatic disease after multiple logistic regression including sex and age as covariates.

We compared allele and genotype frequencies between controls and patients with PsA or PCP (Table 2). The rare *NFKBIA* rs7152376 C was significantly more frequent in the PsA group vs. controls (0.42 vs. 0.36; OR = 2.03 (1.3–3.1), *p* = 0.004). Compared to PCP patients, PsA patients showed a significantly higher frequency of rs7152376 C (0.42 vs. 0.31; OR = 3.2 (2.1–5.1), *p* = 0.001). In reference to the genotypes, carriers of rs7152376 C (CC + CT) were more frequent in PsA patients compared to controls (0.66 vs. 0.58; *p* = 0.06). The frequency of these carriers was significantly higher in PsA vs. PCP groups (*p* = 0.004).

Early-onset psoriasis, female sex, and Cw6 negative were significantly more frequent in PsA patients compared to PCP patients (Table 2). We performed a multiple logistic regression including early onset, sex, Cw6, and *NFKBIA* rs7152376 genotype (CC + CT vs. TT), and the four variables remained significantly associated with PsA vs. PCP (Table 3).

### Table 1: Main characteristics of the total group and different psoriatic disease groups.

|                         | Total PD, *N* = 690 | No PsA, *N* = 503 | PsA, *N* = 187 | PCP, *N* = 309 | *p* value |
|-------------------------|---------------------|-------------------|----------------|----------------|-----------|
| Onset age (mean, years) | 27                  | 27                | 28             | 21             |           |
| Early onset             | 176 (26%)           | 135 (27%)         | 41 (22%)       | 36 (12%)       | 0.002     |
| PASI >10                | 371 (54%)           | 249 (50%)         | 122 (65%)      | 190 (61%)      | 0.40      |
| Male sex                | 381 (55%)           | 292 (58%)         | 89 (48%)       | 182 (59%)      | 0.01      |
| Cw6 positive            | 304 (44%)           | 234 (47%)         | 70 (37%)       | 166 (54%)      | <0.001    |

### Table 2: Genotype and allele frequencies in different psoriatic disease groups and controls.

|                         | Controls, *N* = 550 | Total PD, *N* = 690 | PsA, *N* = 187 | PCP, *N* = 309 | *p* value, OR (95% CI) |
|-------------------------|---------------------|---------------------|----------------|----------------|------------------------|
| *NFKB1* rs230526 A/G    | AA                  | 108 (0.16)          | 30 (0.16)      | 42 (0.14)      | AA vs. AG + GG |
|                         | AG                  | 359 (0.52)          | 95 (0.51)      | 164 (0.53)     | 0.45                   |
|                         | GG                  | 223 (0.32)          | 62 (0.33)      | 103 (0.33)     | 0.73–2.02              |
| *NFKBIA* rs7152376 T/C  | TT                  | 291 (0.42)          | 64 (0.34)      | 147 (0.48)     | 0.004                  |
|                         | TC                  | 314 (0.46)          | 89 (0.48)      | 133 (0.43)     | 1.40–2.54              |
|                         | CC                  | 85 (0.12)           | 34 (0.18)      | 29 (0.09)      | 0.94–5.53              |

*p* and OR values correspond to the putative PsA risk vs. non-risk genotypes. PD: psoriatic disease; PsA: psoriatic arthritis; PCP: pure cutaneous psoriasis (absence of arthritis after 10 or more years of psoriasis onset). OR: odds ratio; CI: confidence interval.

3. Results

We investigated the association between three common variants in *NFKB1* (rs230526), *NFKBIA* (rs7152376), and *NFKBIZ* (rs3217713 indel) and the risk of developing psoriasis/PsA or their main clinical outcomes. A total of 690 psoriatic disease patients, 187 (27%) with arthritis, and 550 controls were genotyped. The main characteristics of the study cohorts are summarised in Table 1.

The genotype frequencies for the three variants are summarised in Table 2. The minor allele frequencies (MAFs) in our population were almost identical to those reported for other populations of European ancestry. Allele and genotype frequencies did not significantly differ (*p* > 0.05) between patients and controls for the three gene variants, and thus, we concluded that none of these polymorphisms contributed significantly to the risk for psoriatic disease in our population. None of the three variants was associated with psoriatic disease after multiple logistic regression including sex and age as covariates.

We compared allele and genotype frequencies between controls and patients with PsA or PCP (Table 2). The rare *NFKBIA* rs7152376 C was significantly more frequent in the PsA group vs. controls (0.42 vs. 0.36; OR = 2.03 (1.3–3.1), *p* = 0.004). Compared to PCP patients, PsA patients showed a significantly higher frequency of rs7152376 C (0.42 vs. 0.31; OR = 3.2 (2.1–5.1), *p* = 0.001). In reference to the genotypes, carriers of rs7152376 C (CC + CT) were more frequent in PsA patients compared to controls (0.66 vs. 0.58; *p* = 0.06). The frequency of these carriers was significantly higher in PsA vs. PCP groups (*p* = 0.004).

Early-onset psoriasis, female sex, and Cw6 negative were significantly more frequent in PsA patients compared to PCP patients (Table 2). We performed a multiple logistic regression including early onset, sex, Cw6, and *NFKBIA* rs7152376 genotype (CC + CT vs. TT), and the four variables remained significantly associated with PsA vs. PCP (Table 3).

4. Discussion

Several published papers have identified genetic factors that might differentiate psoriasis and psoriatic arthritis (PsA) [17, 24, 25]. We performed a genetic association study of psoriatic disease, PCP, and PsA and three common variants in key components of the NF-kB pathway. None of these polymorphisms was significantly associated with the risk of
developing psoriatic disease as a whole in our population. However, we found a significant association between the NFKBIA variant and PsA.

NFKB1 encodes a protein p105 that is further processed to the p50 protein. Previous studies have reported significant associations between common NFKB1 variants and psoriasis, including large-scale genome analysis involving thousands of patients and controls [26]. Most of the studies based on cohorts of limited size studied the NFKB1 −94 ins/delATTG polymorphism and found low significant associations. The deletion allele was initially associated with an increased risk for ulcerative colitis and would be linked to reduced expression of NFKB1 compared to the insertion allele [19]. We did not study this variant; instead, we genotyped NFKB1 rs230526 that was in complete LD with the promoter indel and thus served as a surrogate marker for the functional promoter variant. In reference to the main clinical outcomes, at least one NFKB1 variant has been associated with psoriasis severity [27]. To our knowledge, this SNP has not been previously investigated in other diseases, although it was in high LD with the common rs2836491 (−94 ATTG indel) in the NFKB1 promoter that was associated with differences in gene expression (the deletion would drive less promoter activity) and has been widely studied in cancer and immune-mediated processes [19, 20, 28], including the risk for type 2 diabetes in our population [29]. The promoter indel was also associated with psoriasis in a case-control study of 519 Chinese psoriasis vulgaris patients and 541 matched controls (p = 0.031), but the difference was not still significant after correction for multiple comparisons [30].

In our study, the NFKB1 frequencies did not differ between total psoriatic disease and controls, or the severity, onset age, or disease severity. This variant did not differ between the PsA and PCP groups.

We studied an NFKBIA variant (rs7152376 C/T) that was in complete LD with an SNP (rs12883343 C/G) previously associated with PsA in a meta-analysis of 6 GWAS [17]. The rare G-allele frequency was significantly increased in psoriasis patients vs. controls (0.45 vs. 0.41; meta-OR = 1.16). In the same study, the rare allele was also significantly increased in PsA patients compared to controls (0.46 vs. 0.41; meta-OR = 1.22). The association between this NFKBIA SNP and PsA was recently confirmed by a case-control study in a Chinese cohort [31]. Interestingly, in our study, the rare rs7152376 allele was a disease marker for PsA, with a maximum difference between PsA and PCP patients (p < 0.001). These results provide insights into the pathogenic differences between skin psoriasis and PsA and pointed to NFKBIA as an important determinant of the risk of developing joint disease within the spectrum of psoriatic disease.

Psoriatic disease is associated with high cardiovascular comorbidity. Currently, it is assumed that the link between the two processes is the inflammation itself. We have recently demonstrated that NFKB1 variation was an independent risk factor for developing type 2 diabetes, while the NFKBIZ variant was an independent risk factor for developing early-onset coronary artery disease [29, 32]. Taken together, our data suggest that genetic alterations in the NF-κB pathway play an important role in the pathogenesis of psoriatic disease and its comorbidities, establishing a common link for all these manifestations (arthritis, cardiovascular risk factors, and cardiovascular adverse events).

Our study has several limitations, mainly the limited sample size and the fact that it was based on a single population. In addition, the NFKBIA variants associated with PsA were intronic, and a functional effect on gene expression and/or protein function has not been established. Most likely, these SNPs are in linkage disequilibrium with other variants that have a functional effect and are responsible for the observed genetic associations. Studies focused to characterise these NFKBIA variants are of upmost relevance, as well as functional studies to define the functional differences between the alleles. However, our results support a differential risk in this pathogenic pathway that may help to discern which patients with psoriatic disease have a higher risk of developing PsA.

5. Conclusions

Our study supports a significant effect of the NFKBIA gene (a key component of the NF-κB pathway) on the risk of developing PsA. Additional studies with larger cohorts and from different populations are necessary to confirm these findings.

Abbreviations

PsA: Psoriatic arthritis
NF-κB: Nuclear factor-kappa beta
IkBa: Inhibitor of nuclear factor-kappa B subunit alpha
IkBζ: Inhibitor of nuclear factor-kappa B subunit ζ
IL-17A: Interleukin-17A
HLA- Cw*06: Human leukocyte antigen-Cw*06
NFKB1: Nuclear factor-kappa beta 1
GWAS: Genome-wide association studies
NFKBIA: Nuclear factor-kappa beta IA
NFKBIZ: Nuclear factor-kappa beta IZ
PASI: Psoriasis Area and Severity Index
CASPAR: CLAssification of Psoriatic ARthritis
PCP: Pure cutaneous psoriasis
LD: Linkage disequilibrium
Polymerase chain reaction (PCR) amplification of genomic NFKB1 and NFKBIA variants were genotyped through genotyping the three NFKB pathway gene variants. Supplementary Table 1: primers and PCR conditions for Supplementary Materials. PI16/01792). His work was supported by a grant from the Spanish Instituto de Salud Carlos III-European FEDER funds (no. hisid). This study was approved by the Ethics Committee of Clinical Investigation of Principado de Asturias. All the participants gave their written informed consent. The authors declare no conflicts of interest.

References

[1] B. Hoesel and J. A. Schmid, “The complexity of NF-xB signaling in inflammation and cancer,” Molecular Cancer, vol. 12, no. 1, p. 86, 2013.

[2] M. D. Jacobs and S. C. Harrison, “Structure of an IxBa/NF-kB complex,” Cell, vol. 95, no. 6, pp. 749–758, 1998.

[3] M. S. Hayden, A. P. West, and S. Ghosh, “NF-kB and the immune response,” Oncogene, vol. 25, no. 51, pp. 6758–6780, 2006.

[4] S. Bell, K. Degitz, M. Quiriling, N. Ilig, S. Page, and K. Brand, “Involvement of NF-kB signalling in skin physiology and disease,” Cellular Signalling, vol. 15, no. 1, pp. 1–7, 2003.

[5] A. M. Goldminz, S. C. Au, N. Kim, A. B. Gottlieb, and P. F. Lizzul, “NF-kB: an essential transcription factor in psoriasis,” Journal of Dermatological Science, vol. 69, no. 2, pp. 89–94, 2013.

[6] P. F. Lizzul, A. Aphale, R. Malaviya et al., “Differential expression of phosphorylated NF-kB/RelA in normal and psoriatic epidermis and downregulation of NF-kB in response to treatment with etanercept,” Journal of Investigative Dermatology, vol. 124, no. 6, pp. 1275–1283, 2005.

[7] B. Rebholz, I. Haase, B. Eckelt et al., “Crosstalk between keratinocytes and adaptive immune cells in an IxBa protein-mediated inflammatory disease of the skin,” Immunity, vol. 27, no. 2, pp. 296–307, 2007.

[8] C. Johansen, J. L. Riis, A. Gedebjerg, K. Kragballe, and L. Iversen, “Tumor necrosis factor α-mediated induction of interleukin 17C in human keratinocytes is controlled by nuclear factor κB,” Journal of Biological Chemistry, vol. 286, no. 29, pp. 25487–25494, 2011.

[9] D. Tsuruta, “NF-κB links keratinocytes and lymphocytes in the pathogenesis of psoriasis,” Recent Patents on Inflammation & Allergy Drug Discovery, vol. 3, no. 1, pp. 40–48, 2009.

[10] H. Kitamura, K. Kanaheira, K. Okita, M. Morimatsu, and M. Saito, “MAIL, a novel nuclear IxB protein that potentiates LPS-induced IL-6 production,” FEBS Letters, vol. 485, no. 1, pp. 53–56, 2000.

[11] K. Okamoto, Y. Iwai, M. Oh-hora et al., “IxBC regulates TH17 development by cooperating with ROR nuclear receptors,” Nature, vol. 464, no. 7293, pp. 1381–1385, 2010.

[12] C. Johansen, T. Bertelsen, C. Ljungberg, M. Mose, and L. Iversen, “Characterization of TNF-α and IL-17A-mediated synergistic induction of DEFB4 gene expression in human keratinocytes through IxBC,” Journal of Investigative Dermatology, vol. 136, no. 8, pp. 1608–1616, 2016.

[13] R. Muromoto, T. Hirao, K. Tawa et al., “IL-17A plays a central role in the expression of psoriasis signature genes through the induction of IxBC in keratinocytes,” International Immunology, vol. 28, no. 9, pp. 443–452, 2016.

[14] C. Johansen, M. Mose, P. Ommen et al., “IxBC is a key driver in the development of psoriasis,” Proceedings of the National Academy of Sciences, vol. 112, no. 43, pp. E5825–E5833, 2015.

[15] X. Zuo, L. Sun, X. Yin et al., “Whole-exome SNP array identifies 15 new susceptibility loci for psoriasis,” Nature Communications, vol. 6, no. 1, p. 6793, 2015.
