Possible association between *NOD2* variants and joint surgery in psoriatic arthritis

E. Graell[1], J.I. Arostegui[2], R. Sanmartí[1], F.J. Blanco[3], J. Yagüe[2], J.A. Pinto[3], S. Plaza[2], J.L. Fernández-Sueiro[3], A. González[4], J.D. Cañete[1]

[1]Arthritis Unit, Rheumatology and [2]Immunology Departments, Hospital Clínic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Rheumatology Department, Hospital Juan Canalejo, La Coruña, Spain; [4]Research Laboratory 2 and Rheumatology Unit, Hospital Clinico Universitario de Santiago, Santiago de Compostela, Spain.

Abstract

Background

Psoriatic arthritis (PsA) has been inconsistently associated with common NOD2 *gene variants, although some of these studies did not include patient stratification by clinical phenotype.*

Objective

To analyse the association between the three common NOD2 *variants (R702W, G908R and L1007fs) and clinical phenotypes of PsA, particularly with surrogate markers of severe joint destruction.*

Patients and methods

A total of 183 unrelated PsA patients and 187 controls were included. Demographic, clinical, biological and immunological characteristics were collected. Genotypes for the three common NOD2 *gene variants were obtained by PCR and direct sequencing.*

Results

NOD2 *variants in PsA patients (7.6%) are just as prevalent as in healthy controls (7.5%). 18.5% of PsA patients carrying at least one* NOD2 *variant underwent joint surgery compared with 4.5% of those without these variants (p=0.019). Multivariate analysis confirmed this finding (OR 8.82, CI 1.7-46.3). There was no requirement for early surgery in patients carrying the NOD2 variants but there was an increased possibility of requiring surgery at similar times of disease duration. No other association with clinical features and* NOD2 *status carrier was found.*

Conclusion

Common NOD2 *gene variants are not associated with PsA, but might increase the risk of undergoing joint replacement surgery, suggesting that this autoinflammatory-associated gene could act as a phenotypic modifier gene in PsA patients by increasing the risk of joint destruction. Given the small number of PsA patients with joint surgery included, we consider our findings a new hypothesis that will need further testing.*

Key words

Psoriatic arthritis, *NOD2* gene variants, joint surgery, autoinflammation.

Eduard Graell, MD
Juan I. Arostegui, MD, PhD
Raimon Sanmartí, MD
Francisco J. Blanco, MD
Jordi Yagüe, MD, PhD
José A. Pinto, MD
Susana Plaza, AS
José L. Fernández-Sueiro, MD
Antonio González, MD, PhD
Juan D. Cañete, MD, PhD

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Please address correspondence and reprints requests to:
Juan D. Cañete, MD, PhD,
Unitat d'Artritis, Servei de Reumatologia,
Hospital Clínic,
Villarroel 170,
08036 Barcelona, Spain.
E-mail: jcanete@clinic.ub.es

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Introduction

Psoriatic arthritis (PsA) is an immune-mediated inflammatory disease affecting the skin and joints with a prevalence ranging between 0.3 and 1% in the general Caucasian population (1), and a significant social and economic impact (2). The etiology remains unknown, although it is believed the disease results from the interplay of genetic, immunological and environmental factors. Clinically, PsA is characterised by a wide heterogeneity, which probably reflects the high complexity of PsA susceptibility and makes it difficult to carry out studies with a large number of homogenous clinical phenotypes (3).

With regard to genetic susceptibility to PsA, different loci have been described. There is general consensus on the relevance of genes located on the HLA region, while controversial results from non-HLA loci have been reported (4). Most recently, the *IL23R* gene has been identified as a psoriasis-susceptibility gene in a large-scale genetic-association study (5), although its role in PsA is unclear. In addition, some reports have shown an association between PsA and common genetic variants located on leucine-rich repeat (LRR) domains of the *NOD2* gene, although others have not (6-8). The *NOD2* gene encodes the cytosolic receptor Nod2, which belongs to the growing family of NOD-LRR (NLR) receptors and plays a key role in sensing pathogen-derived compounds and establishing an adequate immune response (9). Interestingly, uncommon gain-of-function mutations of the *NOD2* gene located at the central NACHT domain are associated with the rare autoinflammatory diseases, Blau syndrome and early-onset sarcoidosis, while common genetic variants located at LRR domains have consistently been associated with Crohn's disease (CD) and the need for early and/or recurrent surgery (10-13).

Since PsA could share some genetic susceptibility and inflammatory mechanisms with CD, as may be also suggested by the similar efficacy of methotrexate and TNF-α blocking agents in the two diseases (14), we hypothesised that common genetic variants at LRR domains of the *NOD2* gene might also play a pathogenic role in PsA. Past studies addressing this association did not include patient stratification by clinical phenotypes (6-8).

Among these phenotypes, we believe that the group of PsA patients developing joint destruction may be particularly interesting. We also investigated the possible role of these common *NOD2* gene variants in susceptibility to this severe clinical phenotype, selecting the need for joint surgery as a surrogate marker of advanced joint destruction.

Patients and methods

Patients and controls

A total of 183 unrelated consecutive Spanish patients meeting the CASPAR criteria for PsA (15) from the outpatient clinics of the Hospital Clínic of Barcelona and Hospital Juan Canalejo of La Coruña, and 187 healthy Spanish age and sex-matched controls were included. The clinical, biological and radiological characteristics collected included: sex, age of PsA onset, disease duration, age of psoriasis onset, main clinical pattern (spondylitis, oligoarthritis, polyarthritis), enthesitis, nail psoriasis, tendinitis, dactylitis, erosive disease (presence or absence of an erosion), sacroiliitis (at least unilateral grade II), HLA-B27 typing, previous treatment with DMARDs, previous treatment with TNF-alpha antagonists, and joint surgery due to inflammatory joint destruction. All participants gave written informed consent after approval of the study by the Ethics Committee of the Hospital Clinic. The study was performed in accordance with the principles of the Helsinki Declaration.

NOD2 genotyping

Genomic DNA from whole blood samples was isolated using QIAmp DNA Blood Mini Kit (QIAgen, Hilden, Germany) following the manufacturer's instructions. Specific sequences of exon 4 (missense mutation R702W), exon 8 (missense mutation G908R), and exon 11 (frameshift mutation L1007fs) of the *NOD2* gene were amplified by polymerase chain reaction (PCR) using specific primers and conditions previously published (16). PCR products were purified using QIAquick purification Kit (QIAgen), according to the manufacturer's

instructions, sequenced using an ABI Big Dye Terminator v1.1 Cycle Sequencing Kit (Foster City, CA) and run on an ABI 3100 automatic sequencer. The investigators who determined the *NOD2* genotypes were blinded to the clinical characteristic of the patients. Patients categorised as carrying variants were those with at least one variant of the *NOD2* gene.

Statistical analysis

Results were analysed using the Statistica 7.0 program (StatSoft, Tulsa, OK, USA). For some analyses, the three *NOD2* gene variants were considered both separately and jointly. The Hardy-Weinberg Equilibrium (HWE) was tested in cases, in controls and in the combined data by an exact test using a significance threshold of 0.05. Chi square association tests with Yates continuity correction or Fisher exact tests were performed to compare allele frequencies in 2x2 contingency tables. Multivariate logistic regression analysis was used to evaluate the effect of the *NOD2* gene variants on selected dichotomous clinical features: joint surgery and erosive disease. Carrier and genotype analyses were performed. For the latter, a simplified additive model without interaction parameters was used with codes 0, 1 and 2 for genotypes AA, Aa and aa, respectively. Covariates included in the model were sex, spondylitis, nail psoriasis, enthesitis and years of disease duration. The Student's *t*-test and multiple linear regression were used to evaluate the effect of *NOD2* variants on the age of disease onset, including the same variables in the multivariate model as for logistic regression.

Table I. Clinical characteristics of PsA patients.

Females, % (N/AD)	48.6 (89/183)
Spondylitis, % (N/AD)	33.7 (61/181)
Axial, % (N/AD)	4.4 (8/181)
Mixed, % (N/AD)	29.3 (53/181)
Oligoarthritis, % (N/AD)	13.8 (25/181)
Polyarthritis, % (N/AD)	53.6 (97/181)
Nail psoriasis, % (N/AD)	42.6 (75/176)
Enthesitis, % (N/AD)	35.5 (64/180)
Tendinitis, % (N/AD)	40.4 (55/136)
Dactylitis, % (N/AD)	47.1 (65/138)
Erosive disease, % (N/AD)	60.7 (99/163)
Radiographic sacroiliitis, % (N/AD)	42.3 (66/156)
HLA-B27, % (N/AD)	21.1 (30/142)
DMARDs, % (N/AD)	85.2 (159/183)
TNF-α antagonists, % (N/AD)	20.2 (37/183)
Joint replacement surgery, % (N/AD)	6.6 (12/182)
Age of PsA onset, M (IR)	39.8 (30.5-52.0)
Disease duration (years), M (IR)	10.0 (5.9-17.5)
Age of psoriasis onset*, M (IR)	29.9 (19.1-45)

*This characteristic was available from 134 patients. N/AD = Number/Available data; M (IR) = Median (Interquartile Range).

Table II. Allelic frequencies of *NOD2* variants in PsA patients and healthy controls.

	R702W			G908R			L1007fs			Overall analysis		
	%	(n/N*)	*p*	%	(n/N)	p	%	(n/N)	*p*	%	(n/N)	*p*
PsA (N= 183)	3.82	(14/366)		1.64	(6/366)		2.18	(8/366)		7.65	(28/366)	
Control (N=187)	5.08	(19/374)	ns	1.87	(7/374)	ns	0.80	(3/374)	0.2	7.75	(29/374)	ns
Ref.17 (N=165)	5.8			1.0			1.0			7.8		
Ref.18 (N=454)	5.3			0.7			1.1			7.0		

*n/N: number of this allele/total number of alleles.

Results

Common NOD2 gene variants are not associated with PsA

The clinical characteristics of the PsA patients included are shown in Table I. *NOD2* genotypes for the three common variants were obtained in 183 PsA patients and 187 controls (See Table II). Genotypes were according to HWE except for L1007fs which showed a significant deviation in PsA patients due to the presence of a single patient with the L1007fs/L1007fs genotype, which was unexpected given the sample size and the low frequency of the L1007fs allele. We confirmed the accuracy of this genotyping by twice processing DNA samples carrying at least one *NOD2* variant. As an independent quality check of genotyping, we compared the frequencies obtained in our control group with those of other reports of Spanish populations, revealing a similar overall allelic frequency for the uncommon *NOD2* variants (7.75% in our group *vs*. 7.0% and 7.8% for other Spanish studies) (Table II) (17-19).

The allelic frequencies of each of the three common *NOD2* variants in PsA patients were indistinguishable from those of controls (Table II). Since it is conceivable that small differences had escaped the individual tests for each variant, we also compared the total frequencies of the three variants, in order to correct this problem and increase the power of the study. This analysis was possible because the three variants were not in linkage disequilibrium or in the same haplotype, and the three were assumed to have a similar effect, as has been previously considered in CD. The overall analysis also showed that allelic frequencies were very similar between PsA patients and healthy controls (Table II), reasonably excluding the possibility of any major effect of common *NOD2* gene variants in susceptibility to PsA in our population.

Common NOD2 gene variants are associated with joint surgery

We investigated whether the overall frequency of carriers of the three common *NOD2* gene variants had any effect on the clinical features of PsA, focusing on three features related to PsA severity: erosive disease, requirement for joint surgery due to inflammatory joint destruction, and age at disease onset. Univariate analyses showed no effect of carrying any of the variants in erosive disease (55.6% in carriers *vs*. 61.8%

Table III. Univariate analysis of the association between clinical phenotype and *NOD2* gene carrier status. **A**) Overall analysis of carrier status at any of the three variants. **B**) Analysis of the association between joint replacement surgery and each *NOD2* variant.

A	*NOD2* Carrier		*NOD2* Non-Carrier		
	%	(n/N)	%	(n/N)	*p*-value
Erosive disease	55.6	(15/27)	61.8	(84/136)	ns
Joint replacement surgery	18.5	(5/27)	4.5	(7/155)	0.019
Age of PsA onset, years mean ± SD	40.0 ± 13.3		41.2 ± 13.8		ns

B	R702W			G908R			L1007fs		
	%	(n/N)	*p*	%	(n/N)	*p*	%	(n/N)	*p*
Carrier	14.3	(2/14)	ns	33.3	(2/6)	0.05	14.3	(1/7)	ns
Non-carrier	5.9	(10/168)		5.7	(10/176)		6.3	(11/175)	

Table IV. Frequency of joint replacement surgery according to PsA duration and *NOD2* carrier status.

	NOD2 Carrier		*NOD2* Non-carrier		
Duration of PsA	%	(n/N)	%	(n/N)	*p*-value
< 10 years	0	(0/13)	1.3	(1/77)**	ns
10-20 years	20.0	(2/10)*	6.0	(3/50)**	ns
> 20 years	75.0	(3/4)*	10.7	(3/28)**	0.015

*Replacement surgeries were: 1 hip arthroplasty, 1 knee arthroplasty, 1 wrist and MCP arthroplasty, 1 wrist and DIP arthroplasty, 1 C1-C2 transarticular screw fixation.
**Replacement surgeries were: 6 hip arthroplasty and 1 knee arthroplasty.

in non-carriers) or age at disease onset (40 years in carriers *vs*. 41.2 years in non-carriers). However, there was a statistically significant difference in the prevalence of joint replacement surgery (18.5 % in carriers *vs*. 4.5 % in non-carriers; *p*=0.019) (See Table IIIA). The frequency of joint replacement surgery in PsA patients with *NOD2* for each of the three variants was 14.3, 33.3 and 14.3 % in patients carrying the R702W, G908R and L1007fs variants, respectively, compared with 5.9, 5.7 and 6.3% in non-carriers. (Table IIIB). A similar percentage of replacement surgery was observed for both centres: 19% in carriers *vs*. 5% in non-carriers in the Hospital Clinic of Barcelona and 16.6% in carriers *vs*. 2.6% in non-carriers in the Hospital Juan Canalejo of La Coruña.

The multivariate analysis showed similar results. Logistic regression analysis showed no effect of *NOD2* carrier status on the presence of erosive disease. Covariates in this analysis were sex, years of disease duration, and presence of spondylitis, enthesitis or nail psoriasis. Only the variable years of disease duration was independently and positively associated with erosive disease (*p*=0.0007). Similarly, there was no significant effect of *NOD2* carrier status on age at PsA onset in the multiple linear regressions including all the above-mentioned covariates (with the exception of disease duration, which was dependent on age at disease onset). In contrast, the multivariate analysis confirmed the association between joint surgery and *NOD2* carrier status. The logistic regression model including all the above-mentioned covariates showed a stronger effect of the presence of common *NOD2* variants (OR 8.82, C.I. 1.7-46.3) than that observed in the univariate analysis (OR 4.81, C.I. 1.4-16.6). This stronger association was mainly due to a positive interaction with the variable disease duration, as shown by the increased OR in a logistic regression model that included only this covariate together with *NOD2* carrier status (OR 7.19, C.I. 1.8-29.5, *p*=0.006). The variable disease duration was positively correlated with the presence of joint surgery (*p*=0.00016). In order to visualise the characteristics of this interaction we stratified our population of PsA patients by their disease duration into three levels: <10 years, 10-20 years and >20 years (Table IV). Below 10 years of evolution, joint surgery was rare in general and absent among *NOD2* carriers. After that time, this frequency increased, but more markedly among patients carrying *NOD2* variants (3 of 4 of whom after 20 years). Therefore, the chances of undergoing joint surgery in PsA patients increased with disease duration but much more clearly if the patients carried some of the three common *NOD2* variants. There was no need for early surgery, but there was an increase in the probability of requiring joint surgery at similar times of disease evolution.

NOD2 carrier status was employed in the above analyses to avoid attributing too much weight to the single PsA patient who was homozygote for one of the variant alleles and who underwent joint surgery. However, we also performed analyses with *NOD2* genotypes and the clinical phenotype, and the results were similar with a slightly stronger effect of the *NOD2* variants in the need for joint surgery (OR 11.20, C.I. 2.3-55.9, *p*=0.0027 in the logistic regression model with all the covariates). No association with erosive disease or with early age at disease onset was detected.

Finally, we investigated the type of joints requiring surgery in relation to the presence of *NOD2* variants, finding an interesting and unexpected pattern: only one of the 7 patients undergoing hip replacement carried an *NOD2* variant, whereas 4 of the remaining 5 patients with surgery on other joints (1 knee, 1 wrist with MCP joint, 1 wrist with PIP joint, 1 with C1-C2 transarticular screw fixation) were *NOD2* carriers. The prevalence of hip replacement in NOD2 carriers (3.7%) was as frequent as in non-carriers (3.8%). This suggests that the association of *NOD2* carrier status with joint surgery was less marked for the hip joint. Nevertheless, the small number of patients undergoing surgery precluded further analysis.

Discussion

Genetic studies investigating the possible causal relationship between common

NOD2 variants and PsA have shown controversial results. A Newfoundland population-based study found a higher frequency of these common *NOD2* variants in PsA patients than in controls (6). However, subsequent studies in different countries did not confirm this causal association (7, 8). Nevertheless, the existence of a susceptibility locus for psoriasis and PsA in chromosome 16q, near to where *NOD2* is mapped, adds further interest to this gene (20, 21).

In line with later studies (7, 8), we found no statistically significant difference in the allelic frequency of common *NOD2* variants between PsA patients and controls, excluding a causal relationship between PsA and the *NOD2* gene in our population. However, no previous study included stratification of PsA patients by clinical phenotype. Given the clinical heterogeneity of PsA, we hypothesised that the possible association of these common *NOD2* variants might be restricted only to certain phenotypes. We found that common *NOD2* variants were significantly increased in PsA patients undergoing joint surgery. This finding seems consistent, because it was confirmed in the multivariable analysis with a stronger association than in the univariate analysis. Furthermore, the increased frequency of joint surgery in PsA patients with common *NOD*2 variants was similar in the two participating centers. All patients included had the same access to joint replacement surgery, since the Spanish healthcare system provides universal, free-at-the-point-use care.

After stratification of patients for disease duration, the association of *NOD2* variants with joint replacement surgery was significant only when disease duration was >20 years. This suggests that the presence of at least one of the three common *NOD2* variants is associated with a greater need for joint replacement surgery after very long disease duration.

Recently, *IL4R*-I50V single-nucleotide polymorphism has been associated with erosive joint disease in PsA, but not with PsA *per se*, emphasizing that some gene variants, although not associated with the disease, could act as phenotypic modifiers (22).

We have no evidence-based explanation for the association between the common *NOD2* variants and the need for joint replacement surgery in PsA patients. However, the probable role of *NOD2* as a phenotypic gene modifier has been established for early or recurrent surgery in CD patients (13).

Our results should be interpreted with caution, given the relatively small number of patients with joint surgery. Furthermore, using this kind of surgery as a surrogate marker of joint destruction meant that we probably only included a subgroup of PsA patients with advanced joint destruction.

These results suggest that common *NOD2* variants are not associated with PsA but might increase the risk of joint replacement surgery, probably by acting as a phenotypic modifier gene. Given the lack of association with the qualitative variable joint erosion and the small number of PsA patients subjected to joint surgery included, we consider our findings as a new hypothesis that will require further testing.

References

1. GLADMAN D, ANTONI C, MEASE P, CLEGG DO, NASH P: Psoriatic arthritis: Epidemiology, clinical features, course and outcome. *Ann Rheum Dis* 2005; 64 (Suppl. 2): 14-7.
2. LEUNG YY, TAM LS, KUN EW, LI EK: Impact of illness and variables associated with functional impairment in Chinese patients with psoriatic arthritis. *Clin Exp Rheumatol* 2008; 26: 820-6.
3. RITCHLIN CT: Pathogenesis of psoriatic arthritis. *Curr Opin Rheumatol* 2005; 17: 406-12.
4. RAHMAN P, ELDER JT: Genetic epidemiology of psoriasis and psoriatic arthritis. *Ann Rheum Dis* 2005; 64 (Suppl. 2): 37-9.
5. 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: 273-90.
6. RAHMAN P, BARTLETT S, SIANNIS F *et al*.: CARD15: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. *Am J Hum Genet* 2003; 73: 677-81.
7. LASCORZ J, BURKHARDT H, HÜFFMEIER U *et al*.: Lack of genetic association of the three more common polymorphisms of CARD15 with psoriatic arthritis and psoriasis in a German cohort. *Ann Rheum Dis* 2005; 64: 951-4.
8. GIARDINA E, NOVELLI G, COSTANZO A *et al*.: Psoriatic arthritis and CARD15 gene polymorphisms: no evidence for association in the Italian population. *J Invest Dermatol* 2004; 122: 1106-7.
9. INOHARA N, NUÑEZ G. NODs: Intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol* 2003; 3: 371-82.
10. ROSÉ CD, WOUTERS CH, MEIORIN S *et al*.: Pediatric granulomatous arthritis. an international registry. *Arthritis Rheum* 2006; 54: 3337-44.
11. HUGOT JP, CHAMAILLARD M, ZOUALI H *et al*.: Association of *NOD2* leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411: 599-603.
12. OGURA Y, BONEN DK, INOHARA N *et al*.: A frameshift mutation in *NOD2* associated with susceptibility to Crohn's disease. *Nature* 2001; 411: 603-6.
13. ALVAREZ-LOBOS M, AROSTEGUI JI, SANS M *et al*.: Crohn's disease patients carrying *NOD2*/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence. *Ann Surg* 2005; 242: 693-700.
14. HO P, BRUCE IN, SILMAN A *et al*.: Evidence for common genetic control in pathways of inflammation for Crohn's disease and psoriatic arthritis. *Arthritis Rheum* 2005; 52: 3596-602.
15. TAYLOR W, GLADMAN D, HELLIWELL P, MARCHESONI A, MEASE P, MIELANTS H, CASPAR STUDY GROUP: Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. *Arthritis Rheum* 2006; 54: 2665-73.
16. LESAGE S, ZOUALI H, CÉZARD JP *et al*.: CARD15/*NOD2* mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; 70: 845-57.
17. MENDOZA JL, MURILLO LS, FERNÁNDEZ L *et al*.: Prevalence of mutations of the *NOD2*/CARD15 gene and relation to phenotype in Spanish patients with Crohn disease. *Scand J Gastroenterol* 2003; 38: 1235-40.
18. NÚÑEZ C, BARREIRO M, DOMÍNGUEZ-MUÑOZ JE, LORENZO A, ZAPATA C, PEÑA S: *CARD15* mutations in patients with Crohn's disease in a homogeneous Spanish population. *Am J Gastroenterol* 2004; 99: 450-6.
19. FERREIROS-VIDAL I, BARROS F, PABLOS JL, CARRACEDO A, GÓMEZ-REINO JJ, GONZALEZ A: CARD15/*NOD2* analysis in rheumatoid arthritis susceptibility. *Rheumatology* (Oxford) 2003; 42: 1380-2.
20. KARASON A, GUDJONSSON JE, JÓNSSON HH *et al*.: Genetics of psoriasis in Iceland: evidence for linkage of subphenotypes to distinct loci. *J Invest Dermatol* 2005; 124: 1177-85.
21. KARASON A, GUDJONSSON JE, UPMANYU R *et al*.: A susceptibility gene for psoriatic arthritis maps to chromosome 16q: evidence for imprinting. *Am J Hum Genet* 2003; 72: 125-31.
22. RAHMAN P, SNELGROVE T, PEDDLE L *et al*.: A variant of the *IL4R* I50V single-nucleotide polymorphism is associated with erosive joint disease in psoriatic arthritis. *Arthritis Rheum* 2008; 58: 2207-8.