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Report

CARD15: a Pleiotropic Autoimmune Gene That Confers Susceptibility to Psoriatic Arthritis

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A recent genomewide scan in psoriatic arthritis (PsA) revealed a susceptibility locus at 16q. This region overlaps *CARD15*, a susceptibility gene in Crohn disease. The possibility of a common susceptibility gene between PsA and Crohn disease is further supported by epidemiological studies that note an increased incidence of psoriasis in subjects with Crohn. We screened 187 patients with PsA and 136 healthy controls, all from Newfoundland, for the three common, independent sequence variants of *CARD15* (R702W, leu1007fsinsC, and G908R), which were detected by polymerase chain reaction by use of allele-specific primers and visualized through gel electrophoresis. In total, 53/187 (28.3%) probands with PsA had at least one variant of the *CARD15* gene, compared with 16/136 (11.8%) controls (odds ratio 2.97; 95% confidence interval 1.61–5.47; $P = .0005$). Allele frequencies of R702W, leu1007fsinsC, and G908R were 10.43%, 3.21%, and 1.61%, respectively, in patients with PsA, compared with 3.31%, 2.57%, and 0.37%, respectively, in the control patients. *CARD15* conferred susceptibility to PsA independent of HLA-Cw*0602. Thus, *CARD15* represents a pleiotropic autoimmune gene and is the first non-MHC gene to be associated with PsA.

Psoriasis (MIM 177900) is a chronic inflammatory hyperproliferative skin disorder that affects 1%–3% of the population (Goodfield 1994). Psoriatic arthritis (PsA) has been defined as an inflammatory arthritis associated with psoriasis that occurs in up to one-third of the patients with psoriasis (Wright and Moll 1976; Gladman et al. 1987). The etiology of PsA remains unknown but likely results from an interplay between genetic, immunological, and environmental factors (Gladman 2002). Multiple lines of evidence support a genetic basis of PsA. A large epidemiological study in the United Kingdom reported strong familial clustering of PsA with a relative risk of 55% for affected first-degree relatives (Moll and Wight 1973). Association studies implicate the HLA loci of the major histocompatibility complex (MHC) in psoriasis and PsA (Gladman 2002). The strongest association is between psoriasis and HLA-Cw*0602, with lesser associations with HLA-B13, HLA-B17, and HLA-B57. In PsA, the association with HLA-Cw*0602 is less profound and is most evident in patients with PsA with an early onset of psoriasis (Enerback et al. 1997; Gladman et al. 1999).

Recent completion of the first-ever genomewide linkage scan in PsA, by use of 1,000 microsatellite markers in 178 patients with PsA from 39 Icelandic families, revealed a LOD score of 2.17 on chromosome 16q (Karason et al. 2003). Further analysis, conditional on paternal transmission to affected individuals, resulted in a LOD score of 4.19. The peak of this LOD score is within 20 Mb of the *CARD15* gene (MIM 605956; GenBank accession number 64127). It is interesting that a region overlapping *CARD15* has also been implicated by a genomewide scan in psoriasis, in which a LOD score of 2.5 was reported (Nair et al. 1997). The *CARD15* gene has convincingly been shown to confer susceptibility to Crohn disease (MIM 266600) (Hugot et al. 2001; Ogura et al. 2001). The possibility of a common susceptibility gene between psoriasis/PsA and Crohn disease is further supported by epidemiological studies that note an in-

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Table 1

Genotypes of *CARD15* Variants in Patients with PsA and Healthy Controls

	Allele Frequency of					
	R702W in		leu1007FsinsC in		G908R in	
Genotype	Subjects with PsA	Controls	Subjects with PsA	Controls	Subjects with PsA	Controls
Mutated/mutated	4	0	0	0	0	0
Mutated/wild type	31	9	12	7	6	1
Wild type/wild type	152	127	175	129	180	134
Total	187	136	187	136	186	135

creased incidence of psoriasis and PsA in subjects with Crohn disease (Lee et al. 1990; Nair et al. 1997).

In this study, we set out to determine the prevalence of the three common independent sequence variants of *CARD15* mutations, arg702 to trp (R702W), leu 1007fsinsC, and gly908 to arg (G908R) in PsA.

The study was approved by the local ethics committee, and informed consent was obtained from all patients. All subjects were identified from the province of Newfoundland and were examined by a single rheumatologist (P.R.). PsA was diagnosed as an inflammatory arthritis in patients with psoriasis, in the absence of other etiologies for inflammatory arthritis. Information collected included variables related to disease pattern, joint damage, extent of psoriasis, extra-articular features, medication history, and surgeries. The control subjects were not related and were all examined to ensure that they had no autoimmune disease.

Genomic DNA was isolated from peripheral whole blood collected in EDTA by use of a salting-out technique (Promega Wizard Genomic DNA Purification Kit). For the *CARD15* genotyping, 50 ng of DNA was used for each PCR reaction. All subjects were genotyped for the R702W (SNP8; exon 4), G908R (SNP12; exon 8), and Leu1007fsinsC (SNP13; exon 11) variants. Reaction products were visualized using agarose gel electrophoresis. All samples were scored as wild type/wild type, wild type/mutated, or mutated/mutated. The laboratory scientist was blind to the clinical diagnosis.

The R702W (Hugot SNP8 [GenBank accession number G67950]) variant of the *CARD15* gene was genotyped by use of the PCR-ARMS technique (Vermeire et al. 2002). Appropriate internal, water, and known controls were used for each R702W reaction (Lesage et al. 2002). The primers were wild-type forward (5′-ATCTGAGAAGGCCCTGCTCC-3′), mutated forward (5′-ATCTGAGAAGGCCCTGCTCT-3′), reverse (5′-CCCACACTTAGCCTTGATG-3′), constant forward (5′-GCAGACATTGATTTTACACAG-3′), and constant reverse (5′-TGAGGCAAAACAACTGAGAC-3′). These primers were synthesized by Integrated DNA Technologies. The PCR conditions are available from the authors on request.

The missense mutation G908R (Hugot SNP12 [GenBank accession number G67951]) creates a restriction endonuclease site for *Hha*I. The double-stranded DNA fragment was amplified using primers 5′-CCCAGCTCCTCCCTCTTC-3′ and 5′-AAGTCTGTAATGTAAAGCCAC-3′. The presence of a variant allele produces two bands of 138 bp and 242 bp, and a wild-type allele results in one band of 380 bp following DNA amplification and digestion with *Hha*I.

The Leu1007fsinsC (Hugot SNP13 [GenBank accession number G67955]) was genotyped using multiplex PCR. The primers were 5′-CTG AGC CTT TGT TGA TGA GC-3′ (forward), 5′-TCT TCA ACC ACA TCC CCA TT-3′ (reverse), 5′-CAG AAG CCC TCC TGC AGG CCC T-3′ (wild type), and 5′-CGC GTG TCA TTC CTT TCA TGG GGC-3′ (mutated). The wild-type allele produced a band of 319 bp, and the mutation produced a band of 214 bp.

For HLA genotyping, 200 ng of genomic DNA was amplified using the Dynal RELI SSO HLA-Cw* typing kit. PCR amplicons were identified by a reverse line assay by use of sequence-specific oligonucleotide probes. Assay results were interpreted by use of the Pattern Matching Program provided by Dynal.

Logistic regression was used to calculate odds ratio (OR) estimates and corresponding 95% CIs that relate *CARD15* mutations and PsA, as well as to examine the joint risk associated with *CARD15* mutations and HLA-Cw*0602. Fisher's exact test was used to test for the presence of linkage disequilibrium between all possible combinations of the *CARD15* variants. Cox's semiparametric method (Cox 1972) was used to examine the association between age at onset of psoriasis and age at onset of PsA and the *CARD15* and HLA-CW*0602 genotypes. For the genotype/phenotype correlations, Fisher's exact test was used to examine associations between *CARD15* mutations and selected binary variables. Since the genotype/phenotype correlations were an exploratory analysis for future studies with larger samples of patients with PsA and the appropriate error structure for multiplicity adjustments in the rheumatological context is not well defined (Cook and Farewell 1996), formal adjustments were not made for multiple testing.

Table 2

Logistic Regression for Univariate *CARD15* Variants

Sequence Variant	OR	95% CI	*P*
R702W	3.50	1.51–7.01	.0027
1007	1.26	.48–3.30	.63
G908R	4.47	.53–7.52	.17
CARD15	2.97	1.61–5.47	.0005

Thus, no definitive conclusions can be drawn regarding the genotype/phenotype correlations.

In total, 187 unrelated white patients with PsA (100 males and 87 females) were assessed. For one patient, G908R information was missing. The mean age and disease duration of patients with PsA were 47.93 years (SD 10.83) and 10.94 years (SD 8.50), respectively, and the mean age for the 136 white controls (56 males and 80 females) was 44.87 years (SD 14.01). All subjects were "native Newfoundlanders" who were whites of Northern European ancestry. Allele frequencies of R702W, leu1007fsinsC, and G908R were 10.43%, 3.21%, and 1.61%, respectively, in patients with PsA, compared with 3.31%, 2.57%, and 0.37%, respectively, in the 136 control patients (table 1). There was no evidence against the assumption of Hardy-Weinberg equilibrium in the controls for all *CARD15* variants.

Tables 2 and 3 provide results of univariate logistic regressions of case/control status for models, including variables defined by any *CARD15* mutation and the separate *CARD15* mutations. Only four patients were homozygous for any *CARD15* mutation (all R702W), and none of the controls were homozygous for any *CARD15* mutation (table 1). A formal dominance test provided no evidence of differential risk for heterozygotes and homozygotes. Therefore, all analyses combine individuals homozygous and heterozygous for allele R702W. The OR for the R702W variant was 3.50 (95% CI 1.51–7.01), $P = .0027$; for the leu1007fsinsC variant, the OR was 1.26 (95% CI 0.48–3.30), $P = .63$; and for the G908R variant, the OR was 4.47 (95% CI 0.53–7.52), $P = .17$ (table 2). There was no evidence of any linkage disequilibrium among the three analyzed polymorphisms. Only a single control individual had two *CARD15* mutations (leu1007fsinsC and R702W). There was no evidence, however, that this was inconsistent with independence assumptions in cases or controls for any pair of mutations (minimum *P* value .13). In total, 53/187 (28.3%) patients with PsA had at least one variant of the *CARD15* gene, compared with 16/136 (11.8%) controls (OR 2.97; 95% CI 1.61–5.47; $P = .0005$). The prevalence of the *CARD15* mutation was similar in familial PsA (14/54; 25.9%), defined as at least one other affected first-degree relative, as compared with sporadic PsA (39/133; 29.3%).

In total, 176 subjects with PsA were genotyped for HLA-Cw*0602. Of the 176 subjects with PsA, 60 (34.1%) had at least one HLA-Cw*0602 allele, as compared with 11/90 (12.2%) of the controls for whom HLA information was available (OR 3.72; 95% CI 1.84–7.51; $P < .001$). Table 4 shows the relationship between the presence of HLA-Cw*0602 and the three *CARD15* mutations in patients with PsA. As can be seen from the table, there was no correlation noted between these variables. The ORs associated with the *CARD15* variants in the multivariate models that include the HLA-Cw*0602 variable differ little from those in the univariate models. In addition, there was no evidence of interaction effects on disease incidence between the *CARD15* variables and the HLA-Cw*0602 indicator. In particular, the 15 individuals with both an HLA-Cw*0602 allele and *CARD15* gene were all patients with PsA, but there was no evidence that this was inconsistent with a multiplicative effect of the two factors ($P = .15$).

One of the most consistent associations reported to date on psoriasis and PsA is the early age at onset of psoriasis in subjects carrying HLA-Cw*0602 (Enerback et al. 1997; Gladman et al. 1999). On the basis of a Cox regression model for onset times and explanatory variables coding for HLA-Cw*0602 and the *CARD15* genes, we noted a strong relationship between an earlier age at the onset of psoriasis and the presence of HLA-Cw*0602 ($P < .001$). There is some evidence that G908R is associated with a later age at onset of psoriasis ($P = .045$), with slightly less evidence in the presence of HLA-Cw*0602 ($P = .061$). No other relationship was noted between the *CARD15* mutations and onset of psoriasis or PsA.

With respect to genotype/phenotype correlations, those

Table 3

Logistic Regression for Multivariate *CARD15* Variants

Sequence Variant	OR	95% CI	*P*
R702W	3.91 (3.70)	1.45–10.54 (1.82–7.54)	.0071 (.0003)
1007	1.50 (3.79)	.50–4.47 (1.87–7.68)	.47 (.0002)
G908R	3.68 (3.68)	.43–31.56 (1.82–7.46)	.23 (.0003)
CARD15	3.57 (4.00)	1.69–7.54 (1.96–8.17)	.0009 (.0001)

NOTE.—All values are in comparison with HLAc6.

Table 4

Relationship between HLA-Cw*0602 and *CARD15* Variants

HLACw*0602 Status	No. of Affected Subjects with Variant					
	R702W[a]		1007[b]		G908R[c]	
	Absent	Present	Absent	Present	Absent	Present
Absent	95	21	106	10	111	5
Present	48	12	58	2	58	1

[a] $P = .84$
[b] $P = .22$
[c] $P = .66$

carrying the R702W mutation had greater tendency for corticosteroid use (9/35 vs. 17/152 [$P = .03$]) and joint surgeries related to PsA (5/34 vs. 7/151 [$P = .05$]). No associations were noted between *CARD15* mutations and disease pattern, damaged joints, extent of psoriasis, extra-articular features, and/or use of disease-modifying agents. Of note, Crohn disease was identified in 2/187 (1.1%) probands with PsA, of whom 1 carried a *CARD15* mutation (R702W heterozygote).

To our knowledge, this is the first study to examine the prevalence of *CARD15* mutations in PsA. It is interesting that a recent Italian study failed to demonstrate a strong association between the same three *CARD15* variants and uncomplicated psoriasis (Borgiani et al. 2002). Lack of association between *NOD2* 3020InsC frameshift mutation and psoriasis has also been reported by Nair et al. (2001). It is conceivable that the *CARD15* mutation is more specific to PsA than uncomplicated psoriasis. Alternatively, an enhanced signal-to-noise ratio, like that in Newfoundland, may be necessary to establish this association. Newfoundland is a well-recognized genetic isolate, the homogeneity of which resembles that of religious isolates such as the Hutterites and the Amish (Bear et al. 1988). Thus, the genetic homogeneity of the Newfoundland population, coupled with the relative environmental/cultural homogeneity, may offer the opportunity to detect modest signals. Given the uniqueness of said population, confirmation of our finding in another population or ethnic group is prudent prior to generalizing that *CARD15* confers susceptibility to PsA.

The *CARD15* gene may shed further light on the pathogenesis of PsA, as the *CARD15* mutations have been implicated in altering recognition of the bacterial lipopolysaccharide (LPS) (Ogura et al. 2001). This hypothesis has been supported by functional experiments, as 1007fs mutations decreased the NF-κB activation by the LPS (Hugot and Cho 2002). This is intriguing, in that a temporal relationship has long been noted between bacterial infections (such as *Streptococcus*) and the development and exacerbation of psoriasis and PsA (Vasey 1985).

In summary, *CARD15* is the first candidate gene identified in PsA that resides outside the MHC. The risk for *CARD15* to be associated in our population with PsA (OR 2.97) is comparable with that seen for HLA-Cw*0602 (OR 3.72), the allele most consistently recognized to be associated with psoriasis and PsA. *CARD15* confers its disease risk independent of HLA-Cw*0602, as no relationship between *CARD15* mutations and HLA-Cw*0602 was noted. Finally, our study suggests that *CARD15* is a pleiotropic autoimmune gene, since it confers susceptibility to Crohn disease (Hugot et al. 2001; Ogura et al. 2001), Blau syndrome (Miceli-Richard et al. 2001), and, now, PsA. One of the central genetic factors in autoimmune diseases is the MHC; however, it is unlikely that this locus solely accounts for the similar genetic predisposition. *CARD15* may represent a non-MHC genetic locus that results in susceptibility to multiple autoimmune diseases.

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Electronic-Database Information

Accession number and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/GenBank/ (for *CARD15* [accession number 64127], *CARD15* genomic sequence information [accession number AC007728], Hugot SNP8 [accession number G67950], Hugot SNP12 [accession number G67951], and Hugot SNP13 [accession number G67955])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Crohn disease, psoriasis, and *CARD15*)

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