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Abstract

Objective: To determine whether the three common independent sequence variants of the putative pleiotropic, non-MHC autoimmune gene CARD15 that has been associated with the immunopathogenesis of Crohn´s disease and more recently with joint involvement in psoriasis influence disease susceptibility in large German cohorts of patients with psoriatic arthritis (PsA) and psoriasis vulgaris, before and after stratification to HLA-C.
Methods: DNA was obtained from 375 patients with psoriatic arthritis, 281 patients with psoriasis vulgaris without joint involvement and 376 controls. The three variants of the CARD15 gene (R702W. G908R and leu1007fsinsC), and two SNPs of the HCR gene (HCR-325 and HCR-2327) for HLA-C stratification were genotyped using allelic discrimination Taqman assays.
Results: No significant differences in genotype frequencies were observed between controls and both psoriatic arthritis and psoriasis vulgaris patient populations, even after stratification to HLA-C in both patient cohorts or to the type of joint involvement within the PsA group.
Conclusion: The lack of genetic association between the most common Crohn´s disease alleles of the CARD15 gene and psoriatic joint disease in the present study on large cohorts of Caucasian patients does not support a more recently claimed role of CARD15 as the first non-MHC susceptibility gene in the pathogenesis of PsA, but confirms and extends previous studies in the case of psoriasis vulgaris.

Introduction

Psoriasis is an HLA-associated, chronic inflammatory and hyperproliferative skin disorder that affects about 2 % of the Caucasian population. Psoriatic arthritis (PsA) is an heterogeneous inflammatory joint disease that affects approximately 5-15 % of patients with psoriasis.

CARD15 previously named NOD2, mapping to chromosome 16q12, has convincingly been shown to confer susceptibility to Crohn´s disease (1,2), due to the insertion polymorphism leu1007fsinsC. Patients with Crohn´s disease have a sevenfold greater risk of developing psoriasis (3). Both diseases may lead to similar patterns of inflammatory involvement of the axial skeleton and peripheral joints. Moreover, bowel manifestations have also been demonstrated in a proportion of PsA patients and are discussed as pathogenic factors in locomotor inflammation although they usually present as subclinical forms and thus considerably differ in severity from Crohn´s disease (4). Linkage studies have mapped a disease susceptibility locus in the chromosome 16 interval that contains the CARD15 gene for both psoriasis (5) and PsA (6).

Thus, CARD15 appeared to be an attractive candidate gene for psoriasis and PsA. In the case of psoriasis vulgaris, several studies have failed to find association neither with the insertion leu1007fsinsC (7), nor the other two common independent sequence variants of CARD15 mutations, arg702 to trp (R702W) and gly908 to arg (G908R) (8). In contrast, disease association for PsA was reported in a Canadian cohort of 187 patients, specially with the R702W mutation (9). Since earlier studies on related arthritis conditions e.g. on familial spondyloarthropathies (10) or rheumatoid arthritis (11) had failed to show association, a specific role of CARD15 as the first non-MHC susceptibility gene in PsA was postulated (9). However, a recent Italian study on 193 PsA patients could not confirm the reported genetic association between CARD15 polymorphisms and PsA (12). Based on these contradictory results obtained in relative small PsA cohorts, we decided to analyse the three mutations (R702W, G908R and leu1007fsinsC) in two substantially enlarged German cohorts: one comprising 375 single PsA patients diagnosed by a board certified rheumatologist, the other one consisting of 281 single patients with psoriasis vulgaris without joint involvement.

In order to test whether CARD15 polymorphisms are associated with certain clinical subphenotypes of PsA , we performed a separate analysis upon patient stratification for the type of joint involvement.

Due to the known considerable heterogeneity and well documented genetic association of psoriasis as well as PsA to the MHC locus (PSORS1), we stratified for the risk allele at this locus and tested again for association to the three polymorphisms. For stratification to PSORS1, we used an indirect procedure similar to that described by Asumalahti (13): the PSORS1-genotype is deduced from a risk haplotype (HCR*WWCC) consisting of certain SNP alleles within the HCR gene, which is in strong linkage disequilibrium with HLA-C.

Patients and Methods

Patients and controls

The 281 patients with psoriasis vulgaris were of German (Caucasian) origin and recruited through dermatology clinics at two psoriasis rehabilitation hospitals. Average age of onset was 21.3 $\pm$ 9.4 years. The majority of patients suffered from plaque type of psoriasis vulgaris. We excluded all patients with signs of psoriatic arthritis until the time of recruitment when average age was 48 $\pm$ 11.1 years. 61 % of patients were male.

The 375 patients with psoriatic arthritis were similarly of German descent and recruited through four different rheumatological centres in Germany: one acute clinic, two rehabilitation hospitals and one private practice. The diagnosis of PsA was made by a board certified rheumatologist according to the criteria of Moll and Wright (14). Clinical

examination of all joints was performed to record the pattern of peripheral and axial joint disease in addition to the presence of skin and nail disease. PsA was classified according to the pattern of peripheral joint disease as oligoarticular (<5 joints ever involved) or polyarticular (>5 joints ever involved). Spinal involvement was defined as a history of inflammatory back pain or radiological evidence of sacroiliitis or spondylitis. Patients exhibiting erosions in at least one joint on plain radiographs of hand and feet or any other joint investigated were regarded as to have erosive arthritis.
Average age of onset for psoriasis vulgaris was 30.1 $\pm$ 13.0 years. 59.9 % of patients were male. For 78 % of the patients, the diagnosis of psoriatic arthritis was made $\geq$ 3 years prior to recruitment. Peripheral joint involvement was detectable in the majority of cases (343 or 91.4%), oligoarticular in 85 patients and polyarticular in 258 patients (22.6 and 68.8 % of the entire cohort, respectively). Spinal involvement was observed in 72 patients accounting for 19.2 % of the PsA-cohort. Sacroiliits and/or spondylitis in those patients was partly associated with concomitant peripheral joint disease.
The 376 controls had no psoriasis vulgaris and no history or signs of inflammatory joint disease at the time of recruitment when average age was 32 $\pm$ 10 years. All of them were German (Caucasian) healthy blood donors. 58.7 % of probands were male.

Genotyping

We genotyped the three polymorphisms of the CARD15 gene (R702W or rs2066844, G908R or rs2066845, leu1007sinsC or rs2066847) and the two SNPs of the HCR gene in positions 325 and 2327 (13) using allelic discrimination Taqman assays on a 7900HT Sequence Detection System (Applied Biosystems), with 10 ng of genomic DNA as a template in a 5 µl Taqman reaction.
Sequences of probes and primers are listed in Table 1. Genotyping rate was > 97 % for all polymorphisms. Taqman genotypes for all polymorphisms were verified by direct sequencing in a set of 24 randomly chosen probands.

Table 1: Sequences of primers and TaqMan MGB-probes for each polymorphism assay.

Polymorphism	TaqMan MGB-probes (5´-3´)	Primers (5´-3´)
R702W	wt: CCCTGCTCcGGCGC mut: CCCTGCTCtGGCGC	F: CTGGCTGAGTGCCAGACAT R: GGATGGAGTGGAAGTGCTTG
G908R	wt: CTCTGTTGCcCCAGAAT mut: TCTGTTGCgCCAGAAT	F: CTGTTGACTCTTTTGGCCTTTTCAG R: GCCACCTCAAGCTTGGTGAT
leu1007fsinsC	wt:CTGCAGGCCCTTGA mut: CTGCAGGcCCCTTG	F: CAGGTTGTCCAATAACTGCATCAC R: CAGACTTCCAGGATGGTGTCATT
HCR-325	wt: GGTCcGGCTCCT mut: AGGTCtGGCTCCTG	F: CAGCAGGCTGAGGTGATCGT R: CCTGGGCCTCTAGCCTCATC
HCR-2327	wt: CTCCAGCTcCAATC mut: TGCTCCAGCTgCAA	F: CCTGAGTGAAGCCATTTCCAA R: CTCCAACTGTCAGCTTAGC

F = forward primer; R = reverse primer; wt = wild-type allele probe; mut = mutant allele probe

Statistical analysis

To determine significant differences in allele frequencies between the patient and control groups, a chi-square statistics was used. All SNPs were tested for Hardy-Weinberg-equilibrium. Haplotypes of the HCR gene were reconstructed with the program PHASE (15) to allow stratification for PSORS1.

Results

Association to R702W, G909R and leu1007fsinsC

There was no significant difference in allele frequencies between controls and the cohorts of PsA or psoriasis vulgaris patients for any of the three variants (Table 2). Overall, 82/376 (21.8 %) controls had at least one variant of the CARD15 gene, compared with 43/281 (15.3 %) probands with psoriasis vulgaris ($\chi^2 = 4.42$, p=0.036) and 74/375 (19.7 %) probands with psoriatic arthritis (not significant). Hardy-Weinberg equilibrium for the three mutations was confirmed in all cohorts.

Table 2: Allele frequencies of the three variants of the CARD15 gene in patients and controls and results of chi-square statistics (compared with controls).

		Controls	Psoriasis vulgaris			Psoriatic arthritis		
Polymorphism		n (%)	n (%)	χ^2	p-value	n (%)	χ^2	p-value
R702W	wt mut	666 (94) 46 (6)	584 (95) 28 (5)	1.255	0.263	691 (94) 43 (6)	0.227	0.634
G908R	wt mut	710 (98) 16 (2)	542 (99) 6 (1)	2.263	0.132	726 (98) 14 (2)	0.178	0.673
leu1007fsinsC	wt mut	716 (97) 24 (3)	549 (98) 13 (2)	1.001	0.317	715 (97) 23 (3)	0.019	0.890

Association to R702W, G908R and leu1007fsinsC after stratification for the type of joint involvement

We stratified the PsA patient group for oligoarthritis (85 cases), polyarthritis (258 cases), spinal involvement (72 cases) and radiological evidence of spondylitis (40 cases). No significant differences in the allele frequencies for any of the three polymorphisms between the whole PsA cohort and any of the stratification groups were found (data not shown).

Stratification for PSORS1

We found deviation from Hardy Weinberg equilibrium for the two HCR SNPs due to significant excess of patients heterozygous for the risk alleles. In contrast, Hardy Weinberg equilibrium was confirmed in the psoriatic arthritis and in the control groups.

As expected, we found strong association to the risk alleles of the two HCR-SNPs, although higher in the psoriasis vulgaris group (1.3 E-14 for HCR-325*T and 1.3 E-8 for HCR-2327*G) than in the psoriatic arthritis cohort (1.3 E-7 for HCR-325*T and 4.47 E-4 for HCR-2327*G). We noted as well a significant difference in the number of HCR*WWCC carriers (187 psoriasis vulgaris subjects (66.5 %) versus 198 PsA subjects (53.0 %), $\chi^2 = 12.00$, p= 5.0 E-4).

Association to R702W, G908R and leu1007fsinsC after stratification for PSORS1

Next we stratified controls and both groups of patients for the risk haplotype HCR*WWCC. This resulted in a weak association of the wild type allele C of R702W in the subgroup of

psoriasis vulgaris patients positive for the risk haplotype ($\chi^2 = 5.839$, p=0.016). No association could be observed in any of the other subgroups (Table 3).

Table 3: Allele frequencies of the three variants of the CARD15 gene in patients and controls after stratification for the PSORS1 associated HCR haplotype HCR*WWCC and results of chi-square statistics (compared with controls).

HCR*WWCC positive		Controls	Psoriasis vulgaris			Psoriatic arthritis		
Polymorphism		n (%)	n (%)	χ^2	p-value	n (%)	χ^2	p-value
R702W	wt mut	231 (91) 23 (9)	356 (96) 16 (4)	5.839	0.016	370 (94) 22 (6)	2.819	0.093
G908R	wt mut	252 (98) 4 (2)	357 (99) 5 (1)	0.034	0.853	385 (98) 7 (2)	0.046	0.830
leu1007fsinsC	wt mut	249 (97) 7 (3)	365 (98) 9 (2)	0.066	0.797	383 (98) 7 (2)	0.643	0.422
HCR*WWCC negative								
R702W	wt mut	431 (95) 23 (5)	177 (94) 11 (6)	0.163	0.686	321 (94) 21 (6)	0.431	0.511
G908R	wt mut	454 (97) 12 (3)	187 (99) 1 (1)	2.87	0.09	337 (98) 7 (2)	0.252	0.616
leu1007fsinsC	wt mut	464 (97) 16 (3)	184 (98) 4 (2)	0.676	0.411	333 (96) 15 (4)	0.534	0.465

Discussion

We failed to find any association to any of the three more common variants of CARD15 with PsA or psoriasis vulgaris. Our results in large cohorts of patients confirm previous studies of smaller psoriasis populations that did not find association of the CARD15 alleles to the skin disease (7,8). However, an earlier published association of PsA, specially with the R702W mutation, in a Newfoundland population (9) was not confirmed. This lack of association is in agreement with a recent Italian study that also failed to reproduce the Canadian results (12). Whereas both these earlier contradictory studies suffer from a somewhat limited sample size, the results of our present investigation on twice as large cohorts of PsA patients are clearly much more robust to type I errors. The discordance of our results with the Canadian study might also be explained by the uniqueness of the Newfoundland population as a classical genetic founder population, that might account for different allele frequencies. Alternatively, extended linkage disequilibrium in this special ethnic background between CARD15 and the true susceptibility gene could be envisaged as a more likely alternative explanation.

The proportion of patients positive for the HCR risk haplotype, as an indirect measure of the PSORS1 risk allele, in the present investigation is in the expected range known from other studies on psoriasis (16) and PsA (17). The large overlap of patients with and without arthritis positive for the PSORS1 risk allele suggests similar genetic factors in both groups. Nevertheless, the significant difference in PSORS1 risk allele frequency between the two patient groups might also indicate differential genetic factors contributing to the development of psoriatic arthropathy.

Taken together, our data indicate that none of the three most common independent variants of the CARD15 gene is associated with PsA (including any subphenotype of joint involvement) or psoriasis vulgaris in the German population. Thus, our study does not support a major contribution of the CARD15 polymorphisms to the pathogenesis of psoriatic joint and skin disease, at least in this population.

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Competing interests

All authors declare no competing interests.

Ethics approval

The study protocols were approved by the ethical committees of the University Hospitals of Erlangen and Münster, respectively, and prior to inclusion all individuals gave their informed consent. The investigations were conducted according to Declaration of Helsinki principles.

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