NOD2/CARD15 Gene Mutation Is Not Associated with Susceptibility to Wegener's Granulomatosis

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ABSTRACT. ***Objective.*** Polymorphisms and mutations in the *NOD2/CARD15* gene have been reported to increase susceptibility to Crohn's disease (CD) and the rare Blau syndrome, respectively. Both conditions are characterized by granuloma formation. We assessed the influence of variants in the *CARD15* gene in another disorder characterized by granuloma, Wegener's granulomatosis (WG).

Methods. Direct DNA sequencing of the *CARD15* gene was performed on 25 patients with WG, and an additional 73 patients were genotyped for the 3 CD associated variants, *R702W*, *G908R*, and *fs1007*.

Results. In the WG patients, 10 previously reported single nucleotide polymorphisms (SNP) were identified. No SNP were present in the WG patients at significantly different frequencies than the control population.

Conclusion. Our data provide no evidence to support an association between *CARD15* and WG. (J Rheumatol 2003;30:305–7)

Key Indexing Terms:
WEGENER'S GRANULOMATOSIS *CARD15/NOD2* CROHN'S DISEASE

Wegener's granulomatosis (WG) is a multisystem, necrotizing, granulomatous vasculitis of uncertain etiology. WG affects about 3 per 100,000 population in the United States with no gender preference[1]. The etiology of WG is unknown, but the disease is thought to result from infectious insult of genetically susceptible individuals[2]. WG has been shown in some studies to be associated with polymorphisms in immunologically relevant genes such as the interleukin 10 (IL-10), transforming growth factor-ß, and cytotoxic T lymphocyte associated antigen-4[3] genes and in several other genes, including those encoding angiotensin-converting enzyme[3], proteinase-3, CTLA-4[5] and alpha 1-antitrypsin[6]. The tendency to relapse among patients with WG has also been found to be associated with polymorphisms in the Fc-gamma receptor gene[7]. Despite these data, the molecular pathology underlying WG remains largely undefined.

Recent studies on the genetics of inflammatory bowel disease have identified the association of Crohn's disease (CD) with polymorphisms of the *NOD2/CARD15* gene[8-10], a member of the NOD1/apoptotic protease-activating factor-1 gene family. *CARD15* mutations have also been reported in patients with the rare autosomal dominant condition, Blau syndrome[11]. Because both CD and Blau syndrome are associated with granuloma development and *CARD15* variants have been specifically associated with the presence of granulomata in patients with CD[12], it has been suggested that selected *CARD15* alleles confer susceptibility to granuloma formation. This possibility is supported by data revealing *CARD15* to be expressed predominantly in monocytes[13], cells that can differentiate into the giant and epithelioid cells characteristic of granulomatous lesions. Sharing of given susceptibility alleles in CD and WG is also consistent with suggestions that each of these diseases has an infectious etiology and arise consequent to induction of aberrant immunoresponses in a genetically predisposed individual[14]. *CARD15* therefore represents an attractive candidate susceptibility gene for WG.

MATERIALS AND METHODS

To explore the relevance of *CARD15* polymorphisms to WG, a mutation screen of the entire coding region and intron/exon boundaries of the *CARD15* gene (polymerase chain reaction conditions available on request) was performed in 25 unrelated patients with WG (14 men, 11 women) and 24 age matched healthy individuals with no evidence of granulomatous disease (Table 1). In addition, another 73 patients with WG and 100 controls were screened by allele-specific PCR for the 3 major CD associated *CARD15* variants, *R702W*, *G908R*, and *fs1007*. All patients met American College of Rheumatology guidelines for diagnosis of WG[15].

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Table 1. Primers for allele-specific PCR and exon sequence analysis of *CARD15* gene.

Allele Specific PCR		Primers	PCR product sizes (bp)
R702W	Control-F	GAATTCCTTCACATCACTTTCCAGT	Wild type 330
	Control-R	GTCAACTTGAGGTGCCCAACATT	Mutant 226
	Mutant (T)-F	GCGCATCTGAGAAGGCCCTGTTCT	Control 512
	Wild type (C)-R	CGCCCAGCGGGCACAGGCCTGGCACCG	
G908R	Control -F	GAAAAAGTGTCACAACTGTAAATTACTC	Wild type 211
	Control-R	CCTAACATTGTGGGGTAGAAATAAA	Mutant 351
	Wild type (G)-F	CGGCTTTTGGCCTTTTCAGATTCAGGG	Control 513
	Mutant (C)-R	GCCGCCCCCTCGTCACCCACTCTGTAGCG	
Fs1007	Control-F	CTGAGCCTTTGTTGATGAGC	Wild type 211
	Control-R	TCTTCAACCACATCCCCATT	Mutant 351
	Wild type (-)-F	CAGAAGCCCTCCTGCAGGCCCT	Control 533
	Mutant (C)-R	CGCGTGTCATTCCTTTCATGGGGC	
CARD15 Sequencing primers			
Exon 1	F	GAAGGTGGGGTTGGTAGACA	238
	R	GAAGGCTGAGGATCAAGCTG	
Exon 2	F	CTGCATCTGGCTTCTGGAGA	517
	R	CCTCTGGGACGGTGTGAAGA	
Exon 3	F	TAAGCCTTCCCACATTGCTC	211
	R	ACTGCCCTTCCCTTTCTGAT	
Exon 4a	F	TGCCTCTTCTTCTGCCTTCC	422
	R	AGTAGAGTCCGCACAGAGAG	
Exon 4b	F	TGCACTTGCTGTGGGCTGCA	452
	R	CTCATGATGGCGCTTCCTCA	
Exon 4c	F	GAAGTACATCCGCACCGAG	446
	R	AGCCAAGAGAAATGTCATCAG	
Exon 4d	F	ATGTGCTGCTACGTGTTCTC	456
	R	CAGACACCAGCGGGCACAG	
Exon 4e	F	ACCTTCAGATCACAGCAGCC	494
	R	GCTCCCCCATACCTGAAC	
Exon 5	F	CTGGCACTTCAGGGATGAAT	269
	R	ATCACTCACAGCTTCCCAGG	
Exon 6	F	TCCAATGTGCTTTGCTTCTG	289
	R	CAGCATTAGAGAACCCCTGC	
Exon 7	F	TTCTTCCTGTGTTTCCCTGG	257
	R	GCTGAAGAGTTTCACCTGCC	
Exon 8	F	AAGTCTGTAATGTAAAGCCAC	380
	R	CCCAGCTCCTCCCTCTTC	
Exon 9	F	AAAAAGAAAGAGCACCGCAA	249
	R	CAGAGAATCCCCCAAACTCA	
Exon 10	F	AATTGAGAATCCCCACAACG	289
	R	CTTCCAAAGGCCAGCAATTA	
Exon 11	F	CTGAGCCTTTGTTGATGAGC	533
	R	TCTTCAACCACATCCCCATT	
Exon 12	F	TTGTTTGAAAGCCCTGCTCT	239
	R	GGCTCATTTTGAAGAGGCTG	

RESULTS

A total of 98 patients with WG were evaluated in this study. Among this group, the average age of disease onset was 49 years (range 12–77). Consistent with the low number of multicase WG families documented in reports, only 4 of the WG patients were members of affected sibling pairs. About 11% of

Table 2. Allele frequencies for SNP identified in *CARD15* in patients with WG and controls.

	Amino-acid Substitution					
	P268S	R311W	R702W	G908R	V9551	1007fs
dbSNP	rs2066842		rs2066844	rs2066845		rs2066847
WG patients	C/T	C/T	C/T	G/C	G/A	–/C
	66/34*	96/4	96/4	97/3	80/20	99.5/0.5
Controls	58/42	100/0	95/5	98/2	85/15	97/3

* Data are percentages.

the patients had a first-degree relative with thyroid disease, a finding that supports other data suggesting an autoimmune etiology for WG[16].

Analysis of *CARD15* polymorphisms in this WG population revealed 10 different single nucleotide polymorphisms (SNP) among the 25 individuals in whom the coding region was completely sequenced. All these SNP have been described in conjunction with analysis of patients with CD. As shown in Table 2, 6 of the *CARD15* SNP are associated with nonconservative amino acid substitutions. However, no significant differences were detected by chi-square analysis between the allele frequencies for any *CARD15* SNP in the WG patients compared to controls. Also, the allele frequencies for *R702W, G908R* and *1007fs* were found to be significantly lower in the WG patients than has been reported for patients with CD[9,12]. Importantly, the WG patients showed no changes of the specific *CARD15* residues involved in Blau syndrome. Similarly, previously reported sequence variants within the *CARD15* nucleotide-binding domain, the region of *CARD15* thought to be associated with extraintestinal granuloma formation, were not detected in this WG population[11].

DISCUSSION

Polymorphisms and mutations in the *CARD15* gene have been associated with granulomatous disorders, specifically with increased susceptibility to CD[8-10] and Blau syndrome[11], respectively. However, no previous report assessed the influence of variants in *CARD15* on other forms of granulomatous disease. We report that, although sequence changes that predispose to WG may lie in *CARD15* regulatory regions or intronic sequences not assessed in this study, the data provide no evidence to support an association between *CARD15* and WG. These observations, however, do not preclude the possibility that variants in genes encoding other proteins in the *CARD15* signaling pathway or proteins involved in granuloma formation may contribute to susceptibility to WG.

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