Caspase Recruitment Domain-Containing Protein 15 Mutations in Patients with Colorectal Cancer

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Abstract

The caspase recruitment domain-containing protein 15 (CARD15) plays a crucial role in mediating the innate immune response. Mutations within this protein have been shown to be independent risk factors for the development of Crohn's disease in Caucasians. As Crohn's disease patients are at increased risk of developing sporadic colorectal cancer, it is conceivable that genetic variability within CARD15 may also play a role in determining susceptibility to this gastrointestinal malignancy in individuals without Crohn's disease. This hypothesis is supported by the findings of two case-control studies that found the frequencies of *CARD15* mutations were significantly elevated in Polish and Greek colorectal cancer patients. Given the results of these previous studies, we examined whether the high incidence of sporadic colorectal cancer observed in New Zealand Caucasians was due to mutations within CARD15. To answer this question, we genotyped 133 colorectal cancer patients and 201 Caucasian controls for R702W, G908R, 1007fs, and P268S. χ^2 Testing found that the combined frequency of R702W, G908R, and 1007fs was significantly elevated in colorectal cancer patients compared with controls (P = 0.001; odds ratio, 2.8; 95% confidence interval, 1.5-5.4), but no association was detected between tumor behavior or age of disease onset and *CARD15* mutations in our colorectal cancer cohort. This study is the first to explore the link between *CARD15* mutations and colorectal cancer in New Zealand Caucasians. Our results strongly suggest that CARD15 influences susceptibility to colorectal cancer, but we have found no evidence to indicate that *CARD15* mutations predict the clinicopathologic characteristics of this disease. (Cancer Res 2006; 66(5): 2532-5)

Introduction

Patients with inflammatory bowel disease are known to have an increased risk of developing colorectal cancer (1). In Caucasians, the caspase recruitment domain-containing protein 15 (CARD15) mutations R702W, G908R, and 1007fs have been shown to be independent risk factors for Crohn's disease with up to 50% of patients carrying at least one of these variants (2). All three mutations occur on a background haplotype that is identified by the presence of the single nucleotide polymorphism P268S. This variant is a marker for a novel *CARD15* haplotype (IVS8 + 158) that confers a population-attributable risk of 15.1% for Crohn's disease in Ashkenazi Jews (3). *CARD15* mutations may also increase the susceptibility of developing colorectal cancer in Caucasians without Crohn's disease. Of the three previous case-control studies that have examined the role of CARD15 in colorectal cancer, two report an increased frequency of *CARD15* mutations in colorectal cancer patients compared with healthy controls (4, 5), suggesting that this protein may also play an important role in determining susceptibility to colorectal cancer. The incidence of colorectal cancer in New Zealand is one of the highest in the world and is predicted to increase in the next decade due to an aging population (6). It is possible that one of the factors contributing to the elevated risk of colorectal cancer observed in Caucasians is genetic variability within CARD15. To determine whether *CARD15* mutations play an important role in determining colorectal cancer susceptibility in New Zealand Caucasians, we screened patients diagnosed with sporadic colorectal cancer and healthy controls for R702W, G908R, 1007fs, and P268S.

Materials and Methods

Study participants. Patients with colorectal cancer presenting for surgery at Christchurch Hospital (Christchurch, New Zealand) for a newly diagnosed bowel cancer were consented for banking of tumor tissue and a DNA sample in the Cancer Society Tissue Bank between November 2002 and December 2004 (7). Comprehensive clinical data, including age at disease onset and clinicopathologic characteristics of the tumor, were collected prospectively on each colorectal cancer patient and stored in the Cancer Society Tissue Bank database located in the Christchurch School of Medicine and Health Sciences. Patients were excluded from this study if they had a clinical history of Crohn's disease. Healthy Caucasian controls, living within the catchment area of Christchurch Hospital, were recruited at random from the electoral roll. Details of the recruitment and the demographics of this control cohort have been previously described (8). Informed consent was obtained from all study participants and ethical approval for this research was given by the Upper South B Regional Ethics Committee of New Zealand. The use of colorectal cancer patient DNA was approved by the Cancer Society Tissue Bank Board.

DNA samples. Genomic DNA was collected from the peripheral blood of patients with colorectal cancer and from healthy controls using guanidine isothiocyanate extraction (9) and rapid boiling lysis (10), respectively. The colorectal cancer DNA samples were provided by the Cancer Society Tissue Bank. Patient and control DNA samples were genotyped for the *CARD15* variants R702W, G908R, 1007fs, and P268S using a previously described multiplex amplification refractory mutation system (11).

Statistical analysis. The significance of differences in the *CARD15* allele frequencies between colorectal cancer patients and controls and the association of *CARD15* variants with clinicopathologic characteristics were assessed using χ^2 tests. Odds ratios (OR) were determined with the corresponding χ^2 distribution test and 95% confidence intervals (95% CI). Only P values <0.05 were considered significant.

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Table 1. Allele and genotype frequencies for *CARD15* mutations in colorectal cancer patients and controls

Mutation	Genotype			%Allele frequency	*P*	OR (95% CI)
	−/−	−/+	+/+			
R702W						
CRC	116	15	2	7.1	0.03	2.30 (1.1-5)
Control	189	12	0	3.0		
G908R						
CRC	127	6	0	2.2	0.09	3.10 (7.7-12.7)
Control	198	3	0	0.8		
1007fs						
CRC	127	6	0	2.2	0.19	2.30 (0.64-8.4)
Control	197	4	0	1.0		
P268S						
CRC	86	37	10	21.4	0.06	0.65 (0.41-1.0)
Control	109	80	12	26.0		
702/908/1007						
CRC	104	27	2	—	0.001	2.80 (1.5-5.4)
Control	183	17	1	—		

Results

One hundred and thirty-three Caucasian colorectal cancer patients without Crohn's disease and 201 Caucasian controls drawn from the same region of New Zealand were genotyped for the *CARD15* variants R702W, G908R, 1007fs, and P268S (Table 1). The allele frequencies of *CARD15* variants found in the control cohort did not differ significantly from frequencies reported previously in Australian, United Kingdom, Norwegian, and Belgium Caucasians but did differ markedly from the *CARD15* allele frequencies observed in a number of other Caucasian populations (Table 2). Excluding P268S, R702W was the major *CARD15* variant detected in both New Zealand controls and colorectal cancer patients. A single compound heterozygote carrying R702W and G908R was found in the controls and two R702W homozygotes were identified within the colorectal cancer cohort. χ^2 Testing showed that the combined frequency of R702W/G908R/1007fs was significantly higher in the colorectal cancer patients than in our control cohort [P = 0.001; OR, 2.8; 95% CI, 1.5-5.4]; however, individually, only R702W was found to be associated with colorectal cancer [P = 0.03; OR, 2.3; 95% CI, 1.1-5.0], although a trend toward significance was also observed for P268S [P = 0.06; OR, 0.65; 95% CI, 0.41-1.0] and G908R [P = 0.09; OR, 3.1; 95% CI,7.7-12.7]. No association was detected between colorectal cancer and 1007fs (Table 1).

The distribution of R702W, G908R, and 1007fs within the colorectal cancer cohort for a range of clinicopathologic

Table 2. *CARD15* allele frequencies in Caucasian populations

Mutation	%Allele frequency										
	Poland (*n* = 300; ref. 4)	Finland (*n* = 348; ref. 14)	Greece (*n* = 100; ref. 5)	New Zealand* (*n* = 201)	Australia (*n* = 409; ref. 15)	United Kingdom (*n* = 349; ref. 16)	Italy (*n* = 108; ref. 17)	Spain (*n* = 312; ref. 18)	Norway (*n* = 202; ref. 19)	Germany (*n* = 373; ref. 19)	Belgium (*n* = 95; ref. 20)
R702W											
CRC	—	—	4.8	7.1	—	—	—	—	—	—	—
Control	—	—	1.0	3.0	4.5	5.2	6.0	4.0	2.7	4.8	2.1
G908R											
CRC	—	—	8.7	2.2	—	—	—	—	—	—	—
Control	—	—	3.6	0.8	0.7	1.4	2.3	2.5	1.2	0.7	0.5
1007fs											
CRC	14.4	1.9	12.5	2.2	—	—	—	—	—	—	—
Control	7.0	1.9	6.0	1.0	1.0	1.6	1.4	1.6	1.2	4.1	1.1
P268S											
CRC	—	—	—	21.4	—	—	—	—	—	—	—
Control	—	—	—	26.0	—	—	—	—	—	—	—

*This study.

characteristics was also assessed using χ^2 testing (Table 3). No association was found between carriers of one or two *CARD15* mutations and tumor location, size, or development. Similarly, colorectal cancer patients under 60 years of age at diagnosis were no more likely to carry a *CARD15* mutation than patients ≥60 years. In contrast, a significant association was detected between gender and presence of R702W, G908R, and 1007fs. Female colorectal cancer patients were less likely to carry one of the three *CARD15* mutations than their male counterparts ($P = 0.03$; OR, 0.34; 95% CI, 0.18-0.92; Table 3).

Discussion

Inflammation plays a key role in the development of gastrointestinal disorders, such as ulcerative colitis, Crohn's disease, and colorectal cancer (12). As CARD15 is involved in initiating the innate immune response, it is conceivable that R702W, G908R, and 1007fs, which abolish the normal functioning of this protein, may increase susceptibility to these diseases. Such a link has already been shown for Crohn's disease in Caucasians (13).

The aim of this study was to examine whether *CARD15* mutations increased susceptibility to colorectal cancer in New Zealand Caucasians without Crohn's disease. We found that both the frequency of R702W and the combined frequencies of the three key *CARD15* mutations were significantly elevated in colorectal cancer patients compared with controls (Table 1). These results agree with the findings of Kurzawski et al. (4) and Papaconstantinou et al. (5) who both reported significant associations between colorectal cancer incidence and *CARD15*. However, the frequencies of each *CARD15* mutation differed markedly among the control cohorts, indicating the individual contributions of R702W, G908R, and 1007fs to colorectal cancer susceptibility may vary significantly among Caucasian populations. When the effect of each variant was assessed individually, we only detected a significant association between R702W and colorectal cancer. In contrast, Papaconstantinou et al. (5) found that all three *CARD15* mutations were significantly elevated in Greek colorectal cancer patients. Similarly, Kurzawski et al. (4) found 1007fs to be associated with colorectal cancer susceptibility, whereas Alhopuro et al. (14) found no link between colorectal cancer and the frameshift mutation in a population-based series of 1,042 Finnish colorectal cancer patients. This lack of association between colorectal cancer susceptibility and G908R and 1007fs observed in New Zealand and Finnish colorectal cancer patients may reflect the relative rarity of the alleles in these populations compared with the frequencies reported in Greek and Polish Caucasians (Tables 1 and 2). It is conceivable that an association between CARD15 genetic variability and colorectal cancer incidence may have been detected in the Finnish study if cases and controls had also been genotyped for R702W. Studies investigating the frequencies of *CARD15* mutations in Crohn's disease patients have found R702W to be the most common of the three *CARD15* variants in many European control

Table 3. Clinicopathologic features of colorectal cancer patients with and without *CARD15* mutations

Variables	*n*	Carriers*	Noncarriers	*P*	OR (95% CI)
Gender					
Female	56	6	50	0.03	0.34 (0.18-0.92)
Male	77	20	57		
Age (y)					
≥60	115	23	92	0.83	0.89 (0.26-2.90)
<60	18	4	14		
Tumor location					
Left colon	13	2	11	0.90 (Left vs right)	1.10 (0.43-2.60)
Right colon	25	4	21		
Tumor size (mm)					
≥30	104	23	81	0.16	0.41 (0.11-1.40)
<30	29	3	26		
Duke's stage					
Stage A	28	3	25	0.26 (A vs B + C)	0.48 (0.13-1.80)
Stage B	51	12	39	0.70 (A + B vs C)	0.83 (0.30-2.10)
Stage C	49	8	41		
Vascular invasion					
Yes	40	11	29	0.10	2.1 (0.86-5.00)
No	88	15	73		
Necrosis					
Yes	19	6	13	0.15	2.2 (0.74-6.40)
No	84	15	69		
Lymph node involvement					
Yes	49	10	39	0.94	0.97 (0.40-2.33)
No	81	16	65		
Infiltrative margin					
Yes	48	10	38	0.80	0.86 (0.33-2.21)
No	71	12	59		

*Patients with R702W, G908R, or 1007fs.

cohorts (Table 2). The variability in frequency of *CARD15* mutations in Caucasians illustrates, first, the importance of recruiting cases and controls from the same country and ideally from within the same region and, second, in screening for all common mutations.

As in Crohn's disease—where patients with one or two *CARD15* mutations are more likely to suffer early onset of disease; have inflammation localized to the ileum; and develop granulomas, stenosis, and structuring and fistulizing complications than individuals with no *CARD15* mutation (13)—it is possible that these mutations may also be predictive of age of colorectal cancer onset and tumor behavior. Associations with *CARD15* variants and specific clinicopathologic characteristics were observed in both the Polish and Finnish colorectal cancer cohorts. Within the Polish colorectal cancer cohort, the frequency of the 1007fs variant was found to be significantly elevated in patients that were >60 years of age at diagnosis (4), whereas in the Greek cohort advanced stage tumors were more frequently seen in patients that carried at least one *CARD15* variant (5). Our study did not detect any differences in age of onset or tumor behavior between individuals that carried one or more *CARD15* mutations and those that had a wild-type *CARD15* genotype. However, we did observe a significant association between gender and *CARD15*. Female colorectal cancer patients were far less likely to carry a *CARD15* mutation than their male counterparts. The reason for this significant difference is unknown and has not been reported before.

Unlike the three previous studies examining *CARD15* variants and colorectal cancer, our study also included the analysis of P268S. It is possible that this *CARD15* single nucleotide polymorphism may increase susceptibility to colorectal cancer or act as a marker for a yet to be identified *CARD15* mutation that is relevant to this gastrointestinal malignancy. However, in our study, no significant differences in the frequency of P268S could be detected between patients and controls or among specific clinicopathologic characteristics, suggesting that neither P268S nor a novel variant in linkage disequilibrium with this background variant contribute to colorectal cancer susceptibility in New Zealand Caucasians.

This is the fourth study to explore the link between *CARD15* and colorectal cancer susceptibility and is the first to examine the incidence of *CARD15* mutations in New Zealand Caucasians with colorectal cancer. Our findings and those of Kurzawski et al. (4) and Papaconstantinou et al. (5) strongly suggest that genetic variability within CARD15 plays an important role in determining susceptibility to this cancer. Whether this association is replicated in the majority of Caucasian populations remains to be shown. It is more than likely that the association between *CARD15* mutations and incidence of colorectal cancer will strengthen or weaken depending on which variants are tested and the specific characteristics of the population studied.

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References

1. Gasche C, Carethers JM. NOD2 and colorectal cancer: guilt by non-association. Cancer Res 2004;64:5525; author reply 5525–6.
2. Lesage S, Zouali H, Cezard 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.
3. Sugimura K, Taylor KD, Lin YC, et al. A novel NOD2/CARD15 haplotype conferring risk for Crohn's disease in Ashkenazi Jews. Am J Hum Genet 2003;72:509–18.
4. Kurzawski G, Suchy J, Kladny J, et al. The NOD2 3020insC mutation and the risk of colorectal cancer. Cancer Res 2004;64:1604–6.
5. Papaconstantinou I, Theodoropoulos G, Gazouli M, et al. Association between mutations in the CARD15/NOD2 gene and colorectal cancer in a Greek population. Int J Cancer 2005;114:433–5.
6. Keating J, Pater P, Lolohea S, Wickremesekera K. The epidemiology of colorectal cancer: what can we learn from the New Zealand Cancer Registry? N Z Med J 2003;116:U437.
7. Morrin H, Gunningham S, Currie M, Dachs G, Fox S, Robinson B. The Christchurch Tissue Bank to support cancer research. N Z Med J 2005;118:U1735.
8. Burt MJ, George PM, Upton JD, et al. The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 1998;43:830–6.
9. Ciulla TA, Sklar RM, Hauser SL. A simple method for DNA purification from peripheral blood. Anal Biochem 1988;174:485–8.
10. Talmud P, Tybjaerg-Hansen A, Bhatnagar D, et al. Rapid screening for specific mutations in patients with a clinical diagnosis of familial hypercholesterolaemia. Atherosclerosis 1991;89:137–41.
11. Roberts RL, Gearry RB, Barclay ML, Kennedy MA. Rapid detection of common CARD15 variants in patients with inflammatory bowel disease. Mol Diagn 2004;8:101–5.
12. Macarthur M, Hold GL, El-Omar EM. Inflammation and cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 2004;286:G515–20.
13. Economou M, Trikalinos TA, Loizou KT, Tsianos EV, Ioannidis JP. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. Am J Gastroenterol 2004;99:2393–404.
14. Alhopuro P, Ahvenainen T, Mecklin JP, et al. NOD2 3020insC alone is not sufficient for colorectal cancer predisposition. Cancer Res 2004;64:7245–7.
15. Cavanaugh JA, Adams KE, Quak EJ, et al. CARD15/NOD2 risk alleles in the development of Crohn's disease in the Australian population. Ann Hum Genet 2003;67:35–41.
16. Ahmad T, Armuzzi A, Bunce M, et al. The molecular classification of the clinical manifestations of Crohn's disease. Gastroenterology 2002;122:854–66.
17. Palmieri O, Toth S, Ferraris A, et al. CARD15 genotyping in inflammatory bowel disease patients by multiplex pyrosequencing. Clin Chem 2003;49:1675–9.
18. Ferreira AC, Almeida S, Tavares M, et al. NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. Inflamm Bowel Dis 2005;11:331–9.
19. Hampe J, Grebe J, Nikolaus S, et al. Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. Lancet 2002;359:1661–5.
20. Vermeire S, Louis E, Rutgeerts P, et al. NOD2/CARD15 does not influence response to infliximab in Crohn's disease. Gastroenterology 2002;123:106–11.

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