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Diverticulitis and Crohn's disease have overlapping tumor necrosis superfamily 15 haplotypes

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ABSTRACT

Background: Diverticulitis (DD) and Crohn's disease (CD) have overlapping features including bowel structuring, inflammation, and infection. Tumor necrosis superfamily 15 (TNFSF15) is an immunoregulatory, anti-angiogenic gene. CD has been previously associated with a haplotype of five TNFSF15 single-nucleotide polymorphism alleles: rs3810936 (G allele), rs6478108 (A), rs6478109 (G), rs7848647 (G), and rs7869487 (A). We aimed to determine the TNFSF15 risk haplotype for DD *versus* controls with a subgroup analysis of youthful DD patients (aged ≤55 y) *versus* older controls (aged ≥55 y).

Methods: A total of 148 diverticulitis patients (90 aged ≤55 y) and 200 controls (87 aged ≥55 y) were genotyped using our custom-designed Illumina Veracode microarray chip. Genotypes from rs3810936, rs6478108, rs6478109, rs7848647, rs7869487 and two additional TNFSF15 single nucleotide polymorphisms, rs3810936 and rs11554257, were analyzed. PHASE version 2.1, R with HaploStats and the Broad Institute's Haploview program were used for statistics and imputed haplotype frequency. Permutation corrected for multiple comparisons.

Results: The CD GAGGA haplotype was significantly associated with diverticulitis ($P = 0.03$) in the all DD *versus* all controls comparison. A second haplotype, rs6478108 (A), rs6478109 (G), rs7869487 (A), and rs4263839 (G), was also associated with DD in this cohort ($P = 0.025$). A third haplotype rs6478108 (A), rs6478109 (G), rs7848647 (G) and rs7869487 (A), rs4263839 (G) was demonstrated in the DD < 55 *versus* controls >55 comparison ($P = 0.045$).

Conclusions: Distinct but overlapping TNFSF15 haplotypes were demonstrated in diverticulitis patients *versus* healthy controls when compared with the known Crohn's risk haplotype suggesting similar but distinct genetic predispositions. This study strengthens the role for a genetic predisposition to diverticulitis that involves the TNFSF15 gene.

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Introduction

A genetic predisposition to diverticular disease (DD) has been suggested by (1) the observation of families with multiple affected members, many who present at a young age,[1,2] (2) the association of DD with inherited hypermobility and collagen vascular disorders,[3-5] and (3) large twin studies using extensive familial registries.[6-8] However, thus far, little work has been done to identify genetic markers of DD, particularly those found in the blood. The few preliminary germline genetic studies that have been performed to date include methylation and mitochondrial DNA studies and were limited by small patient numbers.[9-11] Other studies on somatic gene expression focused on genes with roles in gap junction function[12] and neurotransmission[13] and required invasive procedures to obtain tissue for study. The identification of a genetic signature associated with the disease, particularly one that may be obtained via a simple blood draw, may affect surgical decision-making, particularly in young patients diagnosed with the disease.

Tumor necrosis superfamily 15 (TNFSF15) is a gene involved in immunomodulation and vascular endothelial function that has been previously associated with inflammatory bowel disease (IBD), specifically ulcerative colitis that is medically refractory and severe forms of Crohn's disease (CD).[14,15] Interestingly, protein expression levels have been shown to correlate with the severity of both inflammation and fibrostenosis in CD.[16,17] We have previously identified an association between the TNFSF15 single-nucleotide polymorphism (SNP), rs7848647 (the G allele), and diverticulitis requiring surgical intervention. This association appeared to be even more robust than the previously determined and replicated association with IBD.[18]

A TNFSF15 risk haplotype or combination of alleles commonly inherited together has also been demonstrated and replicated in CD. This "GAGGA" haplotype includes five SNPs: rs3810936 (G allele), rs6478108 (A allele), rs6478109 (G allele), rs7848647 (G allele), and rs7869487 (A allele).[19,20] The aim of the present study was to use these and two additional TNFSF15 SNPs to identify a DD TNFSF15 risk haplotype.

Materials and methods

All DD and healthy control patients were identified from the Penn State, Milton S. Hershey Medical Center (HMC) Division of Colon and Rectal Surgery's Internal Review Board–approved Biobank. Established in 1998, this Biobank contains demographic and clinical data and blood and tissue samples from more than 2500 individuals including IBD patients and their unaffected family members, DD patients, colorectal cancer patients, Clostridium difficile patients, and healthy volunteer controls. All subjects were over the age of 18 y and gave informed consent for participation in the Biobank at the time of recruitment. Only index members from recruited families with multiple DD-affected members were included. All patients with a concomitant colorectal disease (i.e., DD and colorectal cancer) were excluded.

Table 1 – Demographics for all controls *versus* all diverticulitis patients.

	All controls, $n = 200$	All diverticulitis patients, $n = 148$	P value
Male	59 (29.5%)	73(49.3%)	0.0002
Smoking status			0.07
Never smoked	125 (62.5%)	77 (52.0%)	
Former smoker	44 (22.0%)	40 (27.0%)	
Current smoker	27(13.5)	31 (20.9%)	
Unknown	4 (2%)	0	
Mean age (y, SD)	51.9 ± 16.3	59.1 ± 13	<0.0001

DNA isolation and genotyping

DNA was extracted from blood or stored B cell samples using a QIAamp DNA Blood Mini kit (Qiagen Inc, Valencia, CA). The concentration of each DNA sample was quantified using a nanodrop spectrophotometer. Working DNA stocks were prepared in 10 mM Tris-HCl, at 10 ng/μL (Invitrogen, Carlsbad, CA).

Genotyping was performed using the Penn State HMC Division of Colon and Rectal Surgery's custom-designed Illumina Veracode microarray chip (Illumina, San Diego, CA) and an Illumina BeadXpress Reader (Illumina) in the Penn State HMC Functional Genomics Core Facility. This chip contains SNPs from more than 150 genes of IBD and colonic disease–related interest. The genotyping results for the seven TNFSF15 SNPs of interest were identified and specifically analyzed separately. Statistical analysis and imputed haplotype frequency were performed using PHASE version 2.1 and R with HaploStats and the Broad Institute's Haploview program.[21,22] Linkage disequilibrium (LD) in the LD plots derived from this program are represented by D′.[23] D′ is a measure of linkage and is represented in decimals. A D′ value of 1 indicates perfect linkage between two SNPs. Demographic values are provided with standard deviation where appropriate.

Two analyses were performed: (1) all DD patients *versus* all controls and (2) DD patients under the age of 55 y at diagnosis (DD < 55) *versus* controls over the age of 55 y (controls > 55) at the time of study. This second analysis was performed for two

Table 2 – Demographics for subgroup analysis, controls aged >55 y *versus* diverticulitis patients aged <55 y at the time of diagnosis.

Subgroup	Controls aged >55 y, $n = 87$	Diverticulitis patients aged <55 y, $n = 90$	P value
Male	28 (32.2%)	50 (55.0%)	0.002
Smoking status			0.013
Never smoked	49 (56.3%)	48 (53.3%)	
Former smoker	25 (28.7%)	20 (22.2%)	
Current smoker	11(12.6%)	22 (24.4%)	
Unknown	2(2.3%)	0	
Mean age (y, SD)	67.2 ± 10.3	53.2 ± 11.1	<0.0001