Prevalence of genetic variants associated with inflammatory bowel disease in a healthy First Nations cohort

Travis B. Murdoch MD MSc, Charles N. Bernstein MD, Hani El-Gabalawy MD, Joanne M. Stempak MSc, Michael Sargent, Brenda Elias PhD, Wei Xu PhD, Saad Pathan DPhil, Mark S. Silverberg MD PhD

ABSTRACT

Background: Inflammatory bowel disease is the result of both genes and environment. Canadian First Nations people, despite living in a region with a high prevalence of inflammatory bowel disease, are relatively protected from this disease. We aimed to compare the carriage of genetic variants associated with inflammatory bowel disease in healthy First Nations and white people.

Methods: DNA was extracted from the venous blood of healthy First Nations (n = 340) and white (n = 285) participants from Manitoba. Genotyping was performed for 69 single nucleotide polymorphisms (SNPs) with known or suspected associations with inflammatory bowel disease. We compared the genotypes between groups by logistic regression, adjusting for multiple testing. We calculated a risk score for the *NOD2* gene by adding the number of risk alleles at three important *NOD2* SNPs (G908R, R702W and 3020insC).

Results: We found genetic variation between white and First Nations participants at 45 of 69 SNPs. Notably, carriage of the *ATG16L1* T300A mutation was lower in First Nations participants (p = 4.1×10^{-30}). Cumulative carriage of important *NOD2* variants was significantly lower among First Nations participants (3.9% v. 15.2%; p < 0.0001 for risk score) than among white participants. Risk variants in *IL23R* (p = 0.014) and *IL12B* (p = 1.2×10^{-16}), among others, were more prevalent among First Nations participants than among white participants.

Interpretation: The low prevalence of variants associated with bacterial processing and handling in First Nations people may explain their relative protection from inflammatory bowel disease. Increased carriage of a number of risk variants, for example in the interleukin-23/Th17 pathway, is especially intriguing given their importance in other inflammatory diseases of high incidence in First Nations populations.

Inflammatory bowel disease is a chronic immune mediated condition of the gut; its major forms include Crohn disease and ulcerative colitis.[1] In people who are genetically predisposed, inflammatory bowel disease is thought to be the result of a loss of tolerance to the gut microflora and consequent aberrant inflammation.

In the past decade, gene association studies have advanced our understanding of the pathogenesis of inflammatory bowel disease (Figure 1).[2–10] The first susceptibility gene discovered for Crohn disease, *NOD2*, encodes a cytoplasmic bacterial pattern-recognition receptor that senses a product of peptidoglycan breakdown, muramyl dipeptide, via its leucine-rich repeat region.[2,3,11] Three polymorphisms in or near this region (3020insC, G908R and R702W) collectively encompass 81% of the variants in *NOD2* associated with Crohn disease.[12] *ATG16L1*, a gene involved in the intracellular pathogen clearance mechanism known as autophagy, was more

recently associated with Crohn disease and further suggests a role for the loss of normal microbial recognition and clearance.[8] Proinflammatory immune responses are likely important, given the association of variants in the interleukin-23 pathway, such as *IL12B* and *IL23R*, with Crohn disease and ulcerative colitis.[4,7] The actions of interleukin-23 have largely been linked to Th17 cells, a recently discovered CD4+ subset of T cells thought to contribute to many immune-mediated inflammatory diseases.[13]

There is heterogeneity in genetic predisposition to inflammatory bowel disease. For example, polymorphisms in *NOD2* are prevalent in white but not Asian patients with Crohn disease.[14] *TNFSF15* has been associated with Crohn disease in both East Asian and white people.[10,14,15] *TNFSF15* encodes a tumour necrosis factor-like cytokine, TL1A, that drives mucosal inflammation by signalling via the transcription factor NF-κB and can enhance the mucosal function of Th1 and Th17 cells.[16]

Despite living in an area with a high prevalence of inflammatory bowel disease, Canadian First Nations people appear to be relatively protected. The term First Nations generally refers to members of a band within the meaning of Canada's Indian Act, encompassing a large proportion of Canada's indigenous population. Studies undertaken in Manitoba in the 1990s found significantly lower prevalence rates of Crohn disease (16/100 000 people) and ulcerative colitis (56/100 000 people) among First Nations people compared with non-First Nations people (209/100 000 people for Crohn disease and 176/100 000 people for ulcerative colitis).[17,18] Nonetheless, this protection from inflammatory bowel disease does not appear to extend to other immune-mediated inflammatory diseases. In particular, many indigenous populations in North America have a high prevalence of rheumatoid arthritis, systemic lupus erythematosus and ankylosing spondylitis (the latter which paradoxically often co-exists with inflammatory bowel disease in other populations).[19] Predisposition to rheumatoid arthritis is thought in part to be secondary to the high prevalence of *HLA-DRB1* (and thus the shared epitope) in some First Nations people.[20]

However, studies of the genetics of inflammatory bowel disease in First Nations people are lacking.

In this study, we explored the prevalence of single nucleotide polymorphisms (SNPs) associated with inflammatory bowel disease in healthy First Nations and white people from Manitoba. We hypothesized that First Nations people have a low prevalence of SNPs associated with inflammatory bowel disease, which possibly contributes to their protection against this disease.

Methods

Participants

We recruited healthy First Nations ($n = 340$) and white ($n = 285$) people from Manitoba. We excluded people if they or any first-degree relative had inflammatory bowel disease, rheumatoid arthritis or another chronic immune-mediated inflammatory disease. We included First Nations people who had at least three grandparents of First Nations origin. We included white people for whom all grandparents were white; the specific country of origin was not requested.

We recruited healthy white and First Nations people by advertisements posted in the University
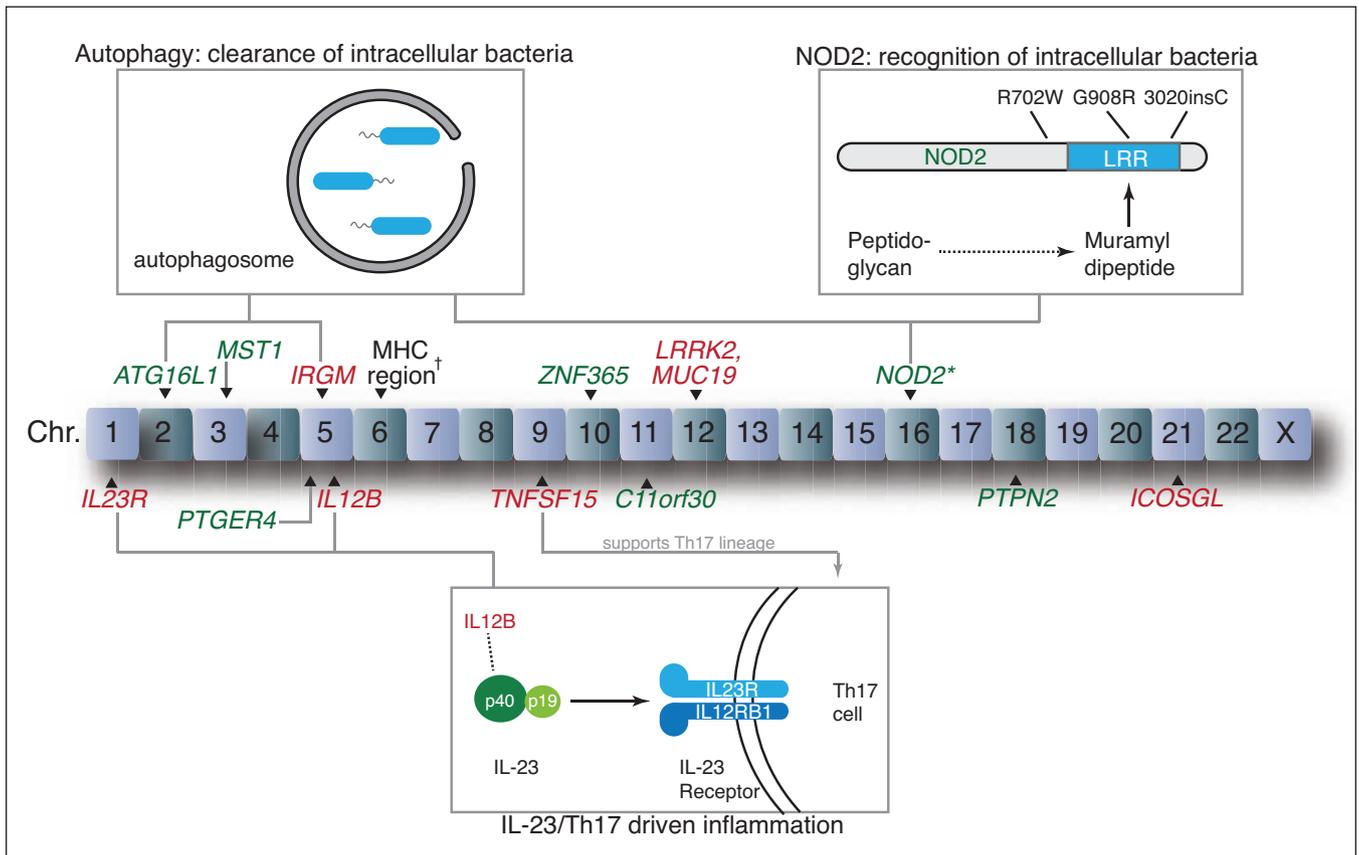
Figure 1: Key loci included in the study and associated biological pathways. The risk variants that we found to be more prevalent among First Nations people are shown in red, and those that we found to be less prevalent are shown in green. *The risk score for *NOD2,* which combined three important variants (R702W, G908R, 3020insC), was lower in First Nations people. †Prevalence of four variants in the region of the major histocompatibility complex (MHC) differed between groups.

of Manitoba's Health Science Centre specialty clinics and in the clinics of participating First Nations communities. The participating communities were Ste Theresa Point, an isolated northern rural community with no road access and an on-reserve population of 2632, and Norway House Cree Nation, a remote semi-isolated northern rural community accessible by road with an on-reserve population of 4071.[21] The participants were all unrelated; however, spouses were invited to enrol.

Ethics approval was granted by the University of Manitoba's research ethics board, and we obtained informed consent from each participant. Samples and data were anonymized. Consistent with the guidelines by the Canadian Institutes of Health Research for conducting research involving Canada's indigenous population, we established an advisory committee (Autoimmune Diseases in First Nations Advisory Committee) to provide oversight, and we entered into research agreements with the two First Nations communities.

DNA processing

We chose 69 SNPs largely based on previous associations with Crohn disease or ulcerative colitis. The list of SNPs is available in Appendix 1 (available at www.cmaj.ca/lookup/suppl/doi:10 .1503/cmaj.110613/-/DC1). We included SNPs associated with inflammatory bowel disease in Asian populations because of the hypothesis that North America became populated as a result of a number of migrations across the land bridge between Eastern Siberia and modern Alaska during the Pleistocene Epoch.[22] For example, we included a number of SNPs from a Japanese ulcerative colitis gene association study.[9]

DNA was extracted from the venous blood of participants and stored at 4 °C. Sixty-eight SNPs were analyzed using the Sequenom iPLEX Gold Assay platform (Génome Québec, Montréal, Quebec). The *NOD2* 3020insC (rs2066847) SNP was genotyped using TaqMan (The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario). The Sequenom genotyping was unsuccessful for 11 First Nations and 28 white samples.

Statistical analysis

We explored for associations between SNPs and ethnic background using logistic regression. We assumed an additive genetic model. We treated the white group as the control group; thus, odds ratios are shown in relation to the white group. *P* values are shown with Bonferroni correction for multiple testing (adjusted for 69 SNPs). We also tested for Hardy–Weinberg equilibrium (Appendix 2, available at www.cmaj.ca/lookup/suppl/doi:10.1503 /cmaj.110613/-/DC1). For important *NOD2* variants (3020insC [rs2066847], R702W [rs2066844]

and G908R [rs2066845]), we created a risk score to increase power because the allele frequencies were low in both populations. The number of risk alleles at these three SNPs were summated and compared by Wilcoxon rank sum test. We considered the results to be significant if the corrected *p* value was less than or equal to 0.05. The number of participants provided power of at least 80% to detect a genetic relative risk of 2.0 at *p* = 0.001, assuming a minor allele frequency of 0.1.

We tested for linkage disequilibrium using the Broad Institute's (Boston, Massachusetts) SNAP Version 2.2 and HapMap Release 22. The resulting correlation coefficient (r^2) is a measure of linkage disequilibrium between two loci, with a range from 0 to 1.

Results

The characteristics of the First Nations (*n* = 340) and white (*n* = 285) participants are shown in Table 1. In total, 336 (98.8%) First Nations participants had four grandparents who were of First Nations ancestry. Of the 69 SNPs tested, 45 were significantly different between First Nations and white participants (Appendix 1). Of these, 28 SNPs were at loci confirmed to be associated with inflammatory bowel disease (Table 2; *NOD2* SNPs are also shown). Table 3 summarizes the putative biological relevance of these loci. After correction for multiple testing (data not shown), we found that all SNPs were in Hardy–Weinberg equilibrium (Appendix 2). The allele frequencies among the white participants were generally similar to those described in HapMap (Appendix 2).

The prevalence of the T300A risk variant of *ATG16L1* (rs2241880) was significantly lower among First Nations participants (*p* = 4.1 × 10^{-30}). This was confirmed in two nearby SNPs in close linkage disequilibrium ($r^2 > 0.75$), rs3828309 (*p* = 3.5 × 10^{-30}) and rs3792106 (*p* = 5.1 × 10^{-28}). A variant associated with Crohn disease (C) at rs11747270 in *IRGM* was enriched in the First Nations group (*p* = 0.01). This variant is in close linkage disequilibrium ($r^2 = 1$) with the *IRGM* SNP (rs13361189) recently shown to be associated with ulcerative colitis.[7]

Table 1: Demographics of study participants

Demographic	First Nations participants *n = 340*	White participants *n = 285*
Age, yr, median (range)	35(18–91)	47 (20–79)
Male, no. (%)	128 (37.6)	107 (37.5)
Participants with four grandparents of reported ancestry, no. (%)	336 (98.8)	285 (100.0)

None of three *NOD2* variants in or near the leucine-rich repeat region that are associated with Crohn disease (R702W, G908R, 3020insC) were independently significantly different between the First Nations and white groups, probably because the risk variants at these SNPs were less prevalent

Table 2: Loci associated with inflammatory bowel disease that were significantly different between First Nations and white participants

Function; SNP	Locus	Risk allele	Risk allele frequency		Odds ratio	p value (corrected*)
			First Nations participants	White participants		
Bacterial sensing and autophagy						
rs3828309	*ATG16L1*	G	0.116	0.516	0.13	3.5×10^{-30}
rs2241880	*ATG16L1*	C	0.115	0.518	0.13	4.1×10^{-30}
rs3792106	*ATG16L1*	C	0.178	0.566	0.16	5.1×10^{-28}
rs11747270	*IRGM*	C	0.165	0.084	2.01	1.1×10^{-2}
rs2066844	*NOD2* (R702W)	T	0.009	0.035	0.25	0.2†
rs2066845	*NOD2* (G908R)	G	0	0.016	NA	> 0.9†
rs2066847	*NOD2* (3020insC)	C (ins)	0.010	0.032	0.34	> 0.9†
rs5743289	*NOD2*	T	0.040	0.158	0.22	1.4×10^{-8}
rs2076756	*NOD2*	G	0.046	0.251	0.13	1.1×10^{-17}
rs10521209	*NOD2*	A	0.875	0.627	4.16	4.0×10^{-17}
Interleukin-23 pathway						
rs11465804	*IL23R*	T	0.987	0.945	4.57	1.4×10^{-2}
rs10045431	*IL12B*	G	0.919	0.695	4.98	1.2×10^{-16}
Immunological synapse/co-stimulatory pathways						
rs762421	*ICOSLG*	G	0.815	0.379	7.26	7.9×10^{-31}
rs3763313	*BTNL2, SLC26A3, HLA-DRB1, HLA-DQA1*	C	0.281	0.169	2.00	4.1×10^{-4}
rs9268480	*BTNL2*	G	0.634	0.749	0.59	4.0×10^{-3}
rs2395185	*BTNL2* to *HLA-DQB1*	G	0.528	0.726	0.42	4.1×10^{-9}
rs9263739	*HLA*	A	0.043	0.191	0.19	2.7×10^{-11}
rs6478108	*TNFSF15*	A	0.826	0.683	2.27	1.6×10^{-6}
rs4263839	*TNFSF15*	G	0.822	0.706	1.97	2.3×10^{-4}
Other						
rs4613763	*PTGER4*	C	0.024	0.105	0.25	9.2×10^{-5}
rs3197999	*MST1*	T	0.096	0.298	0.25	8.5×10^{-14}
rs10995271	*ZNF365*	C	0.146	0.424	0.24	4.5×10^{-19}
rs7927894	*C11orf30*	A	0.111	0.387	0.20	3.7×10^{-20}
rs11175593	*LRRK2, MUC19*	T	0.303	0.035	11.67	8.7×10^{-19}
rs2542151	*PTPN2*	G	0.069	0.165	0.38	5.2×10^{-5}
Unknown‡						
rs9286879	1q24	G	0.806	0.247	11.21	3.4×10^{-37}
rs3806308	1p36	G	0.448	0.621	0.48	4.6×10^{-7}
rs6426833	1p36	T	0.407	0.531	0.63	4.0×10^{-3}
rs2188962	5q31	T	0.095	0.424	0.14	3.2×10^{-26}
rs17582416	10p11	G	0.105	0.305	0.29	1.3×10^{-12}
rs17085007	13q12	G	0.055	0.176	0.28	8.9×10^{-8}

Note: NA = not applicable, SNP = single nucleotide polymorphism.
*Bonferroni correction for multiple testing.
†*NOD2* risk score combining important variants (R702W, G908R, 3020insC) was significantly lower among First Nations participants ($p < 0.0001$). The p values for the individual variants before Bonferroni correction were $p = 0.0034$ for R702W, $p > 0.9$ for G908R and $p = 0.016$ for 3020insC.
‡SNPs associated with inflammatory bowel disease for which no candidate gene has been described, in regions of the genome that lack identifiable genes (gene deserts).

overall (Table 2). To increase the power, we created a risk score by adding the number of risk alleles for each of these three SNPs. The *NOD2* risk score was significantly lower among First Nations participants than among white participants ($p <$ 0.0001, Wilcoxon rank sum test), reflecting lower carriage of any of these three *NOD2* mutations in the First Nations participants (3.9%) compared to white participants (15.2%). Two other *NOD2* risk variants (rs5743289, rs2076756) were significantly decreased in First Nations ($p \leq 1.4 \times 10^{-8}$). One exception was an intronic *NOD2* SNP (rs10521209) identified in a smaller German Crohn disease study;[23] this risk variant was more prevalent in First Nations ($p = 4.0 \times 10^{-17}$).

Inflammatory bowel disease variants in the interleukin-23 pathway were enriched in the First Nations group. The risk allele at rs11465804 (in the interleukin-23 receptor subunit gene *IL23R*)

was more prevalent in the First Nations group (p = 0.014). A risk variant associated with Crohn disease (G at rs10045431) in *IL12B*, which encodes the p40 subunit shared by interleukin-23 and interleukin-12, was more prevalent in the First Nations group ($p = 1.2 \times 10^{-16}$).

The T allele at rs1800896 in the *IL10* promoter, which is associated with lower expression of the regulatory cytokine interleukin-10,[24,25] was more prevalent in First Nations individuals ($p = 1.5 \times 10^{-19}$; Appendix 1); however, it is not in close linkage disequilibrium ($r^2 = 0.19$) with the confirmed inflammatory bowel disease locus at rs3024505.[4,7]

We also found differences in genes involved in the immunological synapse and adaptive immune cell activation (Table 2). A variant associated with ulcerative colitis near *HLA-B* (rs9263739) was less prevalent in First Nations participants

Table 3: Summary of loci associated with inflammatory bowel disease that were significantly different between First Nations and white participants and the putative biological relevance of each locus

Locus	Relative prevalence of risk variant in First Nations participants	Putative biological relevance
ATG16L1	Lower	• Encodes an autophagy scaffold protein important for clearance of intracellular bacteria
NOD2	Lower*	• Encodes cytoplasmic pattern-recognition receptor of peptidoglycan (via muramyl dipeptide) • Important for the recognition of intracellular bacteria • Interacts with ATG16L1 protein to activate autophagy
IRGM	Higher	• Encodes a product involved in autophagy and clearance of intracellular bacteria
IL12B	Higher	• Encodes interleukin-23 subunit shared by interleukin-12
IL23R	Higher	• Encodes a subunit of the heterodimeric interleukin-23 receptor
TNFSF15	Higher	• Encodes tumour necrosis family member TL1A, which drives mucosal inflammation and enhances Th1 and Th17 CD4+ function
MHC region (four SNPS)	Some higher, some lower	• Most polymorphic region of the genome • Encodes a number of products involved in antigen presentation (*HLA*) and co-stimulatory pathways (*BTNL2*)
ICOSLG	Higher	• Encodes inducible T-cell co-stimulator ligand
PTGER4	Lower	• Encodes receptor of inflammatory prostaglandin E2
MST1	Lower	• Encodes macrophage-stimulating protein 1, which is involved in macrophage chemotaxis and regulation of inflammation
ZNF365	Lower	• Encodes zinc finger protein 365
C11orf30	Lower	• Encodes EMSY protein, interacts with *BRCA2*; also associated with eczema
MUC19, LRRK2	Higher	• *MUC19* encodes a mucin-family protein • *LRRK2* encodes a product involved in autophagy
PTPN2	Lower	• Encodes T-cell protein tyrosine phosphatase, which has a role in the modulation of inflammatory response

Note: MHC = major histocompatibility complex, SNP = single nucleotide polymorphism.
*Cumulative carriage of variants in a leucine-rich repeat region important in muramyl dipeptide recognition was lower in First Nations participants.

($p = 2.7 \times 10^{-11}$), while the SNP associated with Crohn disease at rs3763313, near *HLA-DRB1* among others, was enriched in First Nations participants ($p = 4.1 \times 10^{-4}$). Overall, there were significant differences between groups in inflammatory bowel disease SNPs in the vicinity of the major histocompatibility complex (rs3763313, rs9268480, rs2395185, rs9263739). In addition, two risk variants in *TNFSF15* (rs4263839, rs6478108) were enriched in First Nations individuals ($p = 2.3 \times 10^{-4}$), as was one in *ICOSGL* (rs762421) ($p = 7.9 \times 10^{-31}$).

Of the remaining 17 SNPs that were significantly different between the two groups, 10 were nonreplicated from a Japanese ulcerative colitis gene association study[9] (rs12075255, rs885334, rs17351243, rs11583394, rs1862101, rs6746441, rs7591368, rs9553939, rs8004059 and rs379977; Appendix 1). An additional seven SNPs were at loci of interest but were not among the SNPs confirmed to be associated with susceptibility to inflammatory bowel disease in genome-wide association studies, including in the *IL10* promoter (rs1800896), *IL12A* (rs583911), *KIF5A* (rs1678542), *TCF-4* (rs7901695), *PTPRS* (rs17130), *SERPINA1* (rs11832) and rs6128541 (Appendix 1).

Interpretation

We studied the prevalence of SNPs associated with inflammatory bowel disease in First Nations people, using a sample of a Manitoban population in which the low incidence of disease has been previously described.[17,18] As expected, given the disparate anthropologic origins of First Nations and white Canadians, there was significant genetic variation at a large number of SNPs. There is a paucity of research chronicling the genetics of First Nations people in Canada, particularly in relation to immune-mediated inflammatory diseases. However, the unique susceptibility and protection patterns in this population for complex diseases, such as inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis and ankylosing spondylitis, make studying the genetics of First Nations people potentially insightful.

We found that First Nations people had a lower prevalence of a number of variants involved in bacterial recognition and autophagy, perturbations in which are thought to be key in the pathogenesis of Crohn disease (Figure 1).[26] Especially striking was the low prevalence of *ATG16L1* and *NOD2* variants. Replacement of the T allele with C at rs2241880 results in the substitution of threonine with alanine at position 300 (T300A) in *ATG16L1*, which impairs the autophagosomal clearance of intracellular bacteria[27] and confers susceptibility to

Crohn disease[8] and possibly ulcerative colitis.[28] *NOD2* encodes a cytoplasmic pattern recognition receptor (NOD2) that senses the peptidoglycan breakdown product muramyl dipeptide. In First Nations people, combined carriage was lower of three important *NOD2* variants (R702W, G908R, 3020insC) in or near the leucine-rich repeat region responsible for muramyl dipeptide recognition.[11] Notably, the autophagy pathway, a process particularly important in the clearance of intracellular bacteria, was recently shown to involve interaction between NOD2 and ATG16L1 proteins.[29,30]

We found that two variants of *TNFSF15*, a gene associated with inflammatory bowel disease in East Asian people,[14] were enriched in First Nations individuals. This gene's associations have been thus far specific to inflammatory bowel disease[4,7] and ankylosing spondylitis.[31] Its product TL1A enhances Th17 function,[16] and it may thus work together with variants in *IL23R* and *IL12B* to augment this common inflammatory pathway (Figure 1).[13] *IL23R* variants are also associated with anyklosing spondylitis and psoriasis.[32] We postulate that risk variants in the interleukin-23/Th17 axis may predispose First Nations individuals to a number of immune-mediated inflammatory diseases, while an absence of variants in bacterial handling and processing pathways prevents development specifically of inflammatory bowel disease.

Although it is tempting to focus on the analysis of genes for which putative pathogenic mechanisms have been shown, we also found differences between the two populations at a large number of loci whose functions are less understood (Table 2). This highlights the complexity of the pathogenesis of inflammatory bowel disease. Future research elucidating the mechanisms at these loci may shed light on their role in First Nations populations.

Limitations

There are many questions unanswered by our study. For example, although First Nations and white Canadians both live in Canada, there are clearly potential environmental differences (e.g., rural versus urban living, diet, rates of diarrheal illness and *Helicobacter pylori* infection).[33,34] Surely some environmental factors contribute to the unique susceptibility patterns of First Nations people toward immune-mediated inflammatory diseases. However, the low incidence of inflammatory bowel disease among First Nations people makes it difficult to study the environmental and genetic factors affecting susceptibility within this population.

The functional significance of many SNPs included in this study is unknown and, in some cases, may reflect linkage disequilibrium with a causal variant in the population in which they were initially studied. This limits the ability to

make firm conclusions about the contribution of known inflammatory bowel disease loci to the risk of disease in First Nations people. The use of convenience sampling may limit the ability to generalize our study's findings.

Conclusion

We found substantial genetic variation between First Nations and white people at loci associated with inflammatory bowel disease. A paucity of risk variants in bacterial sensing and processing pathways and enrichment of variants in the interleukin-23/Th17 axis may reflect the protection and susceptibility pattern of First Nations people toward inflammatory bowel disease and other inflammatory diseases. However, heterogeneity in the relative prevalence of different variants underlines the complexity of inflammatory bowel disease and the unequal contribution of variants to disease pathogenesis.

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Affiliations: From the Inflammatory Bowel Disease Group (Murdoch, Stempak, Pathan, Silverberg), Mount Sinai Hospital, University of Toronto, Toronto, Ont.; the Inflammatory Bowel Disease Clinical and Research Centre (Bernstein, Sargent), University of Manitoba, Winnipeg, Man.; the Manitoba First Nations Centre for Aboriginal Health Research (El-Gabalawy, Elias), University of Manitoba, Winnipeg, Man.; and the Dalla Lana School of Public Health (Xu), University of Toronto, Toronto, Ont.