Association of the Protein-tyrosine Phosphatase Nonreceptor Type Substrate 1 (PTPNS1) Gene with Inflammatory Bowel Disease

To the Editor:

Genetic risk factors identified for inflammatory bowel disease (IBD) collectively only explain around 10%–20% of the heritability of ulcerative colitis (UC) and Crohn's disease (CD). Therefore, it seems increasingly likely that the missing heritability of IBD will be explained by gene–gene interactions, gene–environment interactions, structurally complex copy number polymorphisms which are poorly tagged by single nucleotide polymorphisms (SNPs), and loci of very modest effect size. A pooled genome-wide association study (GWAS) of 252 unrelated New Zealand (NZ) Caucasians with CD and 187 controls has provided preliminary evidence of association of *PTPNS1* SNP *rs6045210* with CD ($P = 0.03$, odds ratio [OR] = 0.52, 95% confidence interval [CI]: 0.28–0.94). PTPNS1 (also known as: SIRPalpha, SHPS-1, BIT, MFR, CD172a, MYD1, or p84) is a paired cell surface receptor that interacts with the ligand CD47 to control ''homeostatic'' innate immune effector functions, such as host cell phagocytosis. This protein is especially abundant on the surface of macrophages and dendritic cells (DCs).[1] Moreover, PTPNS1-expressing DCs within the lamina propria have recently been demonstrated to promote Th17-biased colitis in a murine model.[2] We hypothesize that *PTPNS1* may be a novel risk locus for IBD, previously undetected, due to its small effect size. The aim of this study was to validate and replicate the initial association of *PTPNS1* with CD and extend association testing to include UC.

The validation sample set comprised 492 independent New Zealand Caucasians (CD $n = 206$, controls $n = 286$)[3] and the two replication sample sets comprised 2133 Dutch Caucasians (CD $n = 933$, UC $n = 664$, controls $n = 536$)[4] and 885 Spanish Caucasians (CD $n = 264$, UC $n = 246$, controls $n = 375$).[5] Diagnosis of CD or UC was based on standard clinical, radiologic, endoscopic, and histopathologic criteria for all sample sets.

DNA was collected from peripheral blood samples by sodium chloride extraction and genotyping for the *PTPNS1* SNP rs6045210 was performed using a predesigned TaqMan SNP genotyping assay (ID: C__27046712_10, Applied Biosystems, Foster City, CA).

No deviations from Hardy–Weinberg Equilibrium were detected (all *P*-values >0.05). Within the NZ GWAS and validation sample sets the minor allele (C)-containing genotypes of *rs6045210* were significantly lower in CD patients than in controls ($P_{combined} = 0.002$, OR = 0.54, 95% CI: 0.36–0.80) (Table 1). A modest protective effect of the TC and CC genotypes was also detected in NZ UC patients ($P = 0.047$, OR = 0.69, 95% CI: 0.48–0.99) (Table 1). No association of *PTPNS1* with CD was observed in either replication sample set ($P_{Dutch} = 0.71$ and $P_{Spanish} = 0.23$; Table 1). Similarly, *PTPNS1* was not associated with UC in the Spanish sample set ($P = 0.79$), but a trend towards significance was observed in Dutch UC patients ($P = 0.10$, OR = 0.76, 95% CI: 0.55–1.06) (Table 1). Combined analysis of the NZ, Dutch, and Spanish sample sets with genotypes imputed from the Wellcome Trust Case Control Consortium (WTCCC) and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) datasets revealed that the *rs6045210* CC and CT genotypes conferred significant protection against UC ($P = 0.002$, OR = 0.73, 95% CI: 0.60–0.89) and IBD ($P = 0.007$, OR = 0.87, 95% CI:0.79–0.96), and a nonsignificant protective effect against CD ($P = 0.092$, OR = 0.85, 95% CI: 0.70–1.03) (Table 1).

PTPNS1 expression and subsequent ligation to the glycoprotein CD47 is crucial to prevent phagocytosis of host cells by macrophages.[2] More recently, PTPNS1 has also been shown to be important in the migration of Langerhans cells, monocytes, and neutrophils; the production of cytokines by immune cells; generation of nitric oxide by macrophages; and the regulation of lipopolysaccharide (LPS)-induced activation of Toll-like receptors.[6] However, the first evidence that PTPNS1 may directly alter susceptibility to inflammatory diseases has come from murine models. In mice expressing a mutant form of PTPNS1 on $CD11c^+$, DCs were found to be highly resistant to the development of experimental autoimmune encephalomyelitis (a model of multiple sclerosis) compared to wildtype mice.[7] Furthermore, in another murine model intestinal $CD103^-$ DCs expressing wildtype PTPNS1 were found, along with CD47, to be crucial for Th-17 response and subsequent development of experimental colitis.[2] While the evidence of association of *PTPNS1* with IBD is preliminary, the role of DC-expressed PTPNS1 in driving TH17-biased responses and colitis makes this gene a candidate worthy of further investigation.

ACKNOWLEDGMENTS

We thank Myriad Genetics (Salt Lake City, Utah, USA), and in particular Dr. Kirsten Timms, for generating the pooled genome-wide association data for the New Zealand sample set. We also thank the patients and healthy controls who generously gave of their time to take part in the study; and Rhondda Brown and Judy Hoar for their assistance in coordinating the recruitment of the New Zealand sample set. This research was supported by a programme grant (08/075) from the Health Research Council of New Zealand. R.L.R. is the recipient of a Sir Charles Hercus Health Research Fellowship and

DOI 10.1002/ibd.21470
Published online in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. *PTPNS1 rs6045210* Allele and Genotype Frequencies in Caucasian IBD Patients and Healthy Unrelated Controls

Sample Set[a]	Phenotype	Genotype Frequency			Allele Frequency		CC + CT vs. TT	Odds Ratio [95% CI]
NZ GWAS		TT	CT	CC	T	C		
	CD	231 (0.917)	21 (0.083)	0 (0.000)	483 (0.958)	21 (0.042)	0.033	0.52 [0.28–0.94]
	controls	159 (0.850)	25 (0.134)	3 (0.016)	343 (0.917)	31 (0.083)		
NZ CD validation[b]		TT	CT	CC	T	C		
	CD	183 (0.888)	23 (0.112)	0 (0.000)	389 (0.944)	23 (0.056)	0.049	0.59 [0.35–0.99]
	controls	236 (0.825)	46 (0.161)	4 (0.014)	518 (0.906)	54 (0.094)		
Combined		TT	CT	CC	T	C		
NZ CD[c] plus UC	CD	414 (0.904)	44 (0.096)	0 (0.000)	872 (0.951)	44 (0.048)	0.002	0.54 [0.36–0.80]
	UC	412 (0.880)	55 (0.118)	1 (0.002)	879 (0.939)	57 (0.061)	0.047	0.69 [0.48–0.99]
	controls	395 (0.835)	71 (0.150)	7 (0.015)	861 (0.910)	85 (0.090)		
Replication 1[d]		TT	CT	CC	T	C		
	CD	784 (0.840)	140 (0.150)	9 (0.01)	1708 (0.915)	158 (0.085)	0.710	0.97 [0.71–1.26]
	UC	578 (0.870)	86 (0.130)	0 (0.00)	1242 (0.940)	86 (0.065)	0.100	0.76 [0.55–1.06]
	controls	445 (0.830)	86 (0.160)	5 (0.01)	976 (0.910)	96 (0.009)		
Replication 2[e]		TT	CT	CC	T	C		
	CD	219 (0.830)	42 (0.160)	3 (0.010)	480 (0.909)	48 (0.090)	0.230	0.77 [0.52–1.15]
	UC	197 (0.800)	47 (0.190)	2 (0.010)	441 (0.896)	51 (0.104)	0.790	0.94 [0.64–1.37]
	controls	296 (0.790)	71 (0.190)	8 (0.020)	663 (0.884)	87 (0.116)		
		TT	CT	CC	T	C		
WTCCC[f]	CD	1499 (0.858)	242 (0.138)	7 (0.004)	3240 (0.927)	256 (0.073)	0.380	0.93 [0.78–1.10]
	controls	2485 (0.848)	431 (0.147)	14 (0.005)	5401 (0.922)	459 (0.078)		
		TT	CT	CC	T	C		
NIDDK[f]	CD	628 (0.782)	161 (0.200)	14 (0.017)	1417 (0.882)	189 (0.118)	0.535	0.93 [0.74–1.17]
	controls	725 (0.770)	203 (0.215)	14 (0.015)	1653 (0.877)	231 (0.123)		
All datasets		TT	CT	CC	T	C		
combined[g]	CD	3027 (0.842)	541 (0.151)	26 (0.007)	6595 (0.918)	593 (0.082)	0.092	0.85 [0.70–1.03]
	UC	804 (0.860)	126 (0.135)	5 (0.005)	1734 (0.927)	136 (0.073)	0.002	0.73 [0.60–0.89]
	IBD	3831 (0.846)	667 (0.147)	31 (0.007)	8329 (0.920)	729 (0.080)	0.007	0.87 [0.79–0.96]
	controls	4346 (0.824)	862 (0.164)	44 (0.008)	9554 (0.910)	950 (0.090)		

[a]All participants gave their written informed consent and ethical approval for this study was given by the Upper South & Otago Regional Ethics Committees (NZ).
[b]New Zealand (NZ) CD patients and controls not included in pooled genome-wide association study (GWAS).
[c]NZ CD patients from GWAS and validation cohort
[d]Dutch IBD patients and controls recruited from the University Medical Centers of Groningen, Amsterdam, Utrecht.
[e]Spanish IBD patients and controls recruited from Hospital Virgen de las Nieves (Granada).
[f]Imputed genotypes *rs6045210* were generated from the Wellcome Trust Case Control Consortium (WTCCC) and the National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) data-sets, using the program IMPUTE and HapMap release NCBI Build 36 dbSNP 130 (May 2009, hg36.3). A quality threshold of 0.95 was set for imputation of both data-sets.
[g]Cumulative evidence for association of *rs6045210* was assessed by the Mantel-Haenszel Method.

J.E.H.M. is the recipient of a National Heart Foundation Research Fellowship. B.Z.A was supported by a grant from the Netherlands Organization for Health Research and Development (ZonMw; grant number 016.096.121).

Rebecca L. Roberts, PhD[1,2]
Jade E. Hollis-Moffatt, PhD[1]
María Gómez-García, MD[3]
Karin Fransen, PhD[4]
Cyriel Y. Ponsioen, PhD[5]
Bart A. Crusius, PhD[6]
Cisca Wijmenga, PhD[7]
Javier Martín, MD, PhD[8]
Rinse K. Weersma, MD, PhD[4]
Tony R. Merriman, PhD[1]
Murray L. Barclay, MD[2,9]
Richard B. Gearry, MB, ChB, PhD[2,9]
Behrooz Z. Alizadeh, MD, PhD[10]

[1]Department of Biochemistry
University of Otago
Dunedin, New Zealand
[2]Department Medicine
University of Otago
Christchurch, New Zealand
[3]Department of Gastroenterology
Hospital Virgen de las Nieves, Granada, Spain
[4]Department of Gastroenterology and Hepatology
University Medical Center Groningen
Groningen, The Netherlands
[5]Department of Gastroenterology and Hepatology, Academic Medical Center
Amsterdam, The Netherlands
[6]Laboratory of Immunogenetics
Department of Pathology
VU Medical Centre, Amsterdam
The Netherlands

[7]Department of Genetics
University Medical Center
Groningen, The Netherlands
[8]Instituto de Biomedicina, CSIC
Granada, Spain
[9]Department of Gastroenterology
Christchurch Hospital
Christchurch, New Zealand
[10]Department of Medical Genetics
University Medical Centre Utrecht
Utrecht, Department of Epidemiology
UMCG, Groningen
the Netherlands

REFERENCES

1. Barclay AN, Brown MH. The SIRP family of receptors and immune regulation. *Nat Rev Immunol*. 2006;6:457–464.
2. Fortin G, Raymond M, Van VQ, et al. A role for CD47 in the development of experimental colitis mediated by SIRPalpha+CD103- dendritic cells. *J Exp Med*. 2009;206:1995–2011.
3. Gearry RB, Roberts RL, Burt MJ, et al. Effect of inflammatory bowel disease classification changes on NOD2 genotype-phenotype associations in a population-based cohort. *Inflamm Bowel Dis*. 2007;13:1220–1227.
4. Weersma RK, Zhernakova A, Nolte IM, et al. ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in the Netherlands. *Am J Gastroenterol*. 2008;103:621–627.
5. Nunez C, Oliver J, Mendoza JL, et al. CD209 in inflammatory bowel disease: a case-control study in the Spanish population. *BMC Med Genet*. 2007;8:75.
6. Kong XN, Yan HX, Chen L, et al. LPS-induced down-regulation of signal regulatory protein {alpha} contributes to innate immune activation in macrophages. *J Exp Med*. 2007;204:2719–2731.
7. Tomizawa T, Kaneko Y, Saito Y, et al. Resistance to experimental autoimmune encephalomyelitis and impaired T cell priming by dendritic cells in Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 mutant mice. *J Immunol*. 2007;179:869–877.