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BRIEF ARTICLE

SLC11A1 polymorphisms in inflammatory bowel disease and *Mycobacterium avium* subspecies *paratuberculosis* status

Lucy C Stewart, Andrew S Day, John Pearson, Murray L Barclay, Richard B Gearry, Rebecca L Roberts, Robert W Bentley

Lucy C Stewart, Andrew S Day, Robert W Bentley, Department of Paediatrics, University of Otago, Christchurch 8140, New Zealand
Andrew S Day, Department of Paediatrics, Christchurch Hospital, Christchurch 8140, New Zealand
John Pearson, Department of Pathology, University of Otago, Christchurch 8140, New Zealand
Murray L Barclay, Richard B Gearry, Rebecca L Roberts, Department of Medicine, University of Otago, Christchurch 8140, New Zealand
Murray L Barclay, Richard B Gearry, Department of Gastroenterology, Christchurch Hospital, Christchurch 8140, New Zealand
Rebecca L Roberts, Department of Biochemistry, University of Otago, Dunedin 9054, New Zealand
Author contributions: Stewart LC and Bentley RW performed the research; Roberts RL, Bentley RW and Day AS designed the research; Pearson J, Stewart LC and Bentley RW analyzed the data; Barclay ML and Gearry RB provided cohort DNA; all authors contributed to the writing of the manuscript.
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Correspondence to: Robert W Bentley, PhD, Department of Paediatrics, University of Otago, PO Box 4345, Christchurch 8140, New Zealand. robert.bentley@otago.ac.nz
Telephone: +64-3-3641558 Fax: +64-3-3640009
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Abstract

AIM: To test for association of *SLC11A1* with inflammatory bowel disease (IBD) and *Mycobacterium avium* subspecies *paratuberculosis* (MAP) status in a Caucasian cohort.

METHODS: Five hundred and seven Crohn's disease (CD) patients, 474 ulcerative colitis (UC) patients, and 569 healthy controls were genotyped for *SLC11A1 1730G>A* and *SLC11A1 469+14G>C* using pre-designed TaqMan® SNP assays. χ^2 tests were applied to test for association of single nucleotide polymorphisms (SNPs) with disease, and the presence of MAP DNA.

RESULTS: *SLC11A1 1730G>A* and *SLC11A 1469+14G>C* were not associated with CD, UC, or IBD. The *SLC11A1 1730A* minor allele was over-represented in patients who did not require immunomodulator therapy (P = 0.002, OR: 0.29, 95% CI: 0.13-0.66). The frequency of the *SLC11A1 469+14C* allele was higher in the subset of study participants who tested positive for MAP DNA (P = 0.02, OR: 1.56, 95% CI: 1.06-2.29). No association of *SLC11A1 1730G>A* with MAP was observed.

CONCLUSION: Although *SLC11A1* was not associated with IBD, association with MAP suggests that *SLC11A1* is important in determining susceptibility to bacteria implicated in the etiology of CD.

Key words: *NRAMP1*; Crohn's disease; Ulcerative colitis; IS900 polymerase chain reaction

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INTRODUCTION

The solute carrier family 11 (*SLC11A1*) gene (also known

as *natural resistance associated macrophage protein 1*, *NRAMP1*)[1] has been associated with susceptibility to intracellular pathogens since its initial identification in mice[2]. *SLC11A1* encodes a divalent cation transporter that is located in endosome and phagosome membranes[3] of macrophages and monocytes within the liver, spleen and lungs[2,4]. This transporter plays a key role in mounting an effective immune response against intracellular pathogens[1,5] through its involvement in the acidification of the phagosomes[6], as well as the regulation of nitric oxide, interleukin-10[7] and vacuolar iron concentrations[8].

Given the pivotal roles that *SLC11A1* plays in innate immunity, it is not surprising that the relationship between polymorphisms in *SLC11A1* and a number of autoimmune and mycobacterial diseases has been explored. Associations have been found with leprosy[9], tuberculosis[10], rheumatoid arthritis[11], visceral leishmaniasis[12], multiple sclerosis[13], type 1 diabetes mellitus[14], and inflammatory bowel disease (IBD)[15-18]. Most of these disease associations have been with a promoter dinucleotide microsatellite (GT_n) that is known to affect *SLC11A1* expression levels[19]. However, *SLC11A1* also contains a number of single nucleotide polymorphisms (SNPs), including *SLC11A1 1730G>A* (*rs17235409*; D543N) and *SLC11A1 469+14G>C* (*rs3731865*; INT4G>C). The non-synonymous SNP 1730G>A is thought to alter the protein function[18], whereas the intronic SNP 469+14G>C has no known functional effect, but has been suggested to be in linkage disequilibrium with functional promoter polymorphisms[12].

SLC11A1 1730G>A and *SLC11A1 469+14G>C* have been tested for association with Crohn's disease (CD) in two European cohorts. Although the smaller of the two studies found no association with CD, Gazouli *et al*[18] have reported a significant association of both SNPs with disease (*SLC11A1 1730G>A* $P_{genotypic}$ = 0.0001, OR: 3.43, 95% CI: 1.95-5.93, *SLC11A1 469+14G>C* $P_{genotypic}$ = 0.006, OR: 15.91, 95% CI: 0.92-273.46). The involvement of SLC11A1 in the handling and elimination of intracellular pathogens, as well as its association with mycobacterial diseases makes it a biologically plausible candidate risk gene for CD. The results of recent genome-wide association studies strongly suggest defects in genes involved in bacterial detection, handling, and elimination are central to CD pathogenesis. Furthermore the assertion, albeit controversial, that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an initial trigger for CD provides an additional rationale to investigate *SLC11A1* as a candidate risk gene for IBD. As a result, this study had two aims. The first was to attempt the first independent replication of the association of *SLC11A1 1730G>A* and *SLC11A1 469+14G>C* with IBD. The second aim was to use previously collected MAP IS900 data[20] to test for association of *SLC11A1* genotypes with occurrence of MAP DNA in peripheral blood.

MATERIALS AND METHODS

Study participants

Patients were selected from a New Zealand Caucasian IBD cohort that had been recruited to investigate genetic and environmental factors that contribute to CD and UC etiology[20-24]. Detailed phenotypic data were available for members of this cohort including ancestry, location of disease, family history of IBD, age of onset, presence of extra-intestinal manifestations, and requirement for surgery. The MAP status of the CD patients in this cohort had been determined previously using IS900 polymerase chain reaction[20]. Randomly selected blood donors (n = 501) from Christchurch (New Zealand), including 180 who had been previously tested for MAP status[20] served as controls.

Genotyping

Genotyping of *SLC11A1 1730G>A* (*rs17235409*) and *SLC11A1 469+14G>C* (*rs3731865*) was performed in 384-well plates using the pre-designed Taqman® SNP genotyping assays C_256352269_10 and C_1659793_10 (Applied Biosystems, Foster City, CA, USA) in a LightCycler® 480 Ⅱ (Hoffmann La Roche, Basel, Switzerland). Cycling conditions for *rs17235409* were 10 min at 95℃, 40 cycles of 15 s at 92℃ and 1 min at 60℃, and 30 s of cooling at 40℃. Conditions were the same for *rs3731865*, but annealing was at 66℃ rather than 60℃. Results were analyzed using Lightcycler® 480 software version 1.5.0. The accuracy of the genotyping assays was confirmed by repeat analysis of 13% of samples. Concordance between original and repeat genotype calls was 99%.

Statistical analysis

A web-based calculator (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl) was used to test for deviations from Hardy-Weinberg Equilibrium (HWE). The χ^2 and OR analyses were performed using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL, USA). Associations were considered significant if P was < 0.05. *Post hoc* power analysis demonstrated that our cohort had 90% power to detect a relative risk of 2.15 for *SLC11A1 1730G>A* ($MAF_{controls}$ = 0.02, α = 0.05) and 99.8% power to detect a relative risk of 1.5 for *SLC11A1 469+14G>C* ($MAF_{controls}$ = 0.30, α = 0.05).

Ethical considerations

All study participants provided written informed consent to be involved in ongoing IBD research, and ethical approval for this study was given by the Upper South Regional Ethics Committee (Canterbury, New Zealand).

RESULTS

Genotyping for *SLC11A1 1730A>G* and *469+14G>C* was successful in 1468 (94.7%) and 1432 (92.4%) of study participants, respectively. No deviations from HWE were detected in cases or controls for either SNP (P > 0.05). The percentage minor allele frequency (MAF) of *SLC11A1 1730G>A* and *SLC11A1 469+14G>C* in our controls was 2% and 30%, respectively. We found no evidence of association of either *SLC11A1* SNP with overall CD, UC or IBD susceptibility (Table 1). Similarly, the minor allele and genotype frequencies of *SLC11A1*

Table 1 Genotype and allele frequencies of *SLC11A1 1730G>A* and *469+14G>C* in New Zealand Crohn's disease and ulcerative colitis patients, and healthy controls *n* (%)

Phenotype	Genotype			MAF	Allelic *P* value	Allelic OR (95% CI)
1730G>A	GG	GA	AA	A		
CD (*n* = 495)	474 (96)	21 (4)	0	21 (2)	0.832	1.07 (0.57-2.00)
UC (*n* = 470)	450 (96)	20 (4)	0	20 (2)	0.827	1.07 (0.57-2.02)
HC (*n* = 503)	483 (96)	20 (4)	0	20 (2)		
469+14G>C	GG	GC	CC	C		
CD (*n* = 495)	265 (54)	192 (39)	38 (8)	268 (27)	0.153	0.83 (0.65-1.07)
UC (*n* = 451)	245 (54)	171 (38)	35 (8)	241 (27)	0.101	0.81 (0.62-1.04)
HC (*n* = 486)	238 (49)	204 (42)	44 (9)	292 (30)		

MAF: Minor allele frequency; OR: Odds ratio; CI: Confidence interval; CD: Crohn's disease; UC: Ulcerative colitis; HC: Healthy controls.

Table 2 Genotype frequencies of *SLC11A1 1730G>A* (*rs17235409*) in inflammatory bowel disease patients who have used/not used immunomodulators *n* (%)

Phenotype/immunomodulator status	Genotype			*P* value	OR (95% CI)
	GG	GA	AA		
CD/never used IM (*n* = 217)	203 (94)	14 (6)	0	0.031	0.38 (0.15-0.95)
CD/have used IM (*n* = 278)	271 (98)	7 (2)	0		
UC/never used IM (*n* = 356)	336 (94)	20 (6)	0	0.010	0.75 (0.71-0.79)
UC/have used IM (*n* = 114)	114 (100)	0	0		
IBD/never used IM (*n* = 573)	539 (94)	34 (6)	0	0.002	0.29 (0.13-0.66)
IBD/have used IM (*n* = 392)	385 (98)	7 (2)	0		

OR: Odds ratio; CI: Confidence interval; CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; IM: Immunomodulator.

Table 3 Distribution of *SLC11A1 469+14G>C* genotype by *Mycobacterium avium* subspecies *paratuberculosis* status[1] in New Zealand Caucasians *n* (%)

MAP DNA in blood	Genotype frequency		*P* value	OR (95% CI)
	GG	GC + CC		
Present (*n* = 150)	66 (44)	84 (56)	0.02	1.56 (1.06-2.29)
Absent (*n* = 351)	193 (55)	158 (45)		

[1]Tested by IS900 polymerase chain reaction to detect the presence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) DNA in peripheral blood[20]. OR: Odds ratio; CI: Confidence interval.

1730G>A and *469+14G>C* did not associate with age of disease onset, disease behavior, disease location, or requirement for resectional surgery (all *P* values > 0.1, data not shown). A significantly higher frequency of the *SLC11A1 1730A* allele was seen in IBD patients who did not require immunomodulator therapy, compared to those who did require this treatment approach (P_{IBD} = 0.002, OR: 0.29, 95% CI: 0.13-0.66, P_{CD} = 0.03, OR: 0.38, 95% CI: 0.15-0.95, P_{UC} = 0.01, OR: 0.75, 95% CI: 0.71-0.79) (Table 2). There was no significant association of *SLC11A1 1730G>A* with MAP status, whereas the *SLC11A1 469+14C* allele was associated with increased incidence of MAP DNA in peripheral blood (*P* = 0.02, OR: 1.56, 95% CI: 1.06-2.23) in our cohort (Table 3).

DISCUSSION

Previous association of *SLC11A1 1730G>A* and *469+14G>C* with mycobacterial infections and preliminary evidence of association with CD[10-12,25] suggest that *SLC11A1* alters susceptibility to IBD. The primary aim of our study was to conduct the first independent replication of the association of *SLC11A1* with CD. In contrast to the original study of Gazouli *et al*[18], we found no evidence of *SLC11A1 1730G>A* or 469+14G>C as risk factors for IBD, CD or UC (all *P* values > 0.8) (Table 1). Comparison of the MAFs for the two *SLC11A1* SNPs revealed the existence of significant heterogeneity between Gazouli *et al*[18] and other studies for *SLC11A1 1730A*, and between populations of Northern versus Southern European ancestry for *SLC11A1 469+14C*. Our cohort and the cohort of Liu *et al*[26], which were composed primarily of individuals of Northern European ancestry, had *SLC11A1 469+14C* frequencies of 30% and 27% respectively. In contrast, the cohorts drawn from Southern European populations (Italian, Greek, and Turkish) exhibited significantly lower MAFs for this SNP. These differences in MAF distribution hint at the existence of a North-South gradient for *SLC11A1*, which could in turn explain the discordance between our study and that of Gazouli *et al*[18]. The occurrence of such gradients is not without precedence. The frequency of the CD-associated SNPs, *R702W*, *G908R* and *1007fs*, within the nucleotide oligomerization binding domain 2 gene (*NOD2*, also known as *CARD15*) exhibits a strong North-South gradient within Europe. A recent meta-analysis of *NOD2* association studies performed on European IBD cohorts has found that the MAFs and thus the contribution of these SNPs to CD risk increased significantly with decreasing latitude[27].

The minor allele of *SLC11A1 1730G>A* was found to be significantly over-represented in the subset of our IBD patients who had never used immunomodulators, and by inference had less severe disease (Table 2). However, we saw no association with other markers of disease severity in our cohort. Due to the very low minor allele frequency (no minor allele homozygotes were observed), this result requires replication in other large cohorts to rule out a type 1 error.

The second aim of this study was to test for association of *SLC11A1* with MAP. The MAP status of 321 CD patients and 180 controls has been determined previously[20]. Combining these patients and controls, we found no association between MAP status and *SLC11A1 1730G>A*, but did find an association with *SLC11A1 469+14G>C* (P = 0.02, OR: 1.56, 95% CI: 1.06-2.29) (Table 3). Earlier studies[14,16] on smaller CD cohorts (n = 37 or 59) did not find any evidence of association of MAP status with *SLC1A11 469+14G>C*. However, this polymorphism has been associated with susceptibility to *Mycobacterium tuberculosis*[10], and additional variation within *SLC11A1* has been associated with susceptibility to other mycobacterial diseases such as leprosy[9]. Our results provide preliminary evidence of an association of the *SLC11A1 469+14C* allele with susceptibility to MAP.

We conclude that although *SLC11A1* could be a risk factor for IBD in some Southern European populations, we did not find an association of *SLC11A1 469+14G>C* or *SLC11A1 1730G>A* with IBD in our cohort that comprised primarily patients of Northern European ancestry. However, the significantly higher incidence of MAP DNA in the peripheral blood of *SLC11A1 469+14C* heterozygotes and homozygotes compared to *SLC11A1 469+14G* within our cohort suggests that this *SLC11A1* SNP, although not directly influencing disease risk, might modify susceptibility to potential CD-causing bacteria.

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COMMENTS

Background

The involvement of *SLC11A1* in the handling and elimination of intracellular pathogens, as well as its association with mycobacterial diseases makes it a biologically plausible candidate risk gene for Crohn's disease (CD). The suggestion that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an initial trigger for CD provides an additional rationale to investigate *SLC11A1* as a candidate risk gene for inflammatory bowel disease (IBD).

Research frontiers

A previous genetic association study has indicated that SLC11A1 is a susceptibility gene for IBD. The authors performed an independent replication of this study in a large population-based cohort of Northern European origin. They also tested for the association of these polymorphisms with MAP status.

Innovations and breakthroughs

This is believed to be the first study to examine the association of SLC11A1 polymorphisms in a well-powered cohort of Northern European origin. These findings indicate that *SLC11A1* polymorphisms do not modify disease risk for IBD, but might influence disease behavior (through indirect markers of severity) and susceptibility to MAP, a putative pathogen in CD. The authors also note the disparity of allele frequency between populations of Northern and Southern European origin.

Applications

By understanding how *SLC11A1* genotype influences the risk of colonization/infection with MAP, the authors might gain some insight into the contribution of this bacterium to IBD, and how defective clearance of MAP and other intracellular bacteria might be associated with modified disease risk.

Terminology

SLC11A1, solute carrier family 11 gene (also known as Natural Resistance Associated Macrophage Protein 1, *NRAMP1*) plays a key role in an effective innate immune response against intracellular pathogens. MAP is an intracellular bacterium that has been cited in several studies as a putative causal agent of CD.

Peer review

This paper provides interesting new results regarding the possible relationship between SLC11A1 polymorphisms and IBD risk. The study has been done carefully and thoroughly, and the paper is very well written. The lack of association of SLC11A1 and IBD risk in the study population (New Zealand Caucasians primarily of Northern European descent) is an important finding. The positive result that shows an association of an SLC11A1 allele and MAP status is novel and interesting.

REFERENCES

1 **Blackwell JM**, Barton CH, White JK, Searle S, Baker AM, Williams H, Shaw MA. Genomic organization and sequence of the human NRAMP gene: identification and mapping of a promoter region polymorphism. *Mol Med* 1995; **1**: 194-205
2 **Cellier M**, Govoni G, Vidal S, Kwan T, Groulx N, Liu J, Sanchez F, Skamene E, Schurr E, Gros P. Human natural resistance-associated macrophage protein: cDNA cloning, chromosomal mapping, genomic organization, and tissue-specific expression. *J Exp Med* 1994; **180**: 1741-1752
3 **Gruenheid S**, Pinner E, Desjardins M, Gros P. Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome. *J Exp Med* 1997; **185**: 717-730
4 **Canonne-Hergaux F**, Calafat J, Richer E, Cellier M, Grinstein S, Borregaard N, Gros P. Expression and subcellular localization of NRAMP1 in human neutrophil granules. *Blood* 2002; **100**: 268-275
5 **Forbes JR**, Gros P. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. *Trends Microbiol* 2001; **9**: 397-403
6 **Cellier MF**, Courville P, Campion C. Nramp1 phagocyte intracellular metal withdrawal defense. *Microbes Infect* 2007; **9**: 1662-1670
7 **Fritsche G**, Nairz M, Werner ER, Barton HC, Weiss G. Nramp1-functionality increases iNOS expression via repression of IL-10 formation. *Eur J Immunol* 2008; **38**: 3060-3067
8 **Gomes MS**, Appelberg R. Evidence for a link between iron metabolism and Nramp1 gene function in innate resistance against Mycobacterium avium. *Immunology* 1998; **95**: 165-168
9 **Abel L**, Sánchez FO, Oberti J, Thuc NV, Hoa LV, Lap VD, Skamene E, Lagrange PH, Schurr E. Susceptibility to leprosy is linked to the human NRAMP1 gene. *J Infect Dis* 1998; **177**: 133-145
10 **Bellamy R**, Ruwende C, Corrah T, McAdam KP, Whittle HC, Hill AV. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; **338**: 640-644
11 **Ates O**, Dalyan L, Müsellim B, Hatemi G, Türker H, Ongen G, Hamuryudan V, Topal-Sarikaya A. NRAMP1 (SLC11A1) gene polymorphisms that correlate with autoimmune versus

infectious disease susceptibility in tuberculosis and rheumatoid arthritis. *Int J Immunogenet* 2009; **36**: 15-19
12 **Mohamed HS**, Ibrahim ME, Miller EN, White JK, Cordell HJ, Howson JM, Peacock CS, Khalil EA, El Hassan AM, Blackwell JM. SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. *Eur J Hum Genet* 2004; **12**: 66-74
13 **Kotze MJ**, de Villiers JN, Rooney RN, Grobbelaar JJ, Manswelt EP, Bouwens CS, Carr J, Stander I, du Plessis L. Analysis of the NRAMP1 gene implicated in iron transport: association with multiple sclerosis and age effects. *Blood Cells Mol Dis* 2001; **27**: 44-53
14 **Paccagnini D**, Sieswerda L, Rosu V, Masala S, Pacifico A, Gazouli M, Ikonomopoulos J, Ahmed N, Zanetti S, Sechi LA. Linking chronic infection and autoimmune diseases: Mycobacterium avium subspecies paratuberculosis, SLC11A1 polymorphisms and type-1 diabetes mellitus. *PLoS One* 2009; **4**: e7109
15 **Hofmeister A**, Neibergs HL, Pokorny RM, Galandiuk S. The natural resistance-associated macrophage protein gene is associated with Crohn's disease. *Surgery* 1997; **122**: 173-178; discussion 178-179
16 **Sechi LA**, Gazouli M, Sieswerda LE, Molicotti P, Ahmed N, Ikonomopoulos J, Scanu AM, Paccagnini D, Zanetti S. Relationship between Crohn's disease, infection with Mycobacterium avium subspecies paratuberculosis and SLC11A1 gene polymorphisms in Sardinian patients. *World J Gastroenterol* 2006; **12**: 7161-7164
17 **Kotlowski R**, Bernstein CN, Silverberg MS, Krause DO. Population-based case-control study of alpha 1-antitrypsin and SLC11A1 in Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2008; **14**: 1112-1117
18 **Gazouli M**, Atsaves V, Mantzaris G, Economou M, Nasioulas G, Evangelou K, Archimandritis AJ, Anagnou NP. Role of functional polymorphisms of NRAMP1 gene for the development of Crohn's disease. *Inflamm Bowel Dis* 2008; **14**: 1323-1330
19 **Searle S**, Blackwell JM. Evidence for a functional repeat polymorphism in the promoter of the human NRAMP1 gene that correlates with autoimmune versus infectious disease susceptibility. *J Med Genet* 1999; **36**: 295-299
20 **Bentley RW**, Keenan JI, Gearry RB, Kennedy MA, Barclay ML, Roberts RL. Incidence of Mycobacterium avium subspecies paratuberculosis in a population-based cohort of patients with Crohn's disease and control subjects. *Am J Gastroenterol* 2008; **103**: 1168-1172
21 **Gearry RB**, Richardson A, Frampton CM, Collett JA, Burt MJ, Chapman BA, Barclay ML. High incidence of Crohn's disease in Canterbury, New Zealand: results of an epidemiologic study. *Inflamm Bowel Dis* 2006; **12**: 936-943
22 **Roberts RL**, Gearry RB, Hollis-Moffatt JE, Miller AL, Reid J, Abkevich V, Timms KM, Gutin A, Lanchbury JS, Merriman TR, Barclay ML, Kennedy MA. IL23R R381Q and ATG16L1 T300A are strongly associated with Crohn's disease in a study of New Zealand Caucasians with inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 2754-2761
23 **Roberts RL**, Hollis-Moffatt JE, Gearry RB, Kennedy MA, Barclay ML, Merriman TR. Confirmation of association of IRGM and NCF4 with ileal Crohn's disease in a population-based cohort. *Genes Immun* 2008; **9**: 561-565
24 **Tarrant KM**, Barclay ML, Frampton CM, Gearry RB. Perianal disease predicts changes in Crohn's disease phenotype-results of a population-based study of inflammatory bowel disease phenotype. *Am J Gastroenterol* 2008; **103**: 3082-3093
25 **Stienstra Y**, van der Werf TS, Oosterom E, Nolte IM, van der Graaf WT, Etuaful S, Raghunathan PL, Whitney EA, Ampadu EO, Asamoa K, Klutse EY, te Meerman GJ, Tappero JW, Ashford DA, van der Steege G. Susceptibility to Buruli ulcer is associated with the SLC11A1 (NRAMP1) D543N polymorphism. *Genes Immun* 2006; **7**: 185-189
26 **Liu J**, Fujiwara TM, Buu NT, Sánchez FO, Cellier M, Paradis AJ, Frappier D, Skamene E, Gros P, Morgan K. Identification of polymorphisms and sequence variants in the human homologue of the mouse natural resistance-associated macrophage protein gene. *Am J Hum Genet* 1995; **56**: 845-853
27 **Hugot JP**, Zaccaria I, Cavanaugh J, Yang H, Vermeire S, Lappalainen M, Schreiber S, Annese V, Jewell DP, Fowler EV, Brant SR, Silverberg MS, Cho J, Rioux JD, Satsangi J, Parkes M. Prevalence of CARD15/NOD2 mutations in Caucasian healthy people. *Am J Gastroenterol* 2007; **102**: 1259-1267

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