LETTERS

Linking risk conferring mutations in NCF4 to functional consequences in Crohn’s disease

We read with interest the paper from Muise et al in which they describe a rare variant in the NCF2 gene, which demonstrates a diminished RAC2 binding capacity.1 The NCF2 encoded protein p67phox is one of the components of the NADPH oxidase complex which drives the production of reactive oxygen species (ROS) during the bactericidal response of innate immune cells. Output of disturbed granulocytic ROS as a result of impaired functioning of this enzyme complex has been shown in a number of diseases, including myeloperoxidase (MDS) and chronic granulomatous disease.2 3 As Muise and colleagues point out, these diseases have been linked to development of a colitis resembling that seen in Crohn’s disease (CD), suggesting a potential role for impaired ROS production in CD pathiology.

Genome-wide association studies (GWAS) are a promising tool to identify genetic variants of genes linked to an increased risk of developing CD. Amongst the single nucleotide polymorphisms (SNPs) identified so far is a T to C substitution in intron 1 of NCF4, encoding the p40phox NADPH oxidase protein. Association of this SNP, rs4821544, with ileal CD was confirmed by Muise et al.1 However, although in general much is made of the importance of GWAS-identified risk-conferring SNPs in patients, it remains as yet unclear how these SNPs affect patient cell function, as functional studies are mostly lacking. Although Muise and colleagues very clearly show that a non-synonymous mutation in NCF2 affects its interaction with RAC2, their paper provides no direct proof that this affects granulocytic ROS production. Although making a good case, the fact that the c.113 G→A mutation is so rare (they were only able to measure ROS in one patient bearing this mutation), impedes conclusions about its general role in CD occurrence.

To further examine the potential of SNP variants in NCF4 to functional consequences in CD, we have investigated granulocyte ROS production in patients with CD bearing either the NCF4 risk allele (C), or patients homozygous for the non-risk allele (T). Patient characteristics (age, treatment, disease location, gender, fistulisation) were identical between the two groups (not shown). We observed no differences in N-formyl-methionyl-leucyl-phenylalanine (fMLP)-triggered intracellular ROS production between carriers and non-carriers of the risk allele. However, fMLP-induced ROS production was significantly lower in granulocyte-macrophage colony-stimulating factor-primed neutrophils from patients with CD with an NCF4 mutation (figure 1).

These results are consistent with previous studies, showing that p40phox is important for intracellular ROS production in response to certain triggers such as phagocytosis, but plays a smaller role in phorbol myristate acetate-induced or fMLP-induced ROS production.3 We observed no differences in granulocyte respiratory burst when patients were stratified according to ATG16L1 (rs10210302, rs2241880), IRGM (rs13631189) or NOD2 (rs2066844, rs2066845, rs2066847) SNP variants (data not shown).

As Muise and colleagues point out, the association between NCF4 and CD is not found in all GWAS studies. However, the rs4821544 SNP may define a subgroup of patients who develop CD in part as consequence of defective granulocyte ROS production. This may also explain why some studies find impaired ROS production in patients with CD, whereas others do not; none of these studies have stratified their patients according to genetic risk factors. Obviously, carrying the rs4821544 cannot be the only factor involved in development of CD, as healthy people also bear this mutation. However, impaired bacterial clearance in patients carrying this risk allele may contribute to the risk of getting CD.

Our results demonstrate for the first time that risk-conferring SNPs within the NADPH oxidase machinery lead to functional alterations in granulocyte ROS production in patients with CD. These data also show that although many of the SNPs found to be linked to CD, including rs4821544, are synonymous, they may nevertheless convey functional consequences.

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Competing interests None.

Ethics approval Ethics approval was provided by the appropriate medical ethical review board of the Erasmus University Medical Center, Rotterdam, the Netherlands.

Contributors RS and JJD performed experiments and interpreted data. CJW, MPP and GMF devised experiments and interpreted data. MPP and GMF wrote the manuscript.

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The Authors’ reply

We read with interest the letter by Somasundaram et al in response to our paper ‘NADPH oxidase complex and IBD candidate gene studies: identification of a rare variant in NCF2 that results in reduced binding to RAC2’ that further demonstrates the importance of the NADPH oxidase complex genes in the pathogenesis of Crohn’s disease.
Their letter provides interesting confirmatory data that complement our findings, which already clearly demonstrate the role of the NADPH oxidase complex in inflammatory bowel disease (IBD). However, there are a number of important issues that require further clarification.

1. Somasundaram et al suggest that our study did not show the association between reactive oxygen species (ROS) production and IBD. Our study clearly shows that ROS production is important in the pathogenesis of IBD. Our index patient with the novel p67phox R38Q variant had low ROS production in multiple tests, as shown in figure 1. Furthermore, the p67phox R38Q variant had previously been shown to clearly reduce RAC2 binding, which would reduce ROS production.

2. Somasundaram et al suggest that our findings are not significant as the c.113G→A NCF2 variant is rare. However, we have clearly shown in our discovery cohort and two independent replication cohorts that the c.113G→A NCF2 variant is significantly associated with very early onset IBD and subsequently found in 4% very early onset IBD patients. While this novel variant is very rare in the general population, its presence is significantly higher in our very early onset IBD patients. Furthermore, the odds ratio of 25 is much higher than the ORs observed for common variants associated with IBD such as the NCF4 rs4821544 single nucleotide polymorphism (SNP) (OR<1.5). The International IBD Genetics Consortium has made significant efforts to deep sequence known IBD loci to identify rare variants that may explain the remaining heritability in IBD. Examining these rare variants will most likely be more important in understanding the pathogenesis of IBD than the common synonymous variant examined by Somasundaram et al.

3. Finally, we showed that the novel genetic variant c.113G→A NCF2 results in a functional protein change that reduces binding to RAC2 leading to decreased ROS production. Somasundaram et al examined a common NCF4 SNP and provided no data to support the functional studies they carried out. In our study, we examined the rs4821544 intronic SNP and found that the genotype of this SNP did not alter gene expression or splicing of NCF4 and imputation analysis did not provide further information regarding the possible casual variants. Therefore, it is difficult to understand how the genotype of the NCF4 rs4821544 intronic SNP functionally results in the changes in ROS production described in their letter. Other important data such as disease location and disease activity of the Crohn’s disease patients, which are important in interpreting their results, are unfortunately lacking.

Our study demonstrates the importance of examining biologically relevant pathways in distinct IBD phenotypes. Overall, we believe our study clearly demonstrates, for the first time, the importance of genetic variants in the NADPH oxidase complex that results in reduced ROS production in the pathogenesis of IBD.

Contributors All authors contributed equally to this letter.

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Prothrombin index slope is an early prognostic marker in patients with severe acute liver diseases

In a recent study published in Gut, Mura et al followed up patients with cirrhosis until their death or liver transplantation (LT), and their results suggest that serum levels of von Willebrand factor can discriminate patients with a high probability of clinical worsening requiring LT. Identifying biomarkers with a high prognostic value is a major challenge, since it can help physicians in their therapeutic decision. While chronic liver disease requires middle- or long-term prognostic biomarkers, very short-term prognostic markers are needed in acute liver disease (ALD) in order to help in deciding whether and when an LT should be performed. In patients with severe hepatic encephalopathy (HE), the outcome can be rapidly determined using validated scores (King’s College and Clichy Criteria and Model for End-Stage Liver Disease); however, these scores do not apply to patients admitted with ALD or early-stage acute liver failure (ALF, HE grade 1 or 2). Prothrombin time and factor V2 are specifically their serial measurements, have been reported to be of prognostic value in patients with ALD/ALF, but series studies were short and/or restricted to acetaminophen-induced ALF. Therefore, we...