

## LAY ABSTRACT

### CHARACTERIZING THE CAUSES OF INFLAMMATORY BOWEL DISEASE

This research is aimed at discovery of the causes of the inflammatory bowel diseases, Crohn's disease (CD) and ulcerative colitis (UC). The key specific objectives of the research are to identify genetic changes that confer risk for CD and UC and then define the pathways whereby such changes lead to disease. Our group has shown that two genes implicated in development of CD (*CARD15* and *SLC22A4*) interact with one another so as to markedly increase the risk for this disease. Over this past year, we have explored the molecular basis for and biological significance of this genetic interaction. We have now verified that the two proteins derived from each of these genes, *CARD15* and *OCTN1*, respectively, physically bind to one another and that a third protein is needed to bring these two proteins together. Our data suggest an important role for one protein (*OCTN1*) in controlling the function of the other (*CARD15*). We are now trying to define the effect of *OCTN1-CARD15* binding on different cell behaviours. This information will help define a pathway that links changes in the *CARD15/OCTN1* genes to development of CD. Our data will also establish the extent to which modulation of this pathway may provide an effective therapy for CD.

Also over this past year, we have explored the genetic basis of both CD and UC. A number of genetic regions have recently been identified as possible risk factors for inflammatory bowel disease. However, these studies do not identify the precise genetic lesions within each region that is responsible for disease. Such information is required so that the genetic data can be used to aid in diagnosis and/or treatment of IBD patients. We have now studied our IBD patient population with respect to these new genetic regions and shown that three such regions are very highly associated with CD and/or UC in our population. Based on these findings, we have begun to intensively study each region so as to define the precise genetic changes that confers risk for disease. This work requires evaluation of the DNA sequence across each region, an analysis we are performing using a very new technology (ultrafast sequencing) that allows DNA sequence to be rapidly defined. In the next year, we hope to have identified the specific genetic lesion predisposing to IBD at each of the three regions of interest.

Results of our studies will provide new knowledge of the genetic lesions and molecular pathways that lead to inflammatory bowel disease. Such information is required to develop improved therapies for CD and UC and for the design of better diagnostic tools enabling earlier diagnosis and intervention and, ultimately, better outcomes for inflammatory bowel disease patients.

## TECHNICAL UPDATE

### Characterization of susceptibility genes/molecules for inflammatory bowel disease

#### INTRODUCTION

Our research program is aimed at defining the molecular pathways underpinning susceptibility to inflammatory bowel disease (IBD). Most specifically, we are focused on elucidating the cellular and signaling events that link a variant in the *SLC22A4* gene encoding organic cation transporter (OCTN1) to Crohn's disease (CD) and at defining the composite of gene variants that confer risk for both CD and ulcerative colitis (UC). Our progress over this past year in achieving both these goals is summarized below.

#### RESEARCH PROGRESS

##### A. Defining molecular pathways linking OCTN1 variants to cell dysfunction and CD.

Over the past year, we have extensively investigated the possibility that OCTN1 effects on intestinal epithelial cell function are realized, at least in part, by modulating function of the CARD15 protein. This work is based on the data from our group and others suggesting that *SLC22A4/OCTN1* and *CARD15* gene variants interact to confer risk for CD. In investigating whether these two proteins physically interact, we previously showed that CARD15 and OCTN1 can be co-immunoprecipitated from SW620 and HEK293 cells. However, more recently, we demonstrated that this interaction is not direct, i.e. that recombinant forms of CARD15 and OCTN1 do not bind to one another *in vitro*. Importantly, we have now shown that both CARD15 and OCTN1 interact with a third protein, a cytosolic adaptor known as PDZK1. PDZK1 is a cytosolic protein containing four "PDZ" domains that enable its interactions with other proteins. We have now shown that PDZK1 uses one PDZ domain to bind the cytosolic domain of OCTN1 and a different PDZ domain to bind to the CARD region of CARD15. Moreover, by overexpressing PDZK1 in HEK293 cells, we have shown that PDZK1 plays a major role in coupling CARD15 to OCTN1. These data suggest that OCTN1 may modulate CARD15 function, a possibility we are now investigating by assessing the effects of PDZK1 and PDZK1/OCTN1 on CARD15-mediated NF $\kappa$ B induction.

In view of our data linking OCTN1 to CARD15, we are also investigating the capacity of OCTN1 to transport peptide/glycopeptide substrates into the cell. OCTN1 is a well-established cation/carnitine transporter, but has recently been implicated in peptide transport by data showing that its orthologue, OCTN2, mediates the transport of a bacterial peptide into the cell. This finding raises the possibility that OCTN1 transports the CARD15-inducer muramyl dipeptide (MDP) or another bacterial peptide into the cell so as to evoke CARD15 activity. We are now using both mass spectrometry and fluorochrome-labeled peptides in conjunction with transporter assays to investigate this possibility. In addition, we are testing the possibility that tumor necrosis factor (TNF $\alpha$ ) or toll-like receptor (TLR) stimuli such as LPS act in concert with OCTN1 to modulate CARD15 activity.

Together our data confirm that PDZK1 enables OCTN1 and CARD15 to interact with one another and strongly suggest that OCTN1 can modulate the function of CARD15. As the CD-

associated OCTN1 variant modulates OCTN1 transporter capacity, these data suggest that this variant also influences CARD15 function and may therefore interact with the CD-associated CARD15 variant to enhance susceptibility to CD. Over this next year, we plan to further explore this hypothesis so as to delineate the basis whereby *CARD15/SLC22A4/OCTN1* variant interaction enhances CD risk and to determine whether targeting this pathway has therapeutic potential in CD.

### **B. Dissecting the genetic basis of inflammatory bowel disease.**

Over the past few years, genome-wide association studies have identified many novel risk loci for CD and UC. A number of these loci have not been replicated and the disease-causal alleles within remain undefined. Thus further replication studies are required to identify the loci most likely to represent valid risk loci for IBD and to define the disease-causal alleles within each locus.

To address these issues and identify valid IBD risk loci, we have studied our IBD case/control cohort for 150 single nucleotide polymorphisms (SNPs) across 49 loci identified as IBD risk loci from genome-wide association surveys published over the past two years. Our data have replicated reported associations of CD with six distinct loci and of UC with two loci. The strongest association was between CD and UC and the *NKX2-3* locus on chromosome 10q24.2. Through the analysis of our Canadian cohort and an additional case/control (300 cases/300 controls) from the UK, we also confirmed the association of a locus at 5p13.1 with CD and identified a novel association between this locus and UC. In addition, we confirmed an association between UC and the *BTNL2* locus on chromosome 6p21, an association that has been suggested, but not validated in other UC cohorts. Based on these data, our group has now initiated deep sequencing of the *NKX2-3*, 5p13.1 and *BTNL2* loci, the goal being to define the disease-causal alleles at each of these loci. These data (now being submitted for publication) pave the way for definition and characterization of novel IBD risk variants, information that is required for dissection of IBD molecular pathophysiology and translation of the genetic data to diagnostic and/or therapeutic tools.

### **SUMMARY**

Our CCFC-funded research over this past year has further elucidated a molecular connection between two CD-risk variants and thus enhanced understanding of the effector pathways linking each of these variants to cell dysfunction and disease. We have also validated several IBD risk loci as being strongly associated with CD and/or UC in our IBD cohort, thus providing a framework for further studies enabling identification of the disease-causal alleles at these loci. Over this next year, we will focus on characterizing the biologic relevance of the OCTN1-CARD15 pathway and on defining the disease-causal alleles within the three major IBD risk loci validated by our recent genetic replication analysis.

**ABSTRACTS/PAPERS**Papers:

- Newman W, Zhang Q, Liu X, Amos CI, **Siminovitch KA**. Genetic variants in IL-23R and ATG16L1 independently predispose to increased susceptibility to Crohn's disease in a Canadian population. *Journal of Clinical Gastroenterology* 43: 444-447, 2009.
- Browning BL, Barclay ML, Bingham SA, Brand S, Buning C, Castro M, Drummond H, Ferguson LR, Fisher SA, Geary RB, Glas J, Henckaerts L, Huebner C, Lakatos L, Lakatos PL, Latiano A, Liu X, Mathew C, Muller-Myhsok B, Newman WG, Nimmo ER, Noble CL, Parkes M, Petermann I, Rutgeerts P, Satsangi J, Shelling AN, **Siminovitch KA**, Torok HP, Tremelling M, Vermeire S, Vito A, Witt H. Gender-stratified analysis of DLG5 R30Q in 4707 Crohn's disease patients and 4973 controls from 12 Caucasian cohorts. *J Medical Genetics* 45:36-42, 2008.

Abstracts:

- Pathan, S. et al. Confirmation of the novel association at the BTNL2 locus with ulcerative colitis. Canadian Genetic Epidemiology and Statistics Meeting. Toronto, May, 2008.
- Pathan, S. et al. Putative promoter region of the NKX2-3 gene at 10q24.2 is associated with inflammatory bowel diseases. Human Immunology Research Day. Toronto, June 2009.

Invited Presentation:

- Gastroenterology Research Day, University of Alberta, Edmonton: Autoimmune inflammatory diseases and the era of genomic medicine. June 2008.

**SUPPLEMENTARY FUNDING**

Ontario Research Funds (ORF) received over this past year provide support for Gene Profiling Facility personnel (technologist and statistician) who contribute to the genetic studies included in this research project. Canadian Foundation for Innovation (CFI) funds received over this past year provide equipment infrastructure (genotyping, PCR, sequencing equipment) required for the genetics research included in this program.

**STAFF SUPPORTED BY CCFC FUNDING**

Postdoctoral Fellow: CCFC funds are supporting the salary of Dr. Elena Fasano, a postdoctoral fellow from Italy, who is working on the CARD15-OCTN1 interaction. Dr. Fasano holds an MD degree.

Postdoctoral Fellow: CCFC funds are also being used to support a second postdoctoral fellow, Dr. S. Pathan. This fellow, who holds a PhD, joined our group 1½ years ago following a one year fellowship with Dr. D. Jewell at Oxford.

Research Technologist: CCFC funds also support 25% of the salary of Dr. Jing Xu, who holds a PhD. Dr. Xu devotes ¼ of her time to this project, working with Dr. Fasano to evaluate the functional roles of the CARD15-OCTN1 pathway.