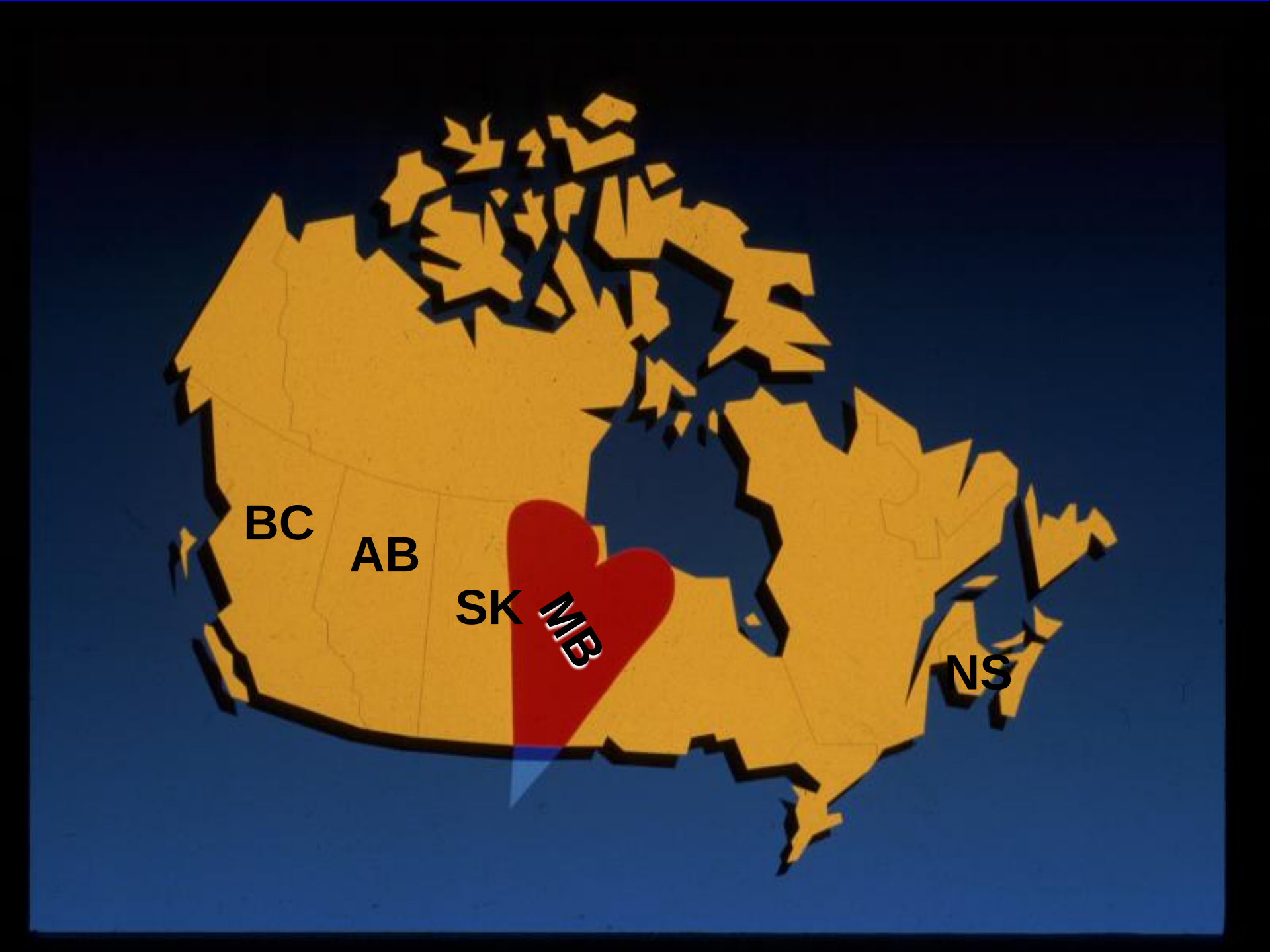
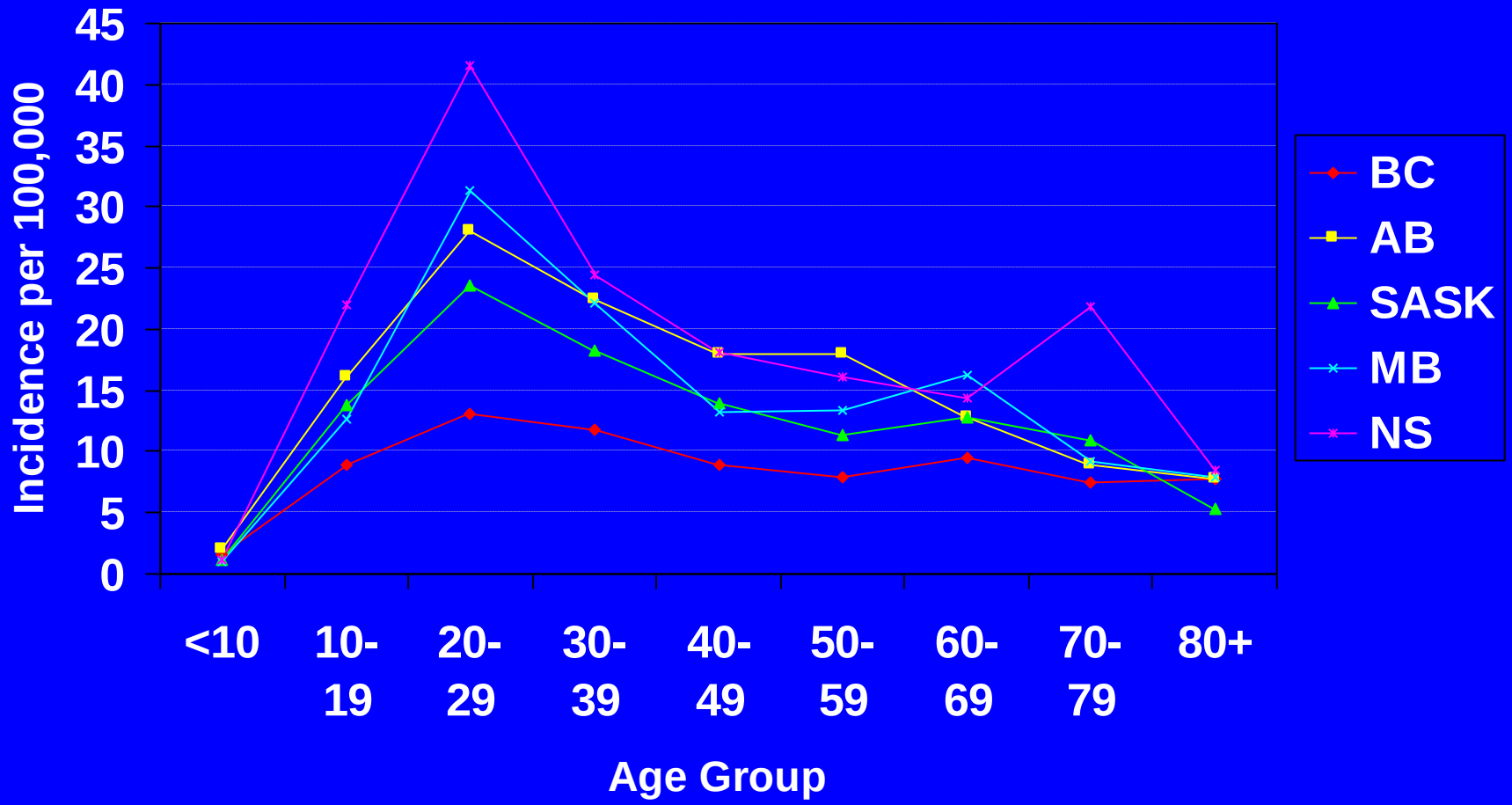
The Environment and IBD

Charles N. Bernstein, MD
University of Manitoba
IBD Clinical and Research Centre

Winnipeg, Manitoba, Canada

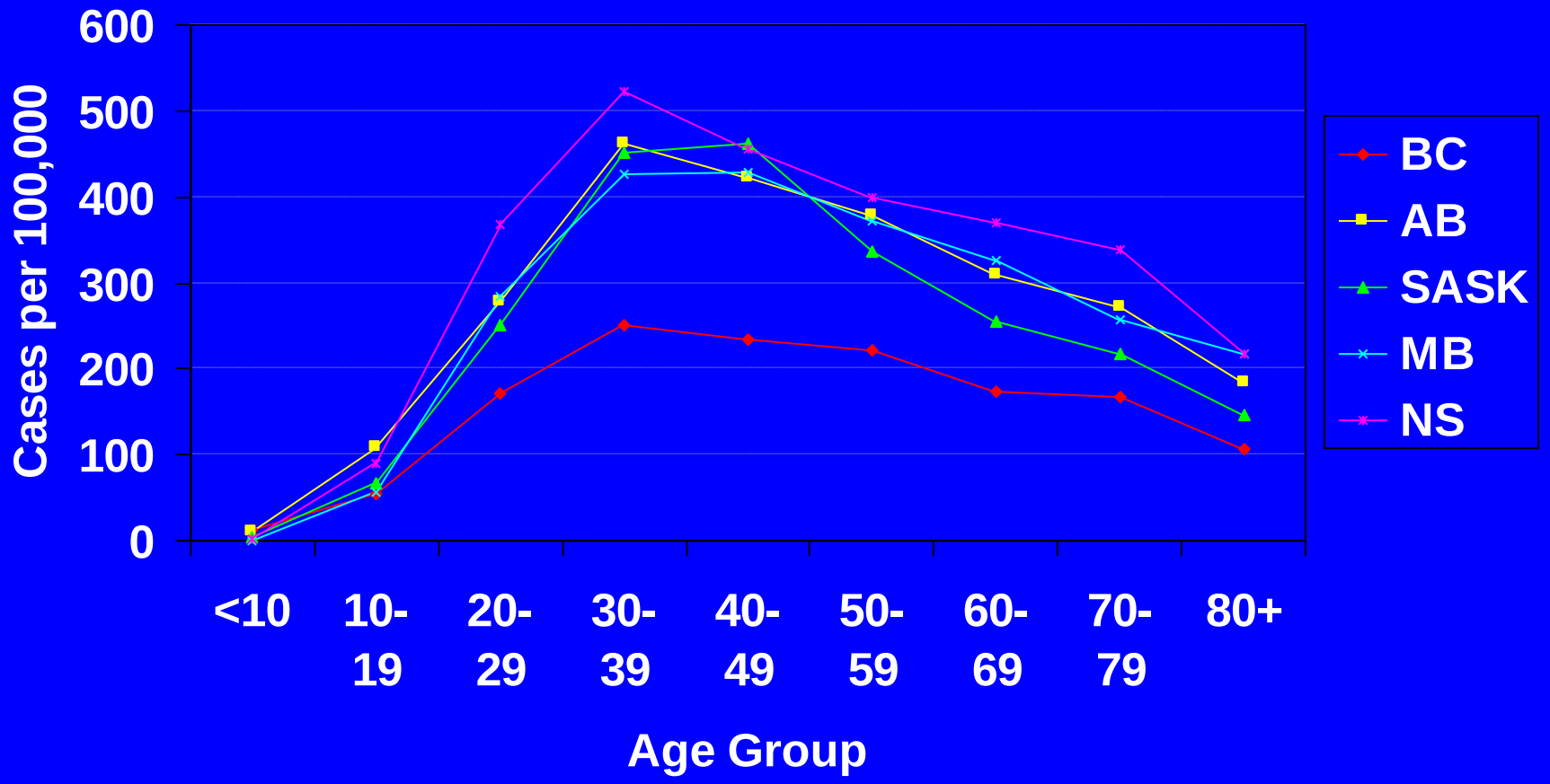

BC
AB
SK
MB
NS

Age-specific incidence of Crohn's Disease: by Province



Bernstein AJGI 2006

Age-specific prevalence of Crohn's Disease: by Province (2000)



Bernstein AJGI 2006

Projected prevalence of IBD in Canada in 2000

Population = 30,750,087

Prevalence of Crohn's disease: 85,854

Prevalence of UC: 64,975

Total IBD: 150,829

?2008: 180,000

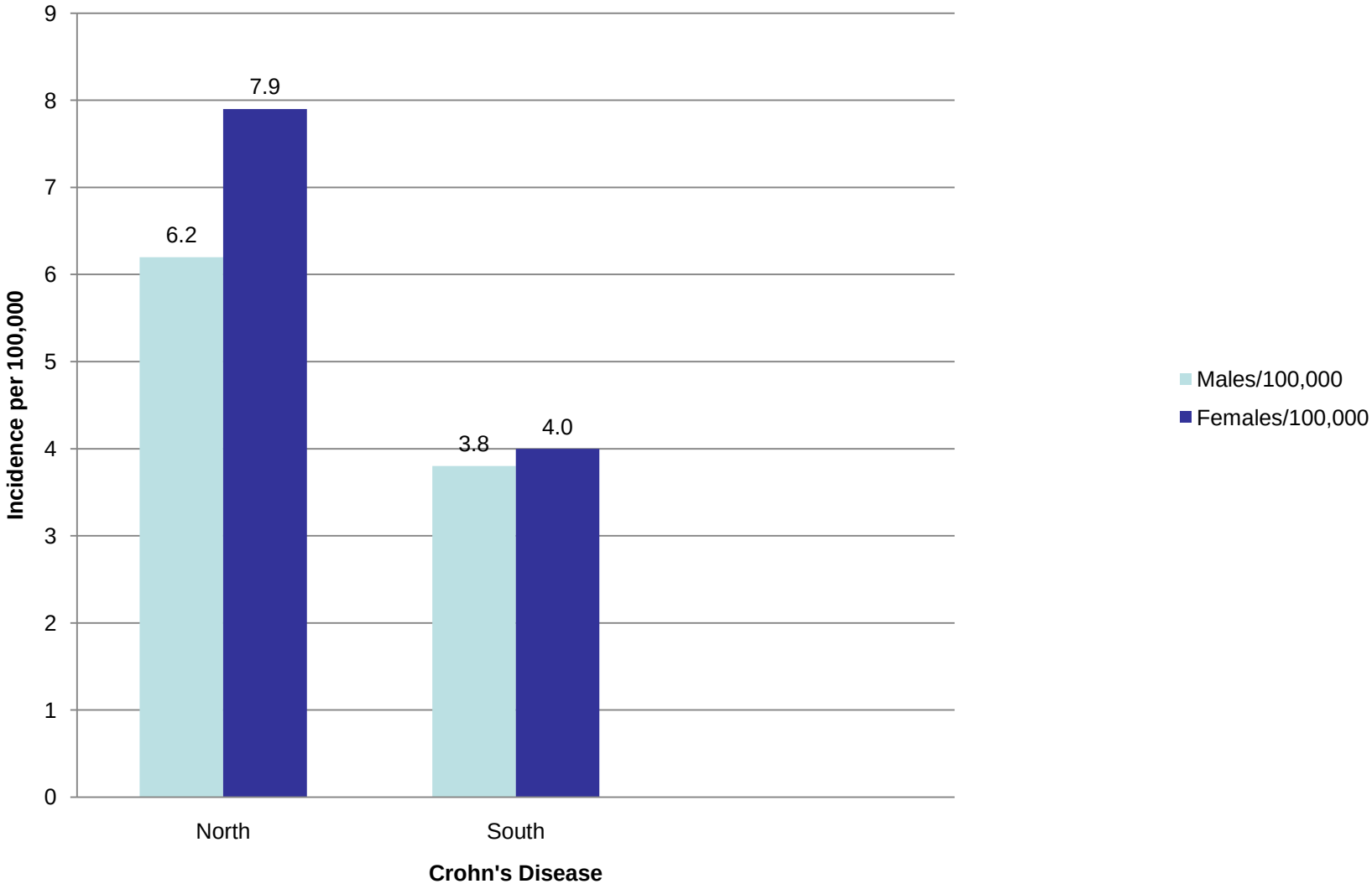
- **MB rates similar to elsewhere in Canada**

- **Low rates of Crohn's disease in BC; 22% visible minorities**

- **High rates of both Crohn's & UC in Canada;**

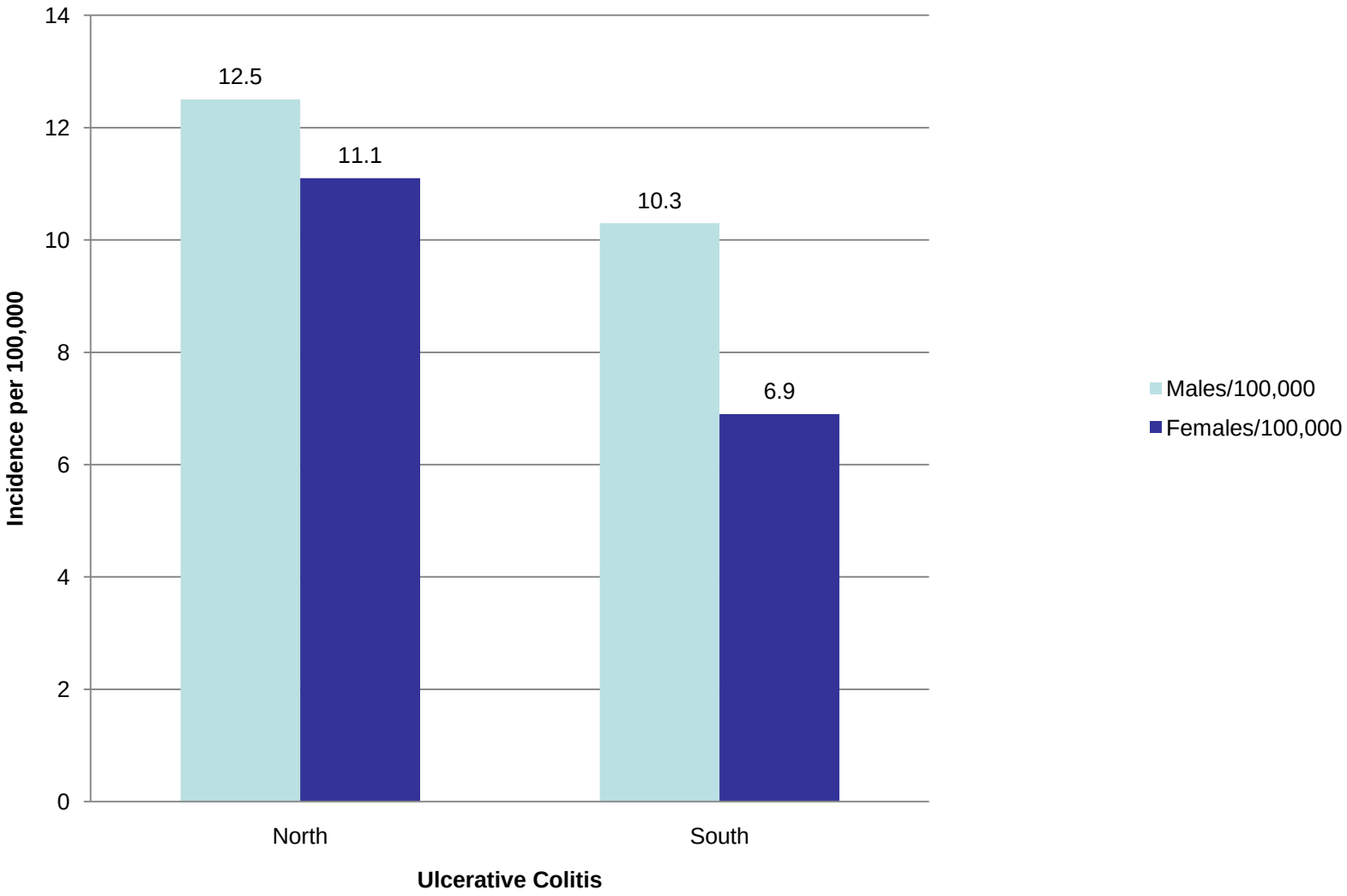
?North-South gradient

European IBD epidemiology data from 1991-1993



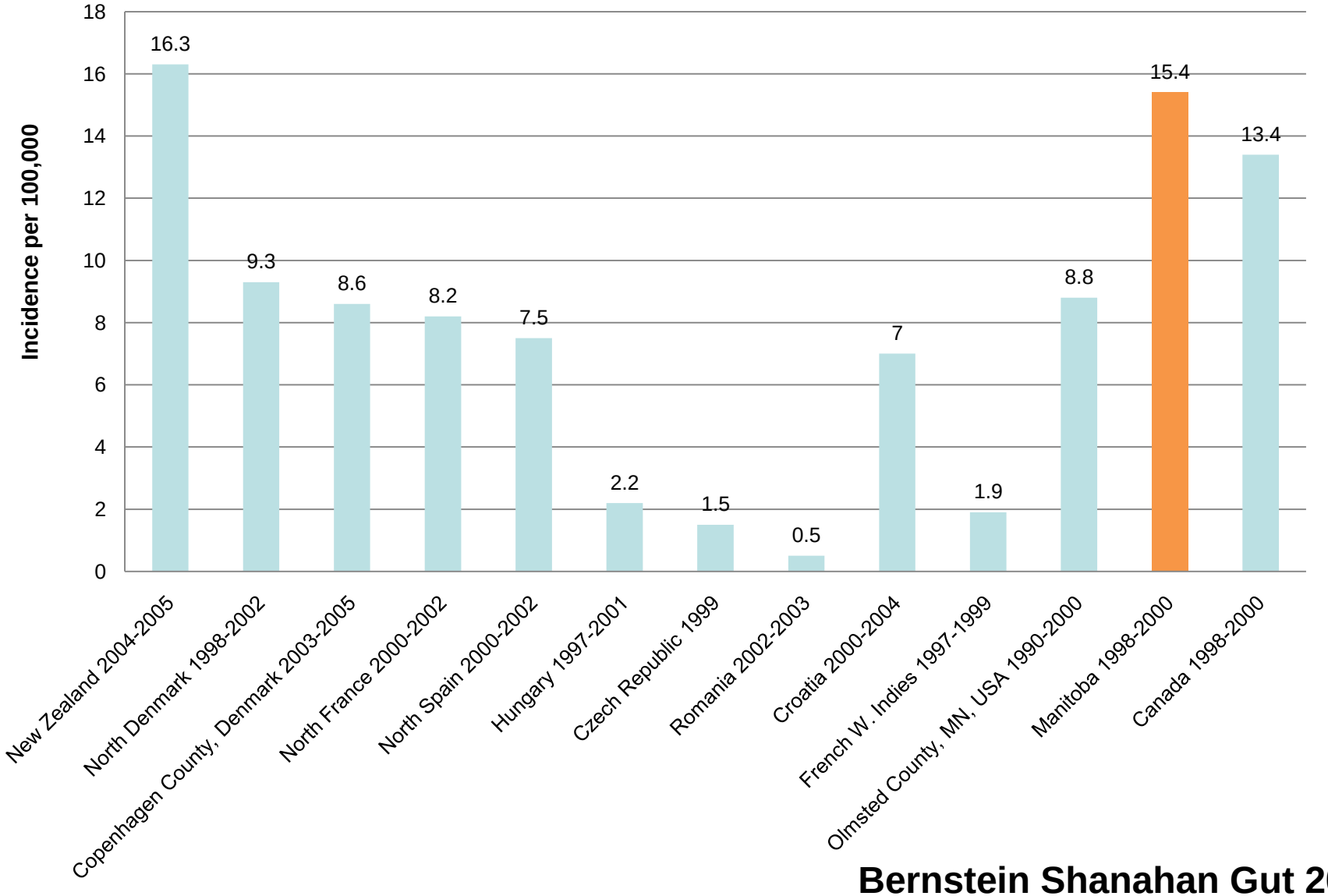
Shivinada Gut 1996

European IBD epidemiology data from 1991-1993

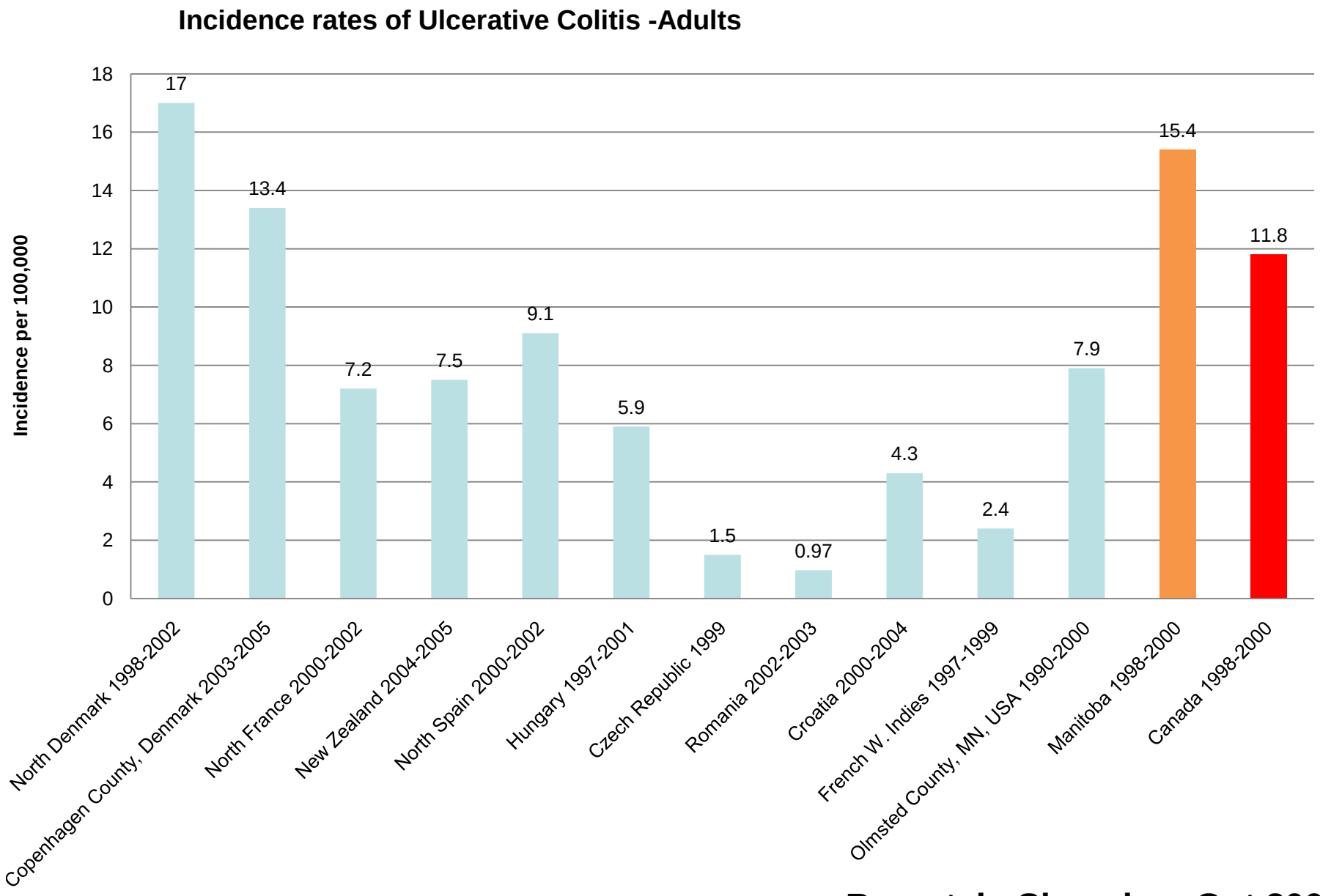


Shivinada Gut 1996

Incidence Rate of Crohn's Disease-Adults

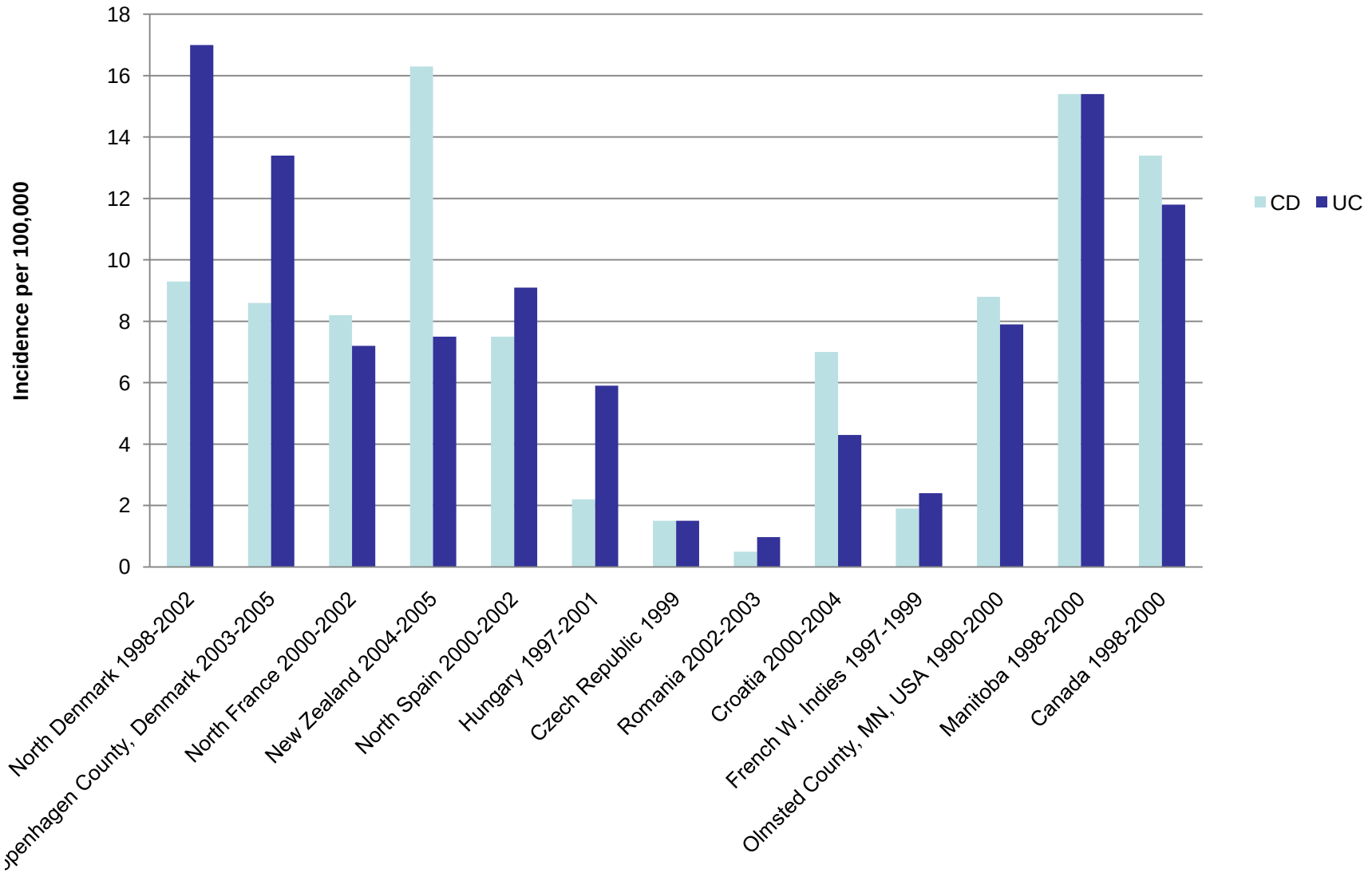


Bernstein Shanahan Gut 2008

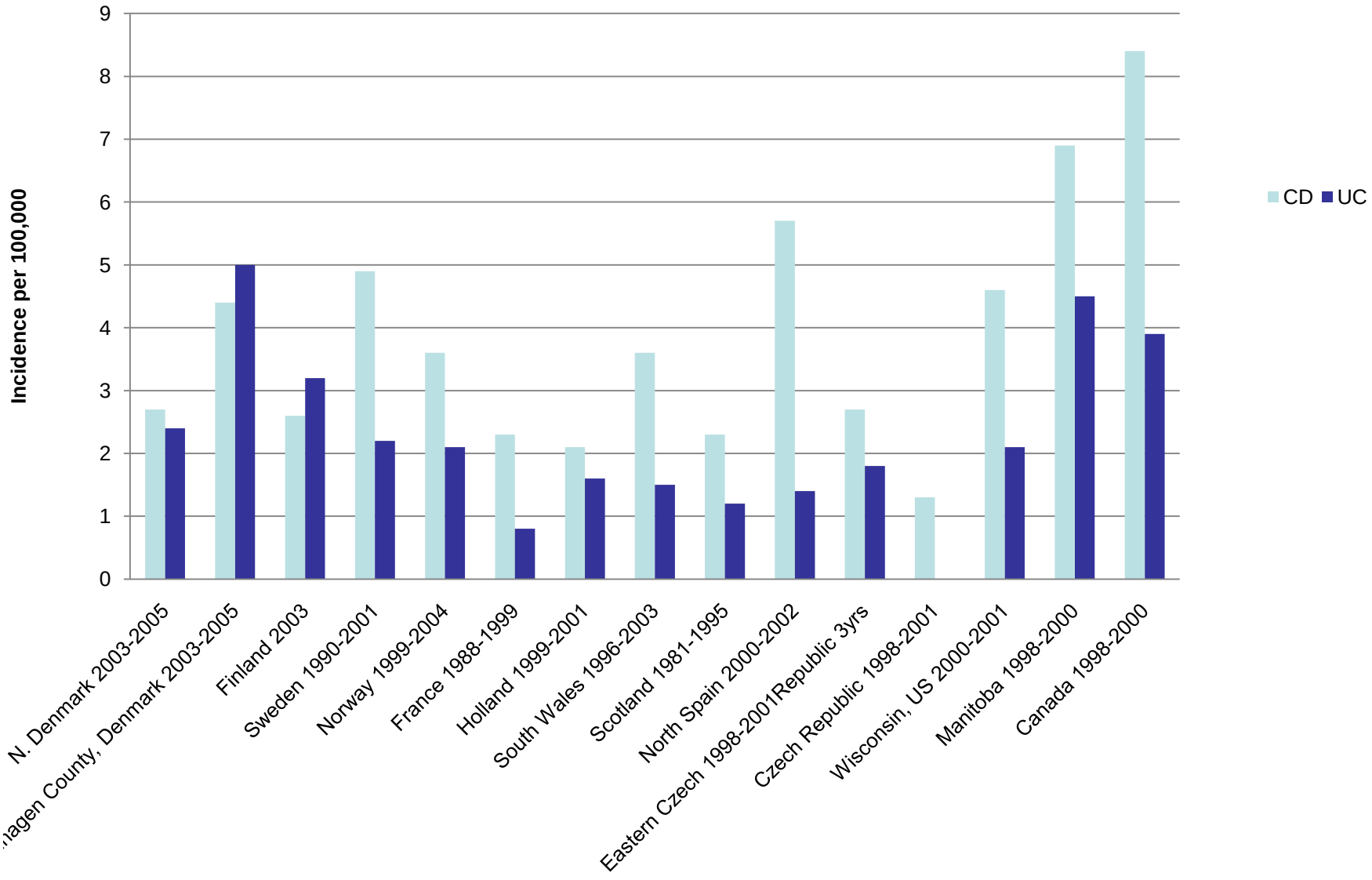


Bernstein Shanahan Gut 2008

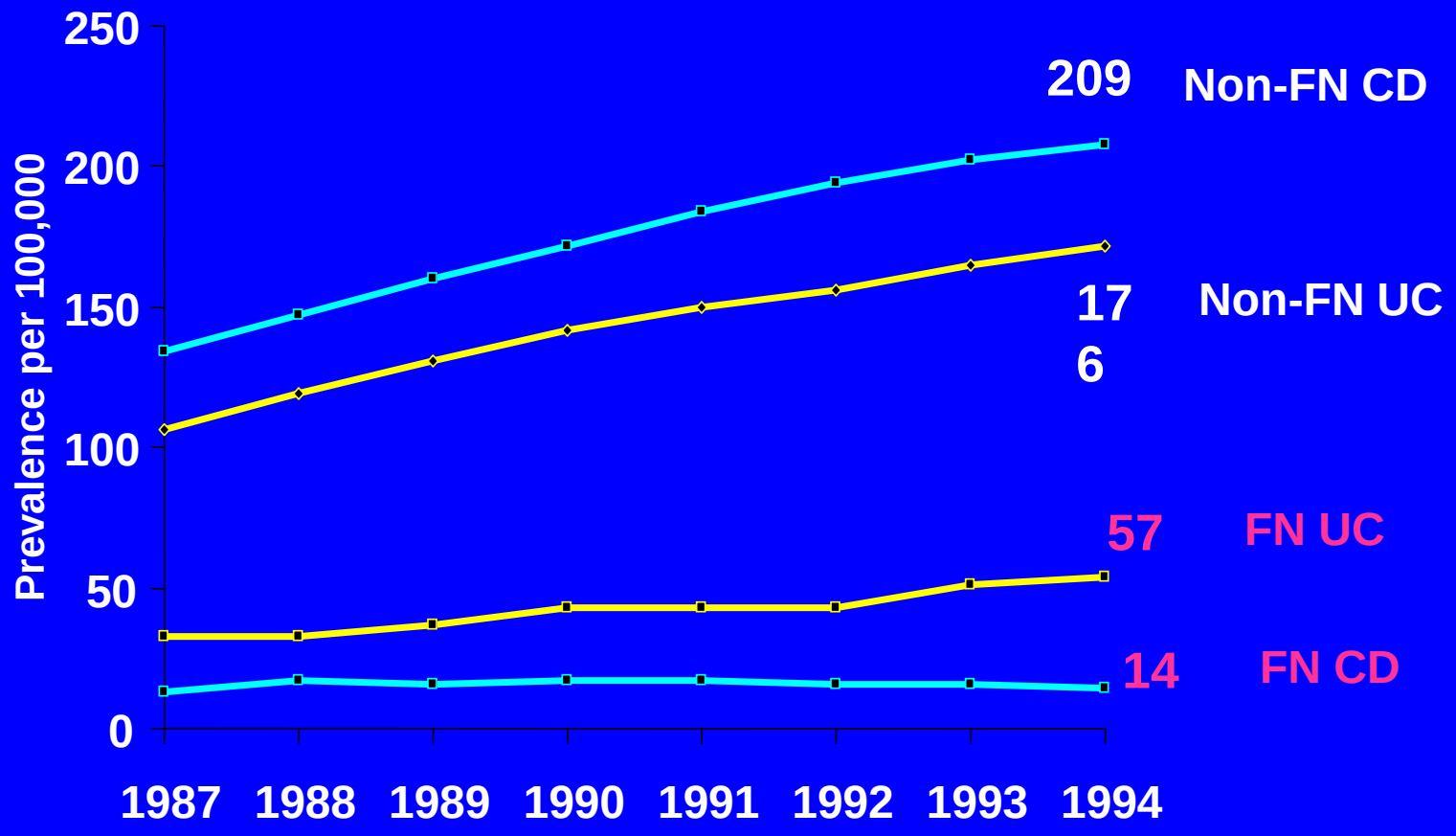
Comparison of Adults with IBD



Comparison of Children with IBD

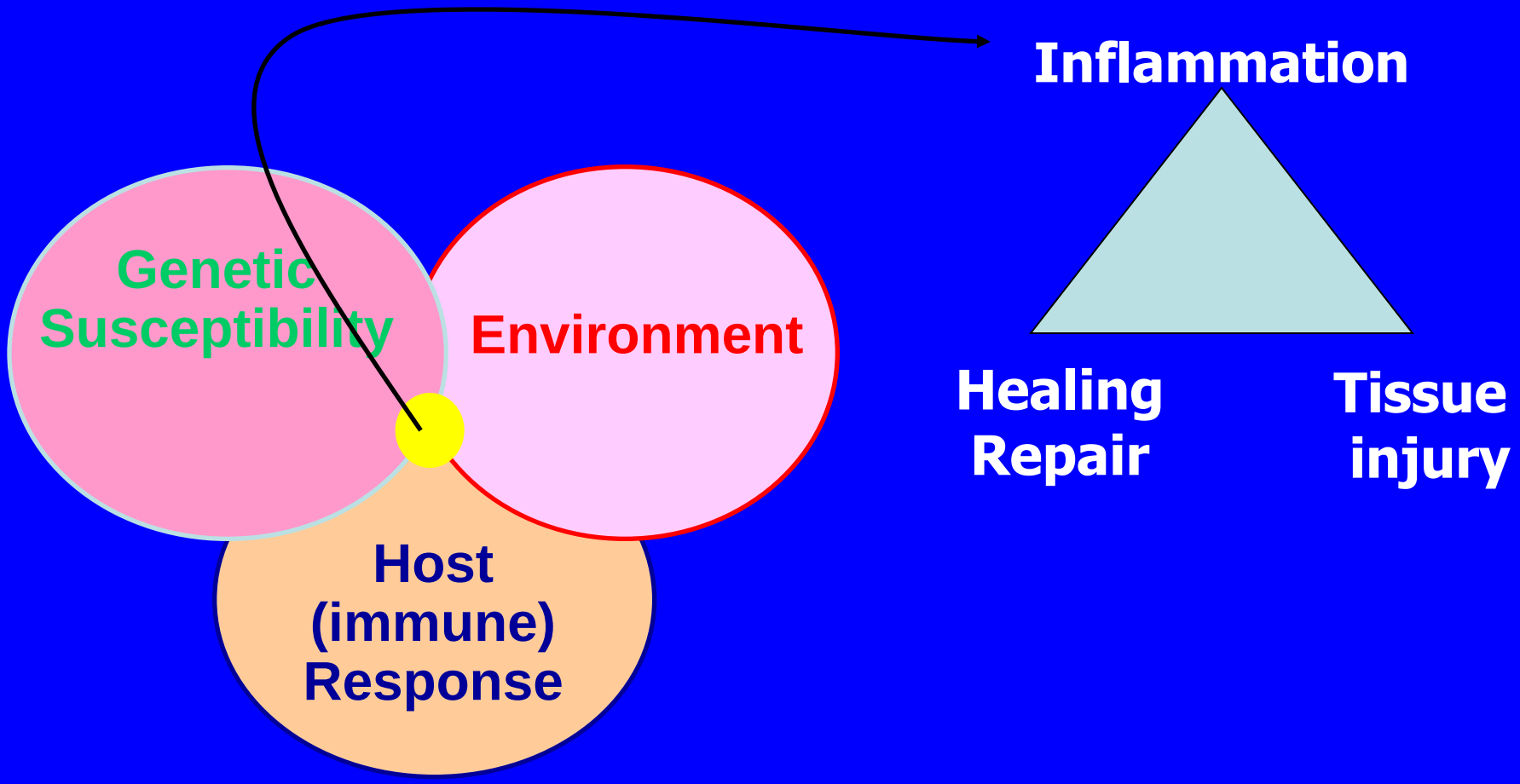


Annual Age-Adjusted Prevalence of CD and UC, by First Nation (FN) Status, Manitoba.



Blanchard Am J Epidemiol 2001

Pathogenesis of IBD



Genes

- Family history
- Twin studies
- NOD-2

The evidence for environment from family/gene studies

- **Monozygotic twins-**

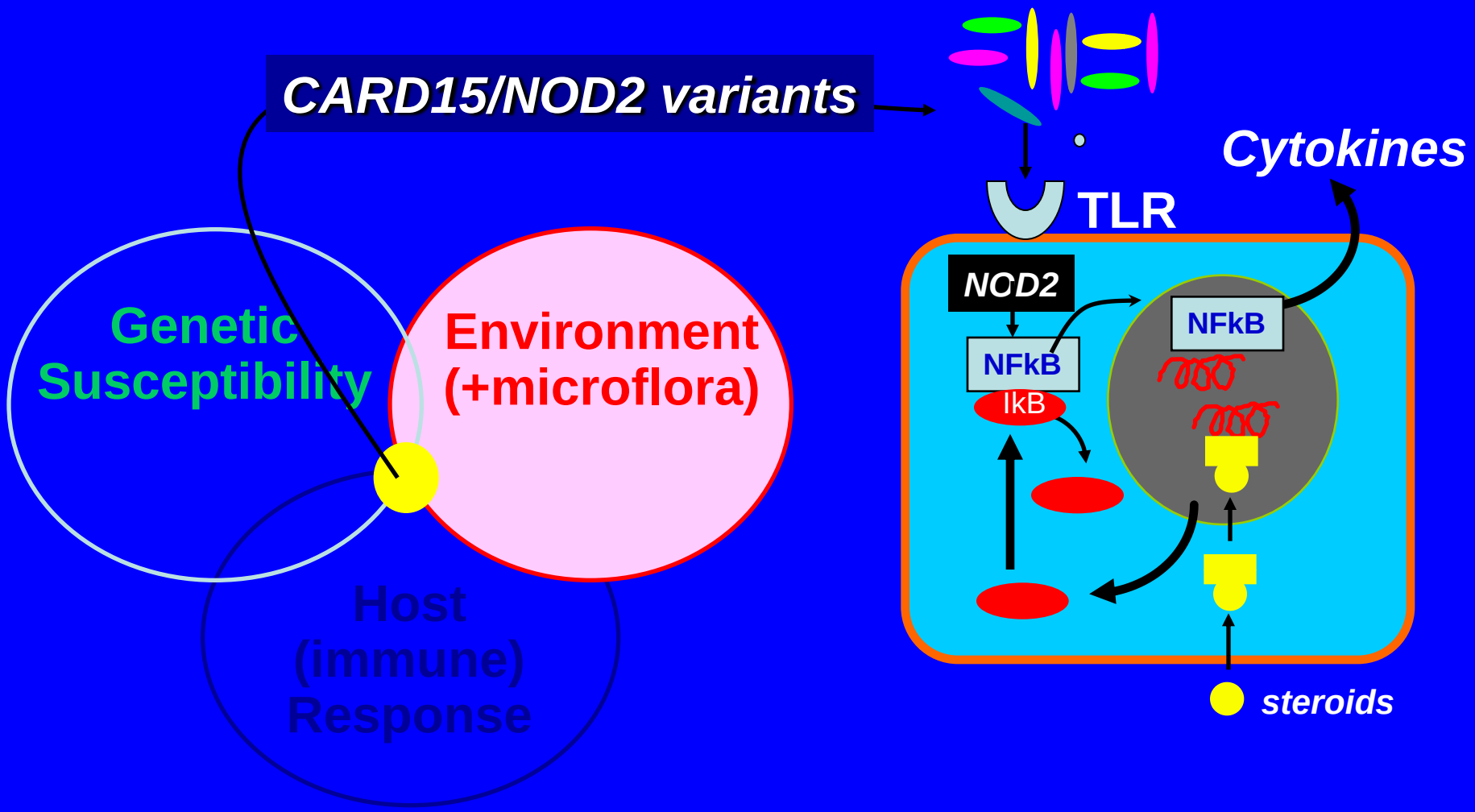
<50% concordance for CD

<10% concordance for UC

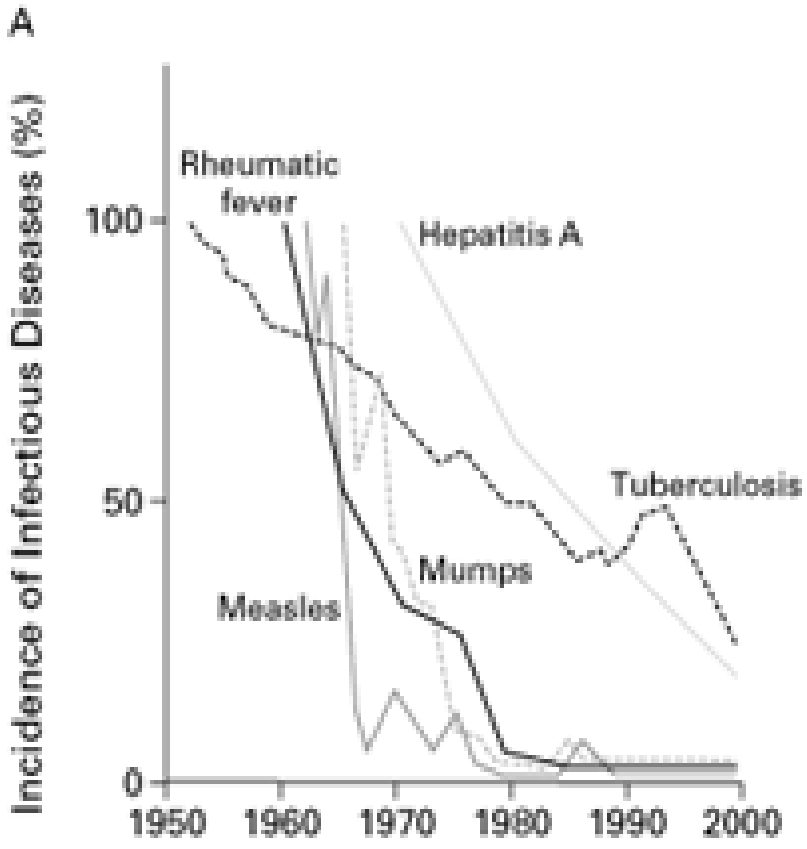
Population based study in Manitoba
NOD-2 mutations (1 or 2):
14% healthy controls (168,000 Manitobans without CD with NOD-2 mutations)
37% CD (1295 Manitobans with CD & NOD-2 mutations)

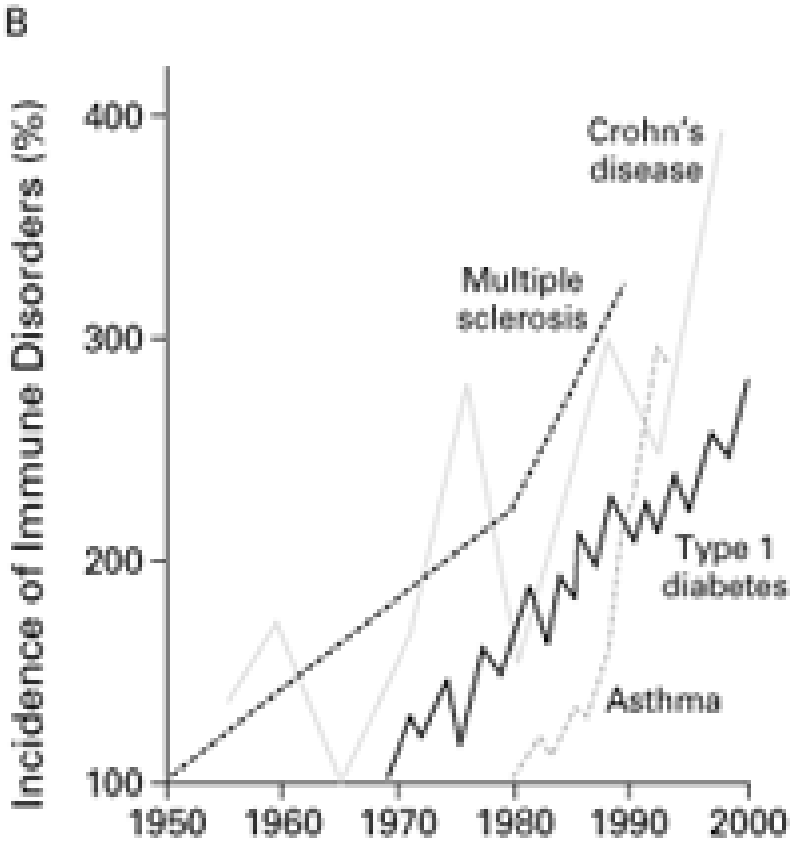
Brant Am J Gastro 2007

Pathogenesis of Crohn's Disease



Inverse relation between the incidence of prototypical infectious diseases (A) and the incidence of immune disorders (B) from 1950 to 2000





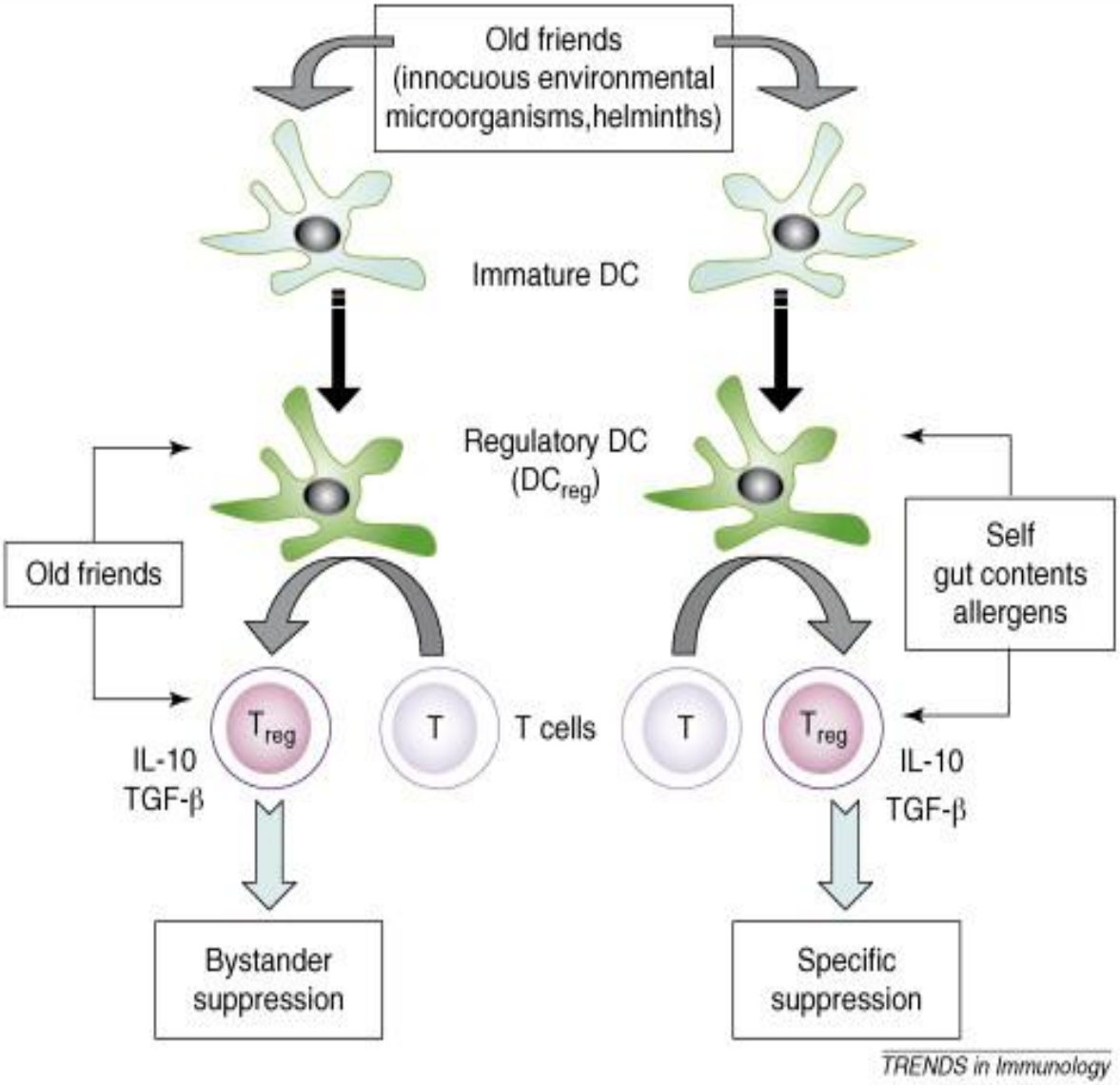
Bach JF et al. N Engl J Med 2002;347:911

Mechanisms of disease: the hygiene hypothesis revisited.

Guarner F Nat Clin Pract Gastroenterol Hepatol 2006

Helminths, saprophytic mycobacteria, bifidobacteria, lactobacilli

Rook and Lowery Trends Immunol 2008



Old friend	Site of inflammation	Animal
Mycobacterium vaccae	Airway	Mice
Mycobacterium vaccae	Skin	Dogs
Heligmosomoides polygyrus	Airway	Mice
Schistosoma mansoni	DM I	NOD mice
Trichuris suis	CD	Humans

Nat Med 2002, Vet Dermatol 2007, J Exp Med 2005, Eur J Immunol 2003, Gut 2005

Features of lifestyle in developed countries

- Improved sanitation
- Decline in endemic parasitism
- Life on concrete (less exposure to soil microbes)
- Decline in *H pylori* and other communicable infections
- Increased antibiotic usage
- Vaccination
- Smaller family size
- Less crowded living conditions
- Refrigeration
- Delayed exposure to mucosal infections
- Sedentary lifestyle and obesity
- Reduced consumption of fermented food products
- Increased consumption of refined sugars and saturated fats

	Bernstein *Am J GI 06*	Amre *Am J GI 06*	Baron *Gut 05*
Study type	Pop-based	Clinic	Pop-based
Age	18-50	<17	<20
Location	Manitoba	Fr Can. PQ	N France
CD (n)	364	222	194
1st Gen Canadian	0.3 (0.2-0.6)	-	
Farm living	0.6 (0.5-0.9)	-	-

	Bernstein *Am J GI 06*	Amre *Am J GI 06*	Baron *Gut 05*
Passive smoking	1.03 (1.01-1.04)	NS	NS
Personal towel	-	0.5 (0.2-0.9)	-
More crowding	0.9 (0.8-0.96)	-	-
Less crowding	-	0.3 (0.1-0.8)	-
Bedroom sharing	-	-	1.6 (1.0-2.4)

	Bernstein *Am J GI 06*	Amre *Am J GI 06*	Baron *Gut 05*
Daycare <6 mos	-	4.5 (1.4-13.7)	-
Pets	<5 y; cat 0.7 (0.5-0.96)	NS	2.0 (0.9-4.5)
Breast feeding	NE	NS	1.6 (1.1-2.4)
Type of water	tap-suggestive	NS	0.5 (0.3-0.8)
MMR vaccine	NE	NS	0.5 (0.35-0.9)

	Bernstein *Am J GI 06*	Amre *Am J GI 06*	Baron *Gut 05*
“Hygiene Hypothesis”	**Supported**	**Refuted**	**Refuted**

Pursuing exogenous microbes

	Crohn's n=24	UC n=28	Controls n=28 (9 sibs)
M. paratuberculosis	0	1	6
Type-2 circovirus	0	0	0
Brachyspira pilosicoli	0	0	0
Brachyspira hyodysenteriae	0	0	0
Coxiella burnetti	0	0	0
Chlamydia psittaci	0	0	0
Bovine viral diarrhea	0	0	0

Bernstein J Clin Micro 2003

Reconciling MAP and IBD epi

Mod to high MAP in cattle

Low MAP in cattle

US, Canada, Denmark → **High IBD** ← **Sweden, Norway**

Chile → **Low IBD**

Map serology (Michael Collins, Univ Wisconsin)

	+ve
Crohn's disease n=297	39%
Ulcerative colitis n=136	30%
Sib controls n=143	36%
Controls n=407	34%

Bernstein J Clin Micro 2004

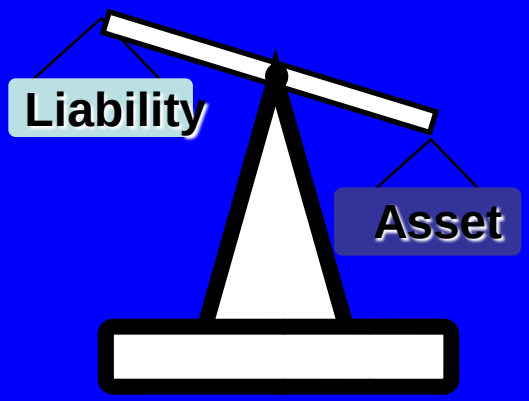
Does M paratuberculosis cause Crohn's?

- Viable organism is difficult to find in human tissue
- Positive and negative studies using PCR in tissue
- Antibiotic studies with agents that could treat MAP do not cure Crohn's
- An immunodiagnostic test to prove Map infections in human is lacking

Does M paratuberculosis cause Crohn's?

- ?
- But no convincing evidence yet
- ?permissive role

Gut Flora as an Asset



- Bacterial antagonism
- Primes mucosal immunity
- Maintenance of peristalsis
- Maintenance of mucosal integrity
- Metabolism of dietary carcinogens
- Synthesis of vits. K & B complex
- Metabolism of prodrugs

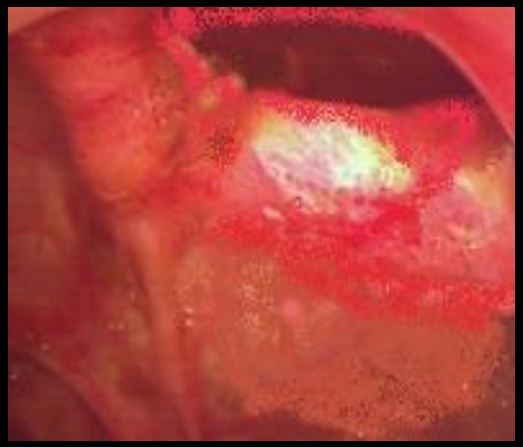
Human distal gut microbiome contains

>100X as many genes as our

2.85 billion bp human genome

Backhed Science 2005

Post operative relapse of Crohn's disease

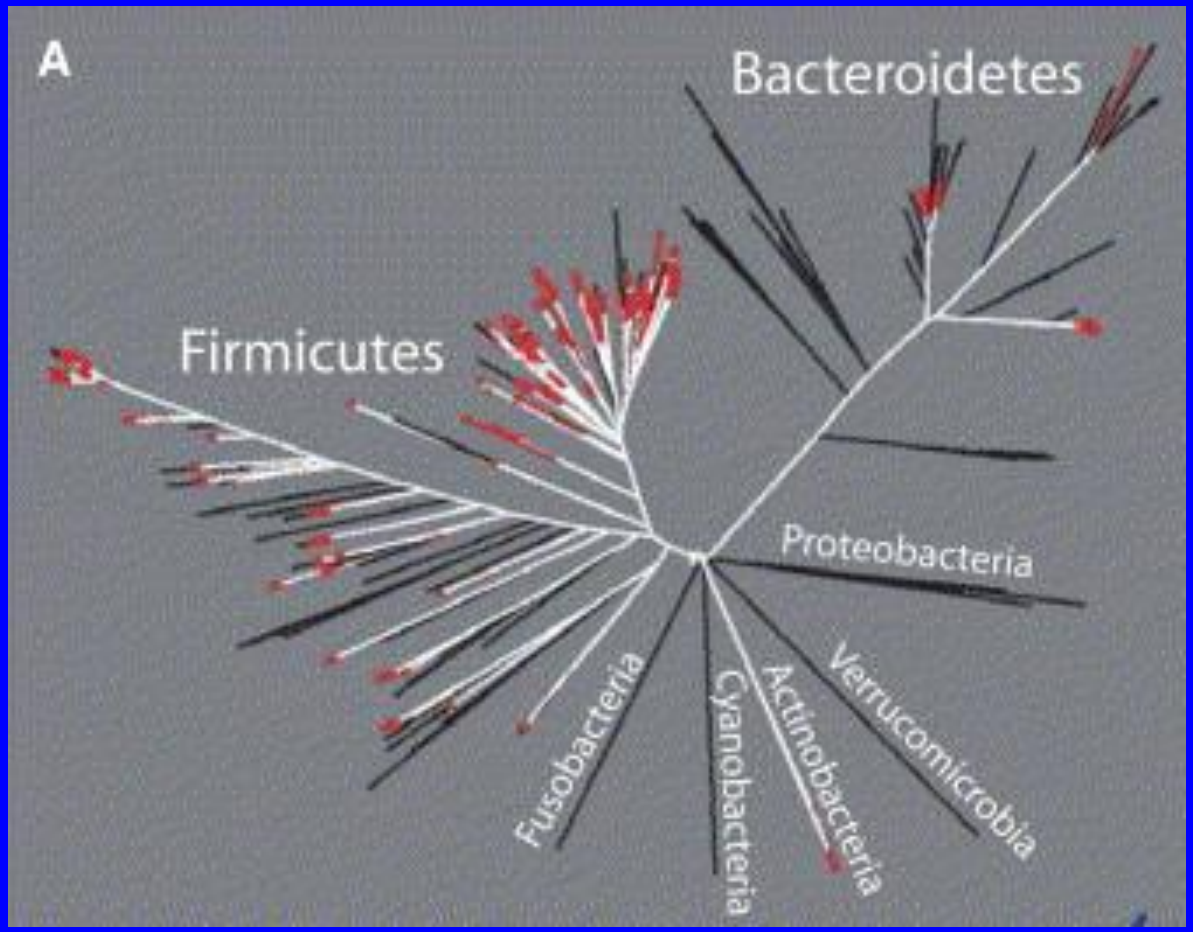


The enemy is within the faecal stream

Characterization of the Intestinal Microflora in Inflammatory Bowel Disease Using Molecular Methods

University of Manitoba + Stanford University collaboration

Bacterial phyla represented in the healthy human gut



Eckburg Science 2005

Eckburg Science 2005

Bacterial 16S rDNA sequences (n=11831)

Archael 16S rDNA sequences (n=1524)

Phylogenetic analysis using 99% minimum similarity as a threshold for any pair of sequences in a phylotype

Bacterial phylotypes (n=395)

Archael phylotypes (n=1) (*methanobrevibacter smithii*)

*** 240 (61%) = novel (not in public databases)**
*** 80% from species that are not cultivated**

*** Most= *Firmicutes & Bacteroidetes***
*** Relatively few sequences of *Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia***

Conclusions of broad sequence PCR in CD, UC, HC

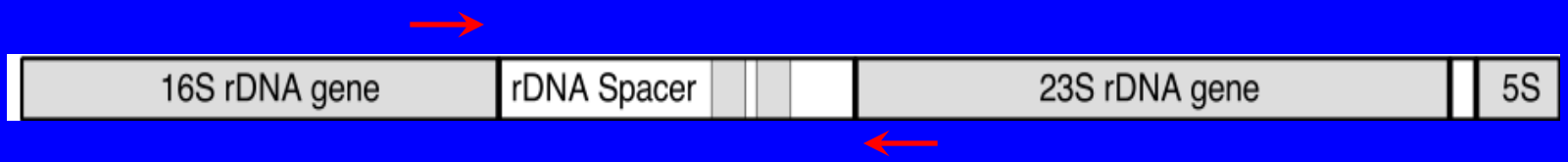
- **This preliminary comparative 16S rDNA analysis reveals differences in the composition and diversity of the colonic mucosal microbiota in CD, UC, and HC.**
- **IBD bacterial microbiota = less diverse than controls**
- **CD microbiota = less diverse than UC or controls**
- **CD microbiota = lack of *Bacteroidetes* & *Firmicutes* predominance of *Proteobacteria* sequences (especially E. coli and Pseudomonas spp.).**
- **Inter-subject differences are the greatest sources of variability between libraries.**

Molecular analysis of IBD tissues at the University of Manitoba

For phylogenetic studies

the following PCR-based methods were used (Krause lab):

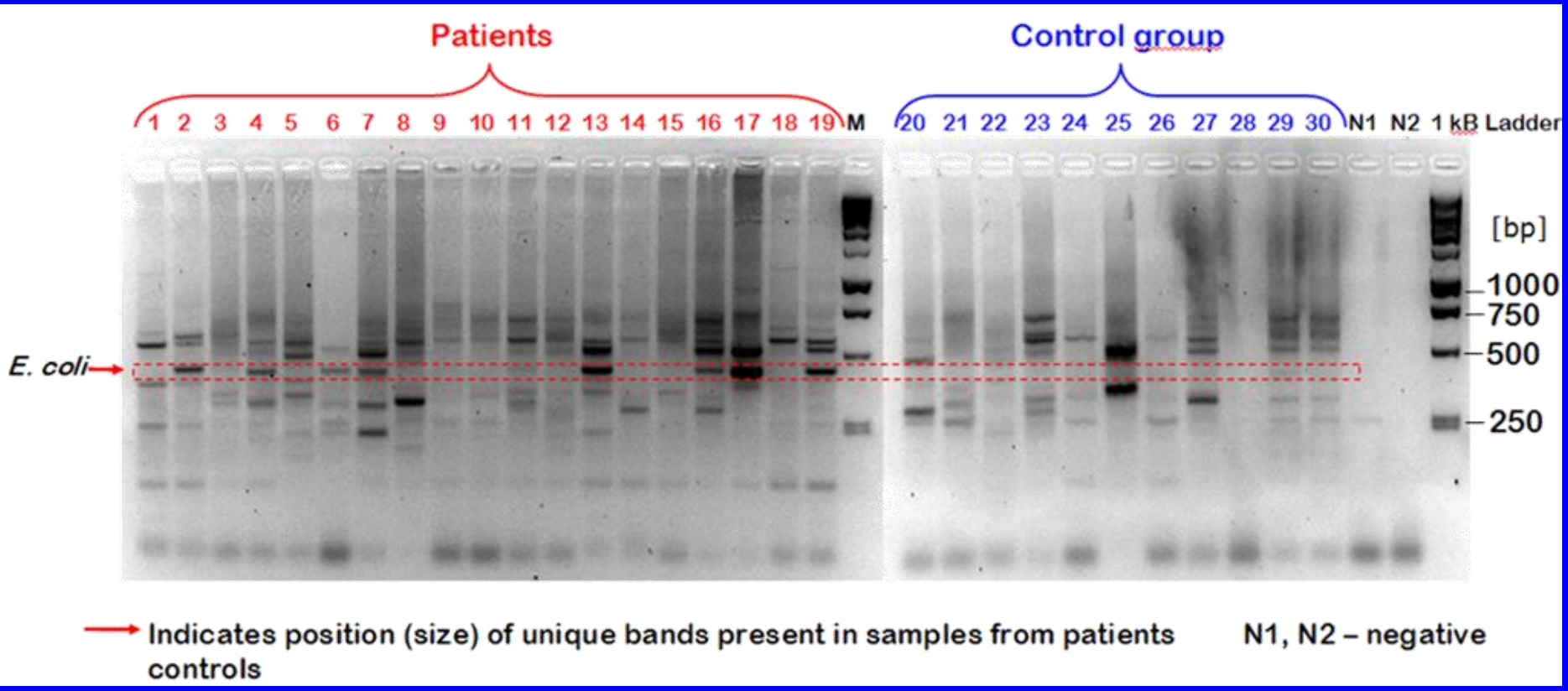
Ribosomal intergenic spacer analysis PCR (RISA-PCR) for amplification of intergenic transcribed spacers between the 16S and 23S rDNA;

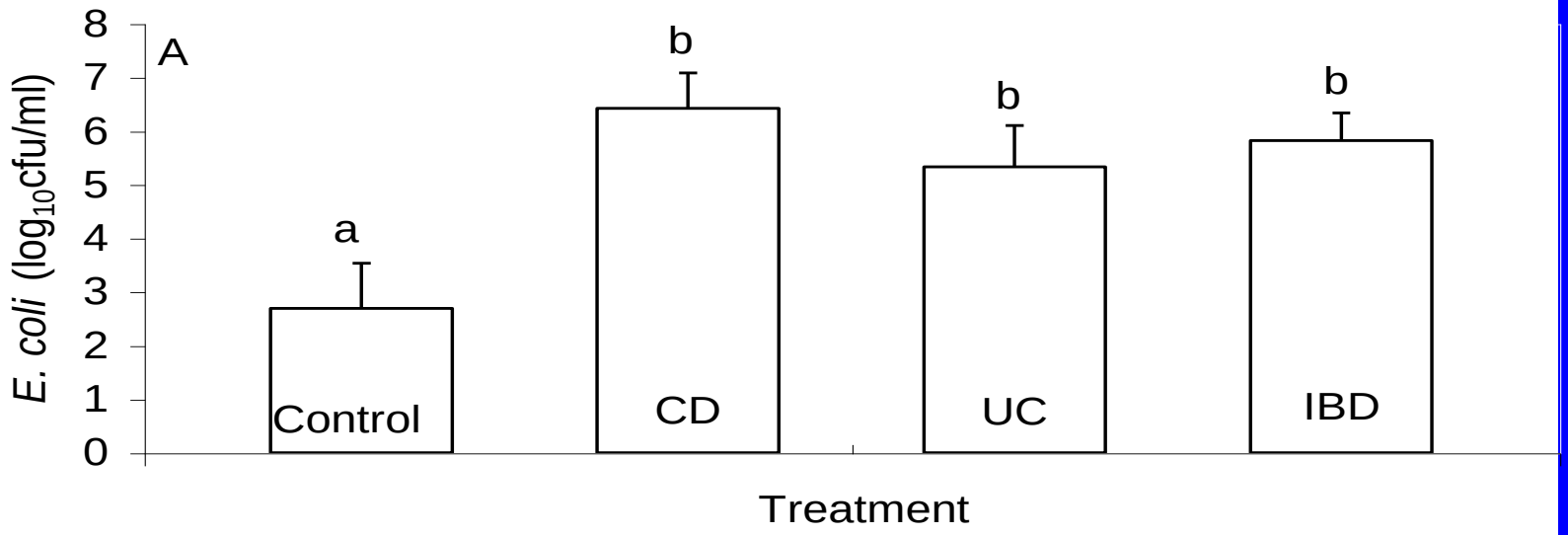


Indices of species richness

	Control	CD	UC
Cecum	81	83	99
Rectum	64	51	99
Total	86	95	92

Identification of unique DNA fragments from tissue samples of IBD patients using RISA-PCR





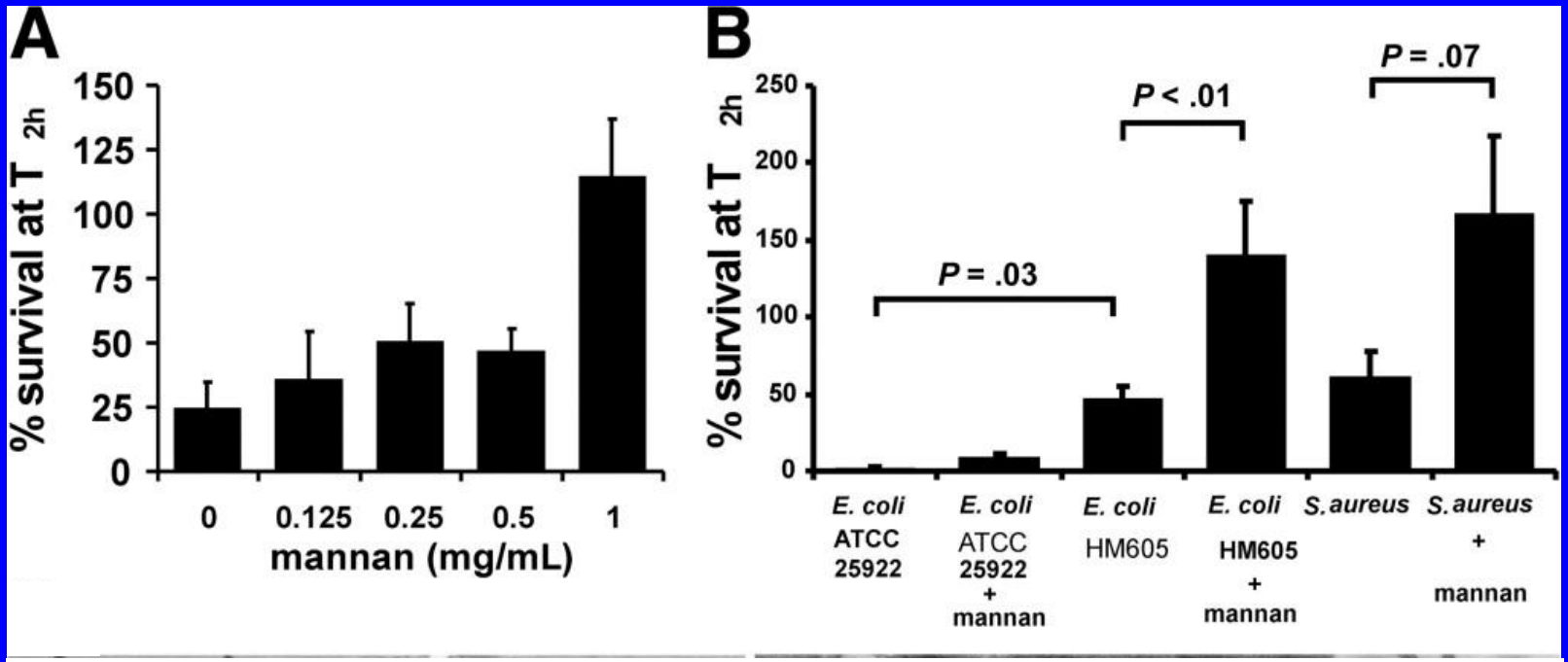
Kotlowski Gut 2006

A hypothesis

- The genetic predisposition to have impaired defense against flora bacteria (?upregulation of CEACAM on enterocytes)
- An E coli species increased in IBD tissue (more easily found in uninflamed tissue)
- The E coli makes proteases in increased quantities and diversities that the host can not defend against
- There is mucosal damage and the immune cascade is initiated

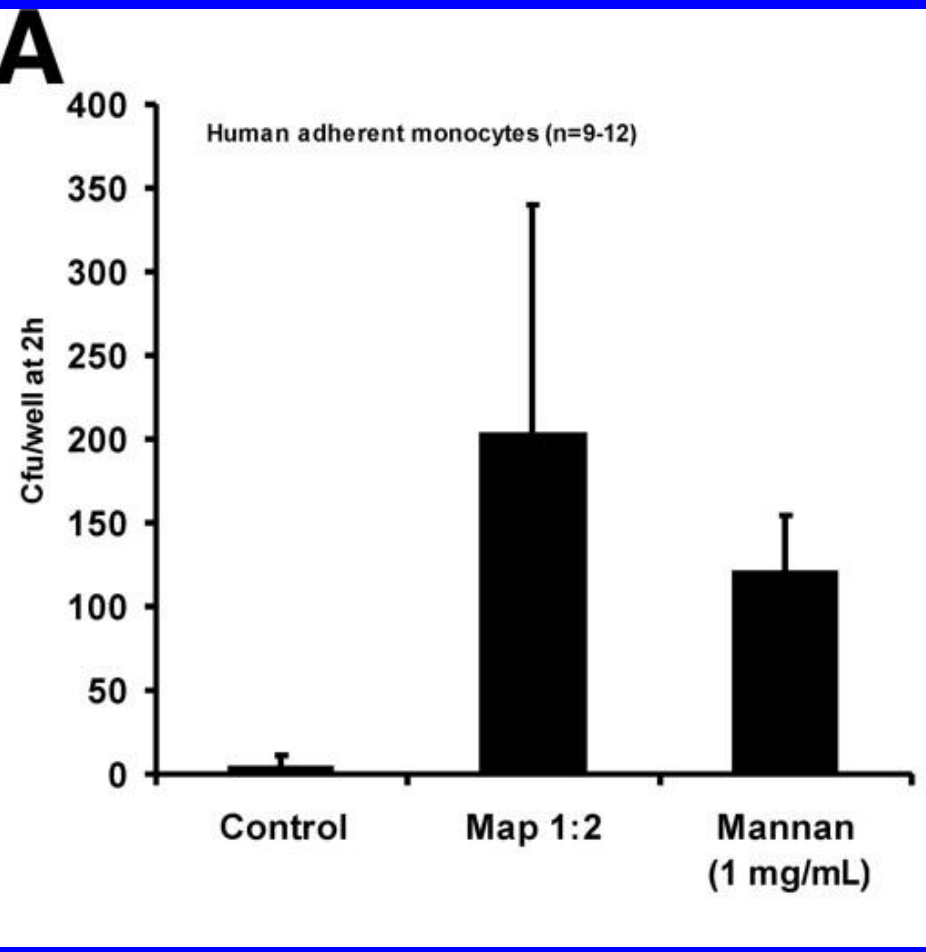
Ability of mannan to inhibit mucosal phagocyte function

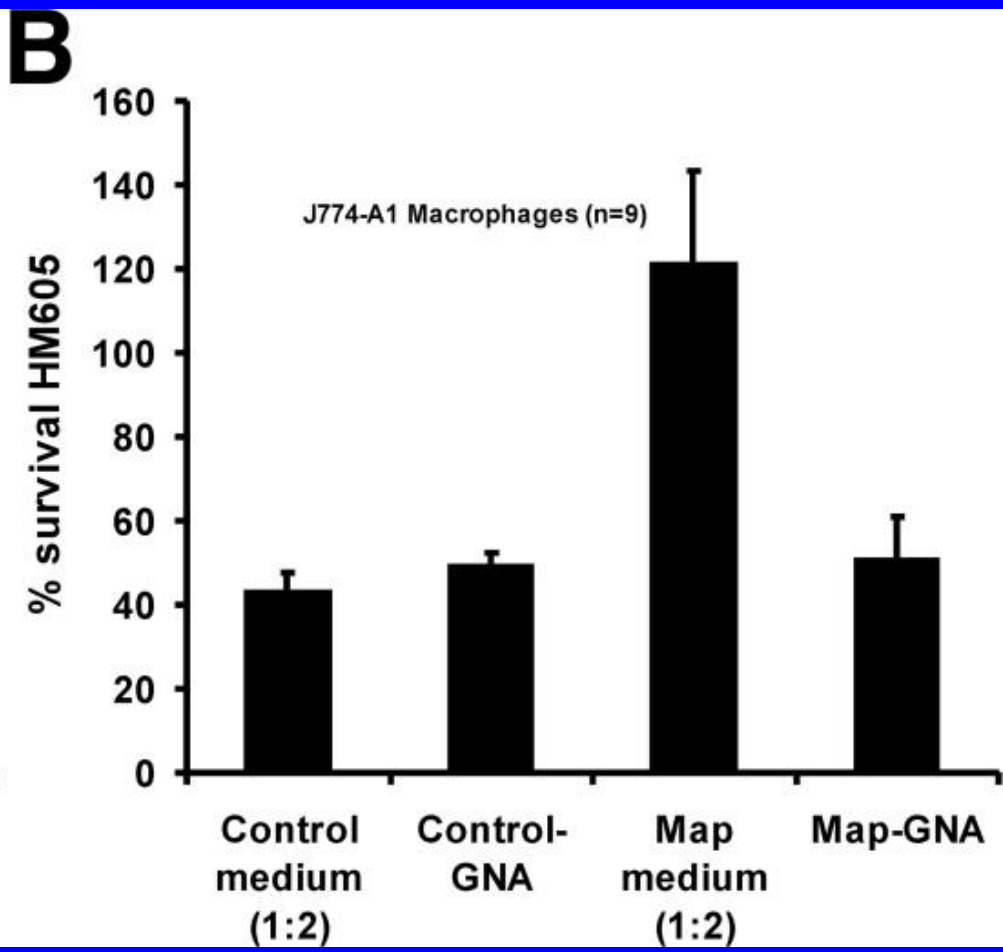
Mpofu Gastroenterology 2007



A.Presence of *S cerevisiae* mannans inhibits monocyte killing of CD assoc. E coli

B.CD assoc E coli survives even better than ATCC E coli

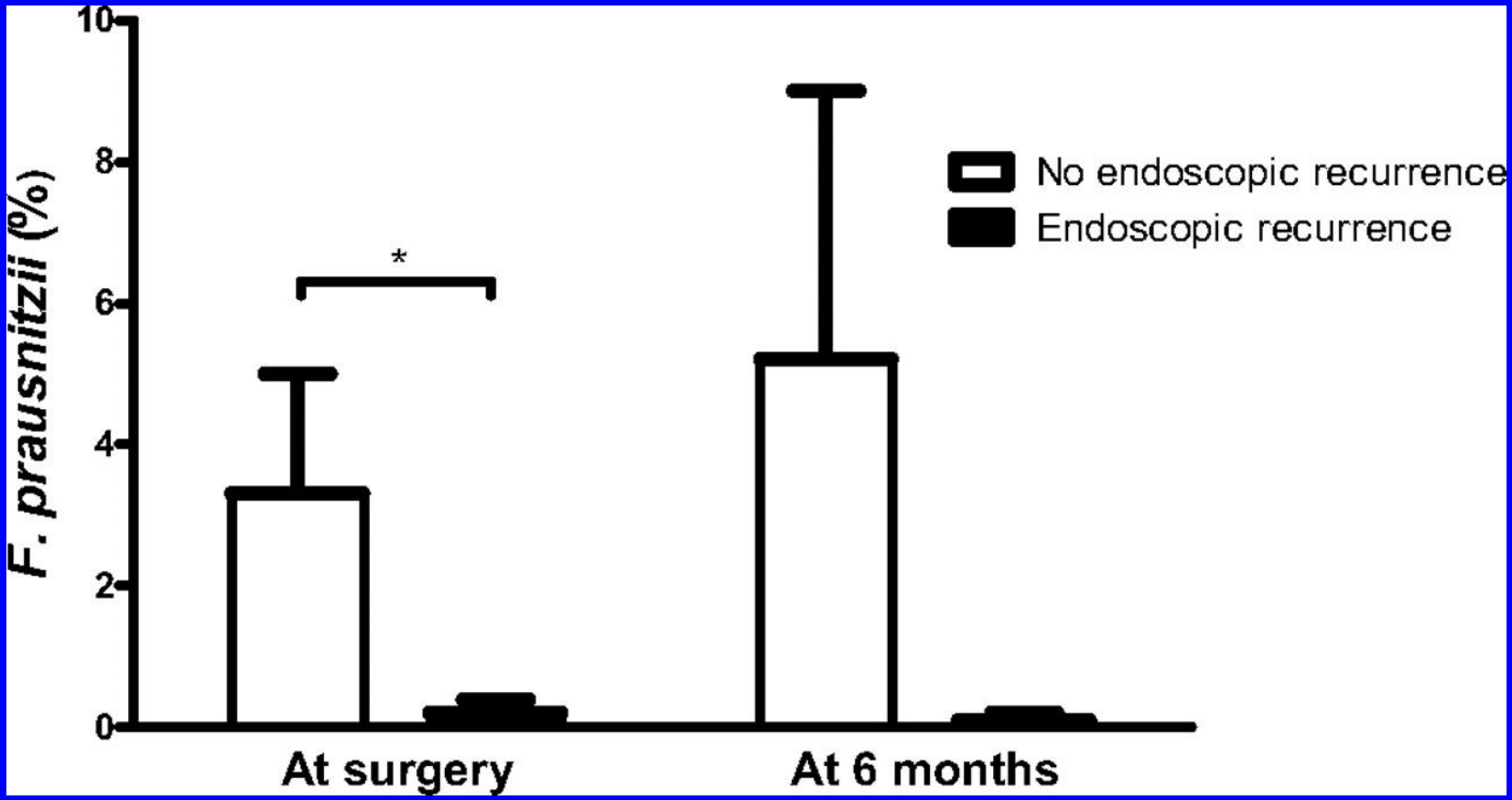




Map culture supernatant increased survival of CD assoc E coli in monocytes

Mpofu Gastroenterology 2007

F. prausnitzii proportions in the ileal MAM using FISH at the time of surgery and at 6 months according to the endoscopic recurrence status. *, P = 0.03.

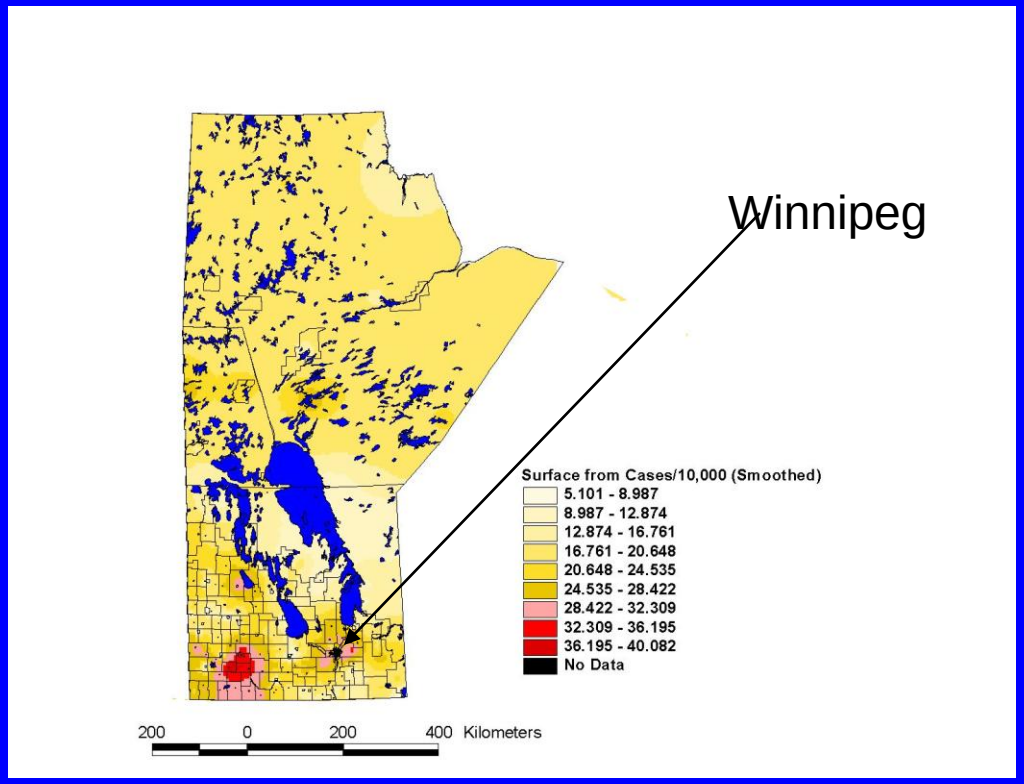


Sokol H. et.al. PNAS 2008;105:16731-16736

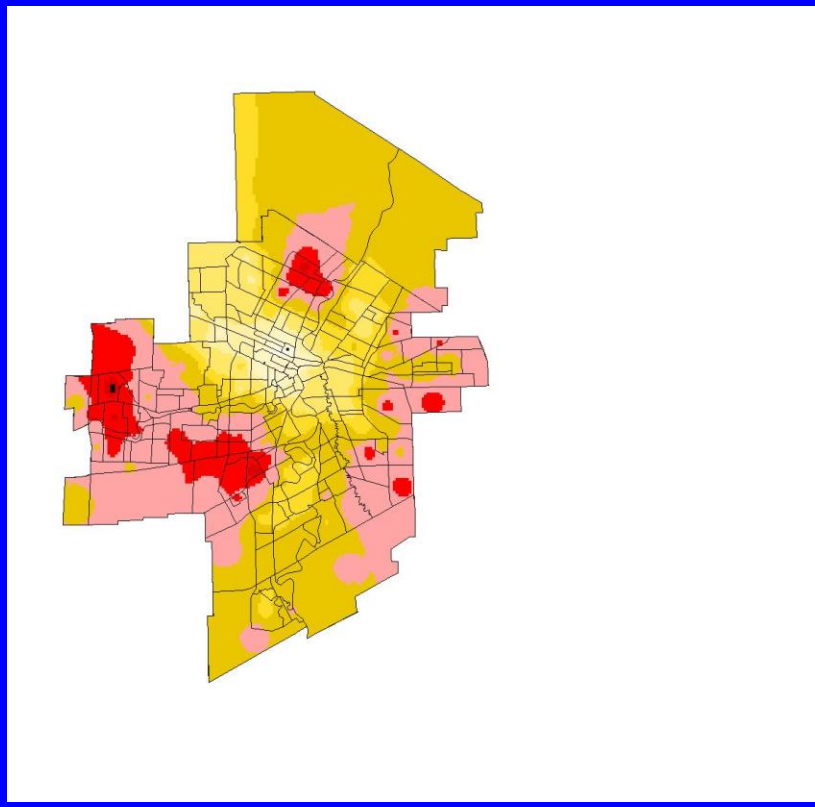
UC Prevalence, 2001, Smoothed Rates

Cases/10,000, Age Standardized to the 1996 Manitoba Population
Smoothing - Mean Nearest Neighbor(10,000)

Hot and cold spots in the province



City of Winnipeg



Diet and IBD
Is this the link?

Newly dx pediatric CD Diet case-control study

Amre Am J Gastroenterol 2006

Fish	OR=0.46 (0.2-1.06)
Fruits	0.37 (0.16-0.86)
Nuts	0.16 (0.04-0.58)
Dietary fiber	0.12 (0.04-0.37)
LC ω–3 FA	0.44 (0.19-1.00)
Sugars	No assoc

Sugar

- Impact on gut flora
- Source of mannans

Associations between CD & sugar consumption

Riordan Eur J Clin Nutr 1998

- **No definite relationship could be identified**
- **No relationship between national sugar consumption and CD incidence**

  (epi studies)

- **CD =greater consumption of sugar vs controls BUT**

- Duration of disease influences recall
- Incomplete recollection of the types of foods eaten and imprecise estimation of portion size
- Estimates of error overlooked since clinical differences between cases and controls are small
- Prediagnosis symptoms may affect intake pre diagnosis; rarely ask for presymptom diet.
- Patient awareness of study aim may lead to under or over reporting
- Ability to separate retrospectively present eating patterns from those in distant past

Current sugar intake during disease

- **?consequence of active disease**
- **?consequence of Rx (decreasing appetite)**
- **?consequence of past dietary advice**

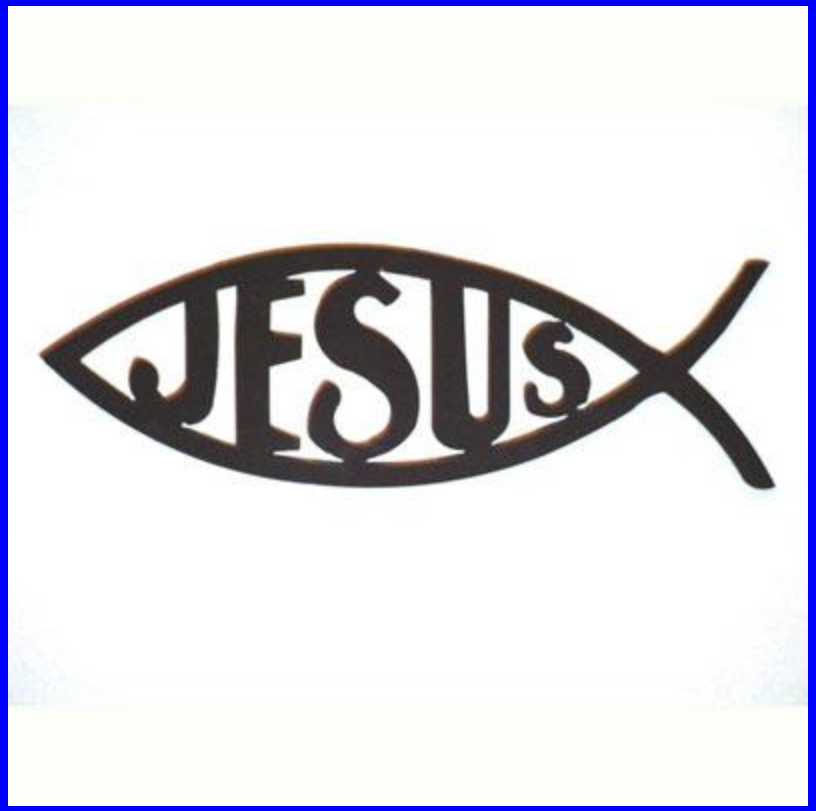
Ecological study of diet in Japan

Shoda Am J Clin Nutr '96

Multivariate model; correlating with CD incidence:

- **Animal protein intake**
- **ω6 fatty acids/ ω3 fatty acids**

Fish oil



The NEW ENGLAND JOURNAL of MEDICINE

Beluzzi et al. June 13, 1996 Volume 334:1557-1560

Effect of an Enteric-Coated Fish-Oil Preparation on Relapses in Crohn's Disease

Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC Randomized Controlled Trials

Feagan et al. JAMA 2008 Apr 9; 299(14):1690-7

- **EPIC-1**

in chronic remission (n=375)

- **EPIC-2**

post steroid induced remission (n=379)

RESULTS: The rate of relapse at 1 year:

- **EPIC-1 fish oil- 31.6% placebo-35.7% (p = 0.30).**
- **EPIC-2 fish oil- 47.8% placebo-48.8% (p = 0.48).**

- **CONCLUSION: Treatment with omega-3 free fatty acids was not effective for the prevention of relapse in Crohn disease.**

The fish oil of 2008

VITAMIN D

Vitamin D deficiency in IBD

- 25-OH vit D <35 nmol/L

18% CD (n=84)

17% UC (n=42)

- 25-OH vit D <75 nmol/L

70% CD

79% UC

Vagianos JPEN 2007

Daily Calcium and Vitamin D Intake by IBD Patients

Study	IBD	Control	Country
Calcium (mg)			
Bernstein	743	–	US
Reed	825	–	US
Silvennoinen	1034	1334	Finland
Bernstein	667	–	Canada
Vitamin D			
Bernstein	128 IU	–	US
Reed	212 IU	–	US
Vogelsang	40 IU	44 IU	Austria
Bernstein	146 IU	–	Canada

% consuming inadequate intake of vitamin D (<66% of recommended)

38% CD

32% UC

Vagianos JPEN 2007

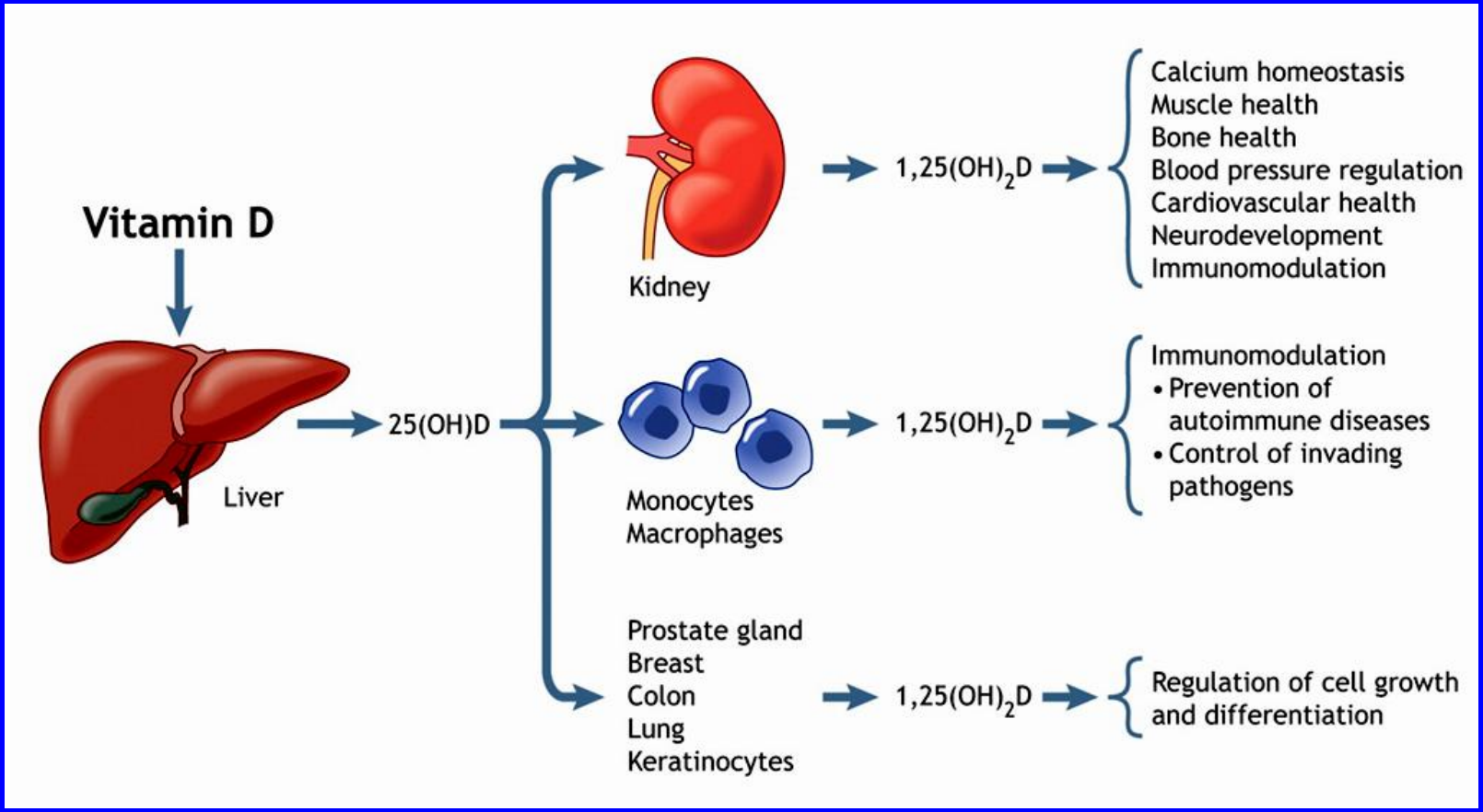
Risk for vitamin D deficiency (< less than 75 nmol/L): Multivariate analysis

controlling for sex, IBD type, IBD status, ileum involvement, medication use, surgical history, duration of disease, use of vitamin/mineral supplementation, serum vitamin B6, B12, carotene, ferritin and zinc.

Vitamin D deficiency higher in UC (OR, 3.40; 95% CI 0.86-13.5).

Vagianos JPEN 2007

The endocrine, paracrine and intracrine functions of vitamin D



Hollis, B. W. et al. CMAJ 2006;174:1287-1290

Tobacco is the most consistent environmental risk factor identified

Tobacco use increases risk for Crohn's disease 2-4x

+ve correlation smoking and Crohn's incidence

Vessey, Br Med J (Clin Res Ed) 1986
Tobin Gastroenterology 1987
Benoni C Scand J Gastroenterol 1987
Lindberg E, Gut 1988
Katschinski B, Gut 1988
Logan RF, Int J Epidemiol 1989
Lashner BA, Gastroenterology 1989
Sandler RS, Epidemiology 1992
Corrao G, Int J Epidemiol 1998

- **+ve correlation between perinatal or childhood passive smoke exposure and Crohn's incidence**

Persson, Gut 1990
Lashner, Am J Gastroenterol 1993
Bernstein, Am J GAstroenterol 2006

- **+ve correlation for smoking and more active Crohn's disease**

Russel MG, Inflamm Bowel Dis 1998

- **+ve correlation for smoking and ileal rather than colonic Crohn's disease**

Lindberg E, Gut 1992
Russel MG, Inflamm Bowel Dis 1998

- **Smokers have higher relapse rates after medically induced remissions**

Duffy LC, Am J Prev Med 1990.
Timmer A, Gastroenterology 1998.
Cosnes J, Aliment Pharmacol Ther 1999.

- **Smokers have higher relapse rates after surgically induced remissions**

Sutherland LR, Gastroenterology 1990.
Cottone M, Gastroenterology 1994.
Breuer-Katschinski BD, Eur J Gastroenterol Hepatol 1996.
Moskovitz D, Int J Colorectal Dis 1999.

- **Quitters have lower relapse rates than those who continue to smoke**

Cosnes J, Gastroenterology 2001.

Environmental-gene interaction

- **Higher relapse rate among smokers post surgical induced remission is more common among females**

Yamamoto T, Br J Surg 2000.

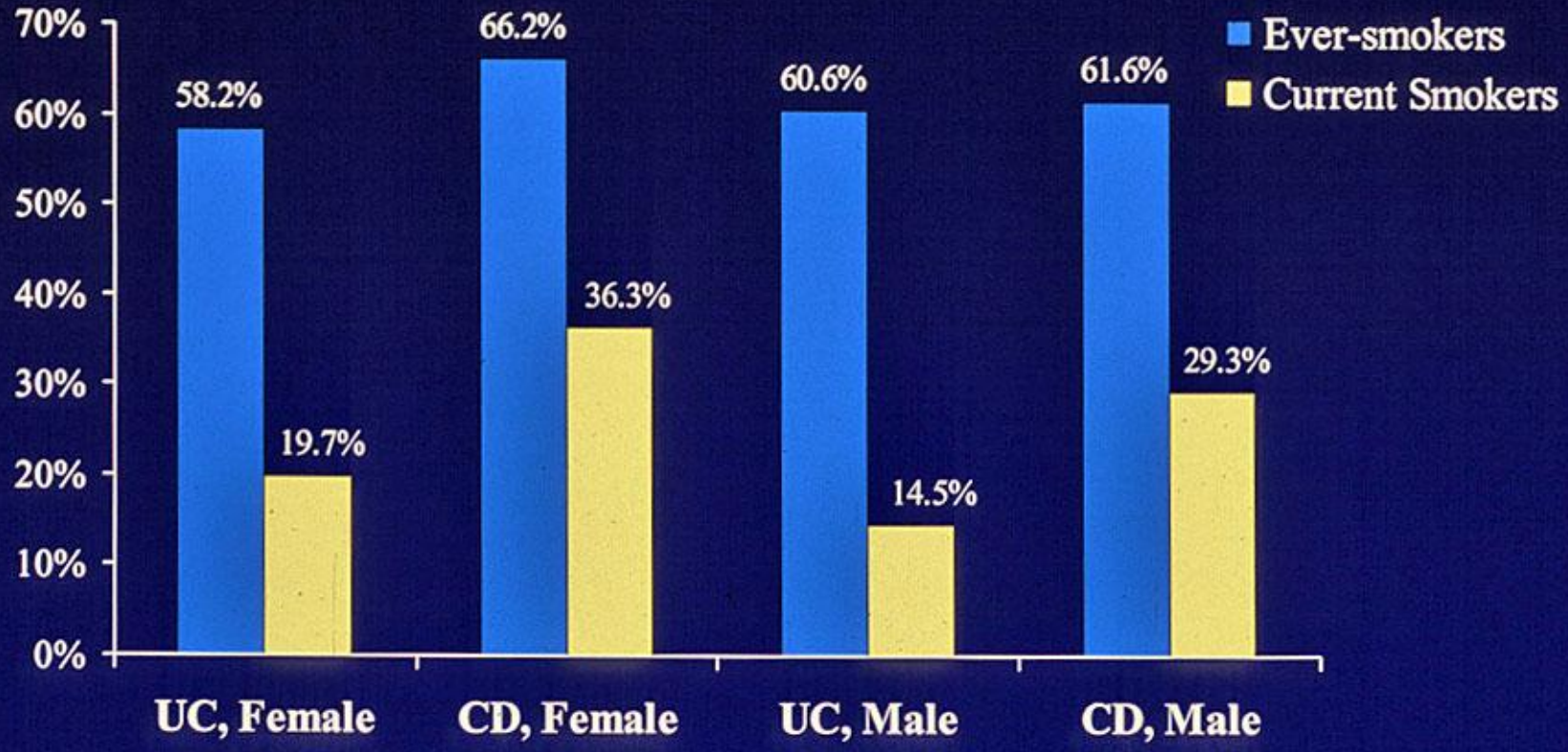
- **Smoking accounts for the discordance between monozygous twins for Crohn's disease incidence in a Danish study**

Orholm M, Scand J Gastroenterol 2000.

- **Patients with familial Crohn's disease were more likely to be smokers than sporadic non familial Crohn's disease**

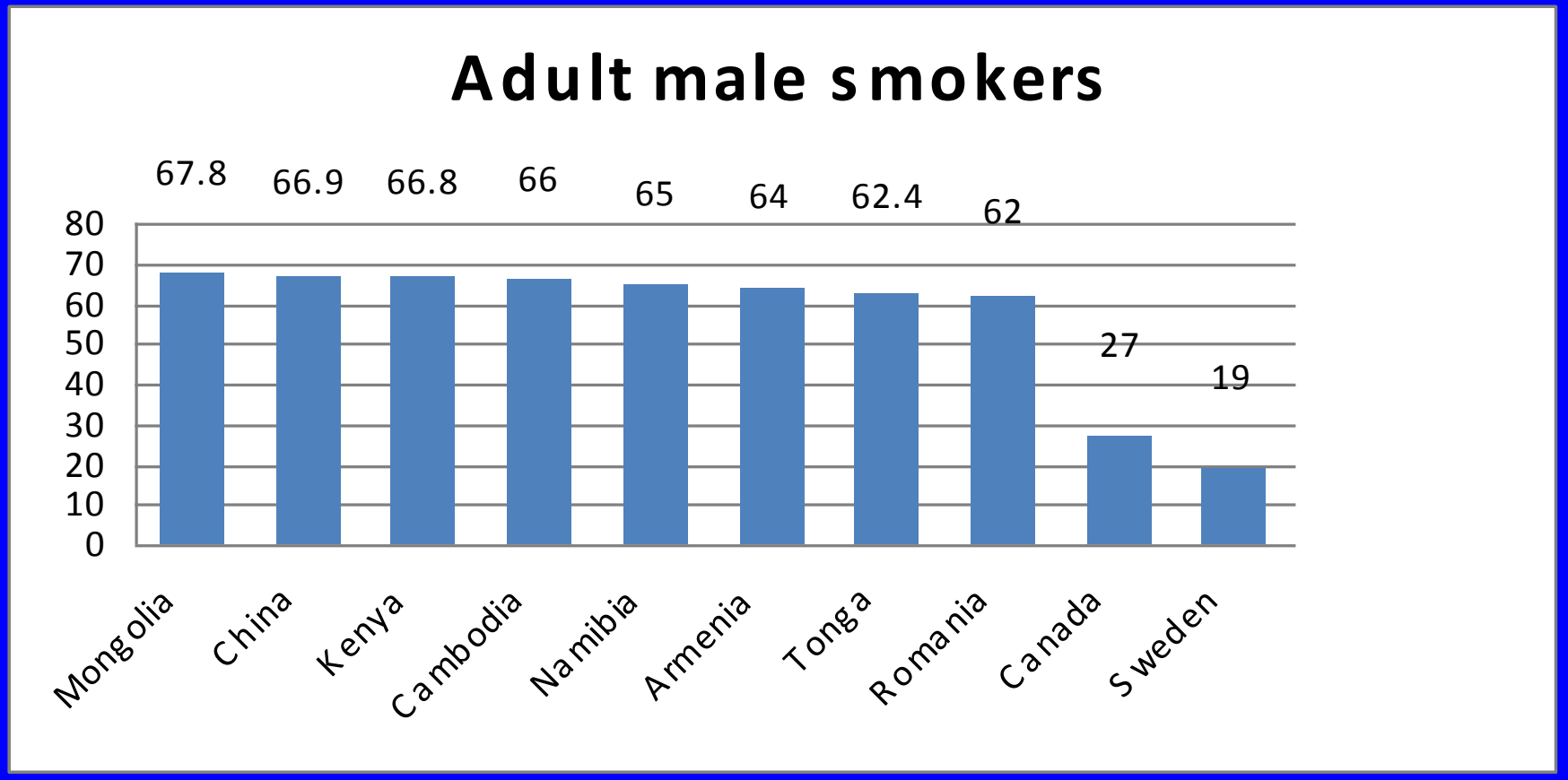
Brignola C, Am J Gastroenterol 2000

Smoking History Among Crohn's Disease (CD) and Ulcerative Colitis (UC) Patients, by Gender



Manitoba data 2001

www.nationmaster.com/graph/hea_tob_adu_mal_smo-health-tobacco-adult-male-smokers



The Passover Seder and 4 sons

Wicked son

Wise son

Simple son

The one who does not know how to ask the question

"ASSESSING ENVIRONMENTAL RISK FACTORS AFFECTING THE INFLAMMATORY BOWEL DISEASES"

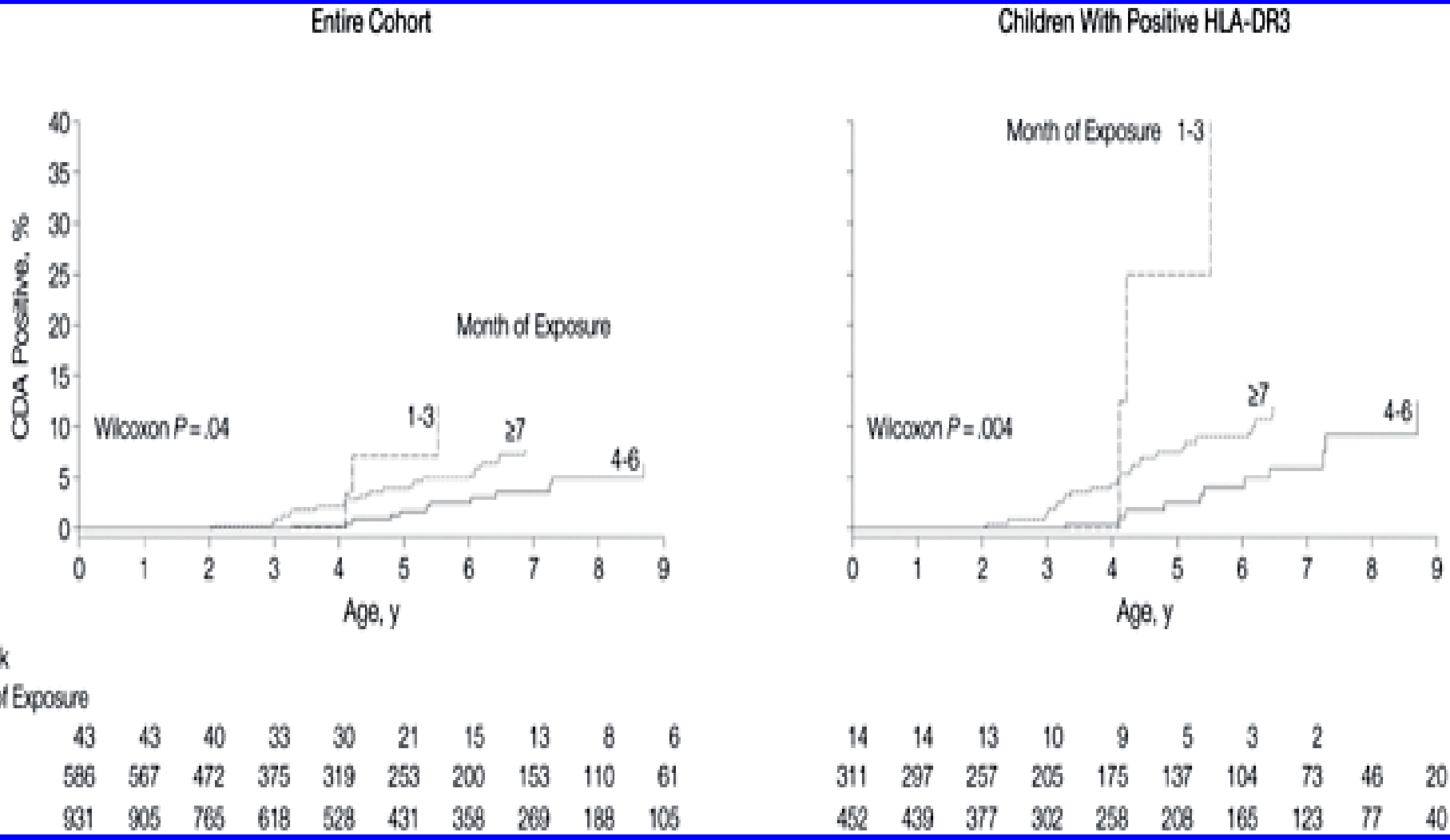
- **A joint symposium of CCFC-CCFA May ’07**
- **Toronto, Ontario**
- **Proceedings in Inflamm Bowel Dis 2008;14:1139.**

DAISY
Diabetes Autoimmune Study in the Young

- Having sibling or parent with DMI or Celiac
- Having high risk HLA type
- Diet collection at 3,6,9,12,15 mos
- 1307 kids enrolled at birth-311 FHX of DMI

996 picked up at newborn HLA screening

- Exposures to gluten 100% by 12 mos,
- Initial +ve TTG=2 yrs (mean age at conversion= 4.7 yrs)



Norris JAMA 2005

Hazard rate of developing CDA by timing of food introduction

Timing of food intro	0-3 Mos	4-6 mos	≥7 mos
Wheats, barley, rye	5.17 (1.44-18.57)	1.00	1.87 (0.97-3.6)
Rice	0.86 (0.4-1.86)	1.00	1.25 (0.58-2.69)

Norris JAMA 2005

- **Similar outcome for development of islet cell Abs and DMI**
- **No protective effect of breastfeeding**

Norris JAMA 2005

Summary

Or where do we go from here?

- Developing world and new IBD “markets”
- Epidemiology in migrant populations
- Pediatric IBD