

**GENETIC AND SEROLOGICAL MARKERS ASSOCIATED WITH
POUCHITIS AND A CROHN'S DISEASE-LIKE PHENOTYPE
AFTER PELVIC POUCH SURGERY FOR ULCERATIVE COLITIS**

by

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**A thesis submitted in conformity with the requirements
for the degree of Masters of Science,
Graduate Department of the Institute of Medical Science,
University of Toronto**

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ABSTRACT

Objective: Study the clinical, genetic and serological factors associated with new onset ileal inflammation in ulcerative colitis (UC) patients who have undergone ileal pouch-anal anastomosis (IPAA).

Methods: Retrospective assessment of UC patients registered in the MSH surgical database, questionnaire, chart review and serological and genetic analysis of blood sample.

Results: There was a positive association between gASCA antibodies, anti-L, smoking status and axial arthritis/ankylosing spondylitis with a Crohn's Disease (CD)-like phenotype post-op in UC/IPAA patients. Associations between alleles of selected genes including ECM1, PTPN2, and CD14 and post-operative phenotype were identified.

Conclusions: gASCA is typically associated with CD rather than UC which raises the possibility that gASCA positivity may delineate a subset of UC patients at risk for developing a CD-like phenotype after IPAA. The identification of genotype/phenotype associations in UC/IPAA will help to find those at risk for poor post-operative outcome. Prospective studies are needed to confirm these findings.

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TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix

CHAPTER I INTRODUCTION AND BACKGROUND

1.1. INTRODUCTION	1
1.2. BACKGROUND	4
1.2.1 Epidemiology	6
1.2.2 Description of Inflammatory Bowel Disease	9
1.2.3 Ulcerative Colitis and Crohn’s Disease	9
1.2.4 Indeterminate Colitis and Inflammatory Bowel Disease – Unclassified (IBDU)	12
1.2.5 Familial Adenomatous Polyposis (FAP)	14
1.2.6 Etiology and Pathogenesis of IBD	15
1.3. SURGERY AND POST-OPERATIVE OUTCOME	17
1.3.1 Ileal Pouch-Anal Anastomosis (IPAA)	17
1.3.2 Diseases of the Pouch	18
1.4. DESCRIPTION OF POUCH OUTCOME	21
1.4.1 History and Definition of Pouchitis	21
1.4.2 Pathogenesis and Etiology of Pouchitis	22
1.4.3 Risk Factors Associated with Pouchitis	25
1.4.4 Diagnosis and Classification of Pouchitis	25
1.4.5 Crohn’s Disease and Crohn’s Disease-like Phenotype of the Pouch	28
1.4.6 Irritable Pouch Syndrome (IPS) and Cuffitis	31
1.5. SEROLOGY OF INFLAMMATORY BOWEL DISEASE	33
1.5.1 Summary of Serological Markers in IBD	33
1.5.2 Anti-Glycan Antibodies	34
1.5.3 Serological Contributions to Pouchitis	36
1.6. GENETICS OF INFLAMMATORY BOWEL DISEASE	40
1.6.1 Summary of Genetic Markers in IBD	40
1.6.2 Gene Identification Techniques in IBD	42
1.6.3 Genome-Wide Association Studies	45
1.6.4 Single Nucleotide Polymorphisms (SNPs)	46
1.6.5 SNP Genotyping	48

1.7.	GENETIC CONTRIBUTION TO INFLAMMATORY BOWEL DISEASE AND POUCHITIS	59
1.7.1.	The Role of the Microbial Environment	50
1.8.	SUMMARY	52
CHAPTER II	AIM, OBJECTIVES, HYPOTHESIS, SIGNIFICANCE AND JUSTIFICATION	54
2.1.	AIM	54
2.2.	OBJECTIVES	54
2.3.	HYPOTHESIS	54
2.4.	SIGNIFICANCE	55
2.5.	JUSTIFICATION	55
CHAPTER III	METHODS	57
3.1.1.	Study Design	57
3.2.1.	Subject Selection and Recruitment	57
3.2.2.	Clinical Data Collection	60
3.3.	PATIENT GROUPING	61
3.3.1.	Group 1 – No/Acute Limited Pouchitis	62
3.3.2.	Group 2 – Chronic Relapsing Pouchitis	63
3.3.3.	Group 3 – CD-like Phenotype	64
3.3.4.	Inclusion and Exclusion Criteria	66
3.4.1.	Study Variables and Definitions	66
3.5.	LABORATORY DETAILS	67
3.5.1.	Biospecimen Collection and Storage	67
3.5.2.	Serum Assays	67
3.5.3.	Serotyping	68
3.5.4.	DNA Genotyping	68
3.6.	STATISTICAL ANALYSIS	69
3.6.1.	Serum Analysis	69
3.6.2.	Genetic Analysis	69
CHAPTER IV	RESULTS	70
4.1.	PATIENT CHARACTERISTICS	70
4.1.1.	Patient Characteristics and Pouchitis Prevalence	70
4.1.2.	Reasons for Exclusions	72

4.2.	CLINICAL RESULTS	73
4.2.1.	Overview of Patient Characteristics and Post-IPAA Grouping	73
4.2.2.	Extent of Disease Pre-Colectomy	79
4.2.3.	Association between Smoking Behaviour and CD-like phenotype Group	80
4.2.4.	Extra-intestinal Manifestations	81
4.2.5.	IPAA Surgery: Early and Late Post-operative Complications	82
4.2.6.	Functional Outcome Post-IPAA and Medications	84
4.3.	SEROLOGICAL RESULTS	86
4.3.1.	Multivariate Analysis	87
4.4.	GENETIC RESULTS	92
4.4.1.	Three Level Outcome – Group 1 vs. Group 2 vs. Group 3	92
4.4.2.	Two Level Outcome – Group 1/Group 2 vs. Group 3	93
4.4.3.	Two Level Outcome – Group 1 vs. Group 2/ Group 3	94
CHAPTER V	DISCUSSION	96
5.1.	DISCUSSION – CLINICAL	96
5.1.1.	Extra-intestinal Manifestations	99
5.1.2.	Post-operative Medications and Complications	101
5.1.3.	Smoking Behaviour	102
5.2.	DISCUSSION – SEROLOGICAL	105
5.3.	DISCUSSION – GENETICS	111
5.4.	LIMITATIONS OF THE STUDY	116
5.5.	CONCLUSIONS	119
5.6.	FUTURE DIRECTIONS	121
5.6.1.	The Microbial Environment	122
CHAPTER VI	REFERENCES	124
CHAPTER VII	APPENDIX	147
7.1.	Letter of Participation for Patients in MSH Surgical Database	147
7.2.	Questionnaire	153

LIST OF TABLES

CHAPTER I

Table I – 1	IBD Linkage Regions	44
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CHAPTER III

Table III – 1	Breakdown of Patients in Databases and Request for Study Participation Sent to Following Pouch Patients	59
Table III – 2	Diagnosis, Classification and Treatment	66
Table III – 3	Classification of Patient Population	66
Table III – 4	Study Variables and Definitions	67

CHAPTER IV

Table IV – 1	Patients Excluded from Study	73
Table IV – 2	Demographic Characteristics of Patients by Post-IPAA Group	77
Table IV – 3	Extent of Disease Pre-Colectomy of Patients by Post-IPAA Group	80
Table IV – 4	Association between Smoking Behaviour and Post-IPAA Group	81
Table IV – 5	Surgical Characteristics and Post-operative Complications	83
Table IV – 6	Functional Outcome and Medications of Groups Post-IPAA and Patient Cohort	85
Table IV – 7	Association between Serological Outcome and Post-IPAA Classification and Patient Cohort	86
Table IV – 8	Summary of Chromosome, Gene/Region, SNP and Association to IBD Analyzed	89
Table IV – 9	Genotypes, P-values and Odds Ratio of the CD14 Gene Polymorphism in Subgroups of Patients	90
Table IV – 10	Minor Allele Frequency of the CD14 Gene Polymorphism in Subgroups of Patients	90
Table IV – 11	Genotypes, P-values and Odds Ratios of the ECM1 and CD14 Gene Polymorphisms in Subgroups of Patients	91
Table IV – 12	Minor Allele Frequency of the ECM1 and CD14 Gene Polymorphisms in Subgroups of Patients	91
Table IV – 13	Genotypes, P-value and Odds Ratio of the PTPN2 Gene Polymorphism in Subgroups of Patients	92
Table IV – 14	Minor Allele Frequency of the PTPN2 Gene Polymorphism in Subgroups of Patients	92

LIST OF FIGURES

CHAPTER I

Figure I – 1	Schematic Depiction of the Large Intestine	10
Figure I – 2	Schematic Depiction of J Shaped Pouch With Closed Stoma After IPAA	18
Figure I – 3	Classification of Diseases of IPAA in Patients with UC	19

CHAPTER III

Figure III – 1	Study Recruitment Diagram	60
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CHAPTER IV

Figure IV – 1	Patient Recruitment and Final Study Inclusion	72
Figure IV – 2	Classification of 355 IPAA Patients According to Occurrence of Post-Surgical Outcome During Follow-up	74
Figure IV – 3	Classification of 355 IPAA Patients According to Racial Distribution	75
Figure IV – 4	Histogram Depicting Modest Association between Post-IPAA Grouping and Race	76
Figure IV – 5	Histogram of Serum Collection Date From STC and Duration of Years with Pelvic Pouch between Groups	79
Figure IV – 6	Pre-Colectomy Perianal Disease and Backwash Ileitis Prevalence between Post-IPAA Groups	80
Figure IV – 7	Histogram of Smoking Behaviour and Post-IPAA Group	81
Figure IV – 8	Prevalence of Axial Arthritis/Ankylosing Spondylitis between Groups	82
Figure IV – 9	Post-operative Complications between Groups	83
Figure IV – 10	Prevalence of the Anti-Glycan Antibodies and Post-IPAA Groups	87
Figure IV – 11	Prevalence of the Anti-Glycan in Each Post-IPAA Group	87
Figure IV – 12	Relationship between Genotype and Disease Phenotype	110

LIST OF ABBREVIATIONS

ABCB1	ATP-Binding Cassette, subfamily B (MDR/TAP), member 1
ACCA	Anti-Chitobioside Carbohydrate
AD	Autoimmune Disorders
ATG16L1	Autophagy Related 16-Like 1
ALCA	Anti-Laminaribioside Carbohydrate
AMCA	Anti-Mannobioside Carbohydrate
ASCA	Anti- <i>Saccharomyces cerevisiae</i> Antibody
Anti-C	Anti-Chitin
Anti-CBir1	Antibody to <i>Clostridium</i> -related flagellin
Anti-I2	Antibody to a <i>Pseudomonas fluorescens</i> -related peptide
Anti-L	Anti-Laminarin
Anti-OmpC	Antibody to Outer Membrane Porin C
BTNL2-HLA-DQB1	Butyrophilin-like 2 to Histocompatibility Complex, class II, DQ Beta 1
CARD15	Caspase-Activation Recruitment Domain 15
CCNY	Cyclin Y
CD	Crohn's Disease
DZ	Dizygotic
ECM1	Extracellular Matrix Protein 1
EIMS	Extra-intestinal Manifestations
FAP	Familial Adenomatous Polyposis
GWA	Genome Wide Association
HERC2	Hect domain and RLD2
IBD5	Inflammatory Bowel Disease 5
IBD	Inflammatory Bowel Disease
IBDU	Inflammatory Bowel Disease – Unclassified
IC	Indeterminate Colitis
IL-1RA	Interleukin 1 Receptor Antagonist
IL12B	Interleukin 12B
IL23R	Interleukin 23 Receptor

IPAA	Ileal Pouch-Anal Anastomosis
IRGM	Immunity Related GTPase Family M
J-IPAA	J structured IPAA (see above)
MAF	Minor Allele Frequency
MST1	Macrophage Stimulating 1
MZ	Monozygotic
MSH	Mount Sinai Hospital
NKX2-3	NK2 Transcription Factor Related, locus 3
NOD	Nucleotide-binding Oligomerization Domain containing 2
NSAID	Non-steroidal anti-inflammatory drug
OCTN	Organic Cation Transporter
pANCA	Perinuclear anti-neutrophil cytoplasmic antibodies
PDAI	Pouchitis Disease Activity Index
PLA2G2E	Part of Phospholipase A2 Group IIE
PRRs	Pattern recognition receptors
PSC	Primary Sclerosing Cholangitis
PTPN2	Protein Tyrosine Phosphatase Non-Receptor, type 2
PTPRS	Protein Tyrosine Phosphatase, Receptor type, S
RPC	Restorative Proctocolectomy
SNP	Single Nucleotide Polymorphism
STC	Subtotal colectomy
TCPTP	T cell Protein Tyrosine Phosphatase
TLR4	Toll-like Receptor 4
TNF	Tumor necrosis factor
TNFSF15	Tumor Necrosis Factor (ligand) Superfamily member 15
UC	Ulcerative Colitis

CHAPTER I

INTRODUCTION AND BACKGROUND

1.1. INTRODUCTION

Inflammatory bowel disease (IBD) is a spectrum of disease characterized by a heterogeneous family of multigenic inflammatory disorders where clinical phenotype is determined by the complex interplay of genetic and environmental factors.¹ IBD is comprised of two major diagnostic subtypes: Crohn's disease (CD), and ulcerative colitis (UC) which have similar patterns of injury despite their distinct immunological and clinical differences.² Additional, types of IBD include inflammatory bowel disease of the colon-unclassified (IBDU) also known as indeterminate colitis. UC patients manifest a continuous pattern of disease localized to the colon whereas CD patients have patchy, often transmural inflammation, that appears anywhere along the digestive tract (mouth to anus). The chronic inflammatory bowel diseases represent a group of diseases with both common and unique characteristics³ and are a significant cause of morbidity in the developed world. Each entity is characterized by intestinal inflammation that affects different locations throughout the gastrointestinal (GI) tract and demonstrates a range of patterns and severity.⁴⁻⁶ In particular, it appears that the location of intestinal inflammation may be a genetically mediated trait as demonstrated by the association of NOD2 variants with ileal inflammation in CD.¹ In addition to this observation, it is known that expression of some of the IBD susceptibility genes (e.g. IL23R) is found throughout the gastrointestinal tract raising the question of why inflammation occurs only in particular areas.⁷ Unlike other forms of intestinal inflammation in which an underlying etiological agent or factor is identifiable, the cause or causes of IBD remains unknown.

IBD has a profound impact in both adult as well as pediatric populations and is a common cause of gastrointestinal morbidity in these groups.⁸ Growth, pubertal development, education, employment potential, and quality of life all suffer as a result of these chronic illnesses.⁸ Although the pathophysiological basis of IBD is largely unknown, significant heterogeneity in UC and CD suggests that distinct phenotypic subgroups exist based on features

such as behaviour, location, natural history and response to drugs.¹ Approximately, one third of UC patients will eventually require surgery due to medically-refractory disease and dysplasia.⁹ Patients with UC and familial adenomatous polyposis (FAP) patients are ideal candidates for surgical treatment due to location of disease in the colon.¹⁰ The surgical procedure of choice for this group of patients is restorative proctocolectomy (RPC) with ilea pouch-anal anastomosis (IPAA), which involves removal of the diseased colonic mucosa. The most frequent complication post-operatively is onset of nonspecific inflammation of the ileal reservoir, referred to as pouchitis. UC patients with a pouch are an important group to study because the development of disease in the ileal reservoir after surgery serves as a human model of IBD initiation and progression.¹¹ Identifying specific biomarkers which are associated with pouchitis patients may shed light on variants associated with IBD.¹² In addition, comparing marker profiles between UC/IPAA with pouchitis, UC/IPAA patients with a CD-like phenotype of the pouch, IBD, FAP and healthy individuals may provide further insight into the inflammatory pathways implicated in disease. The majority of FAP patients undergo IPAA following RPC and serve as an interesting control cohort because, despite the same post-surgical anatomy, these patients show a very low incidence of pouchitis (3-14%) compared to their IBD-affected counterparts (7-59%).^{11, 13} Surgery is usually contraindicated in patients with Crohn's colitis before or during colectomy due to poor results observed retrospectively.¹⁴ Nevertheless, *de novo* CD of the pouch can develop post-IPAA which was initially performed in patients with a diagnosis of UC or indeterminate colitis (IC) pre-operatively. The true incidence of CD of the pouch in patients who initially undergo surgery for UC is not known, with reported cumulative frequencies ranging from 2.7%-13%.¹⁵

Overall, inflammatory disorders of the pouch including pouchitis, CD of the pouch and cuffitis, greatly affect outcomes and patients' health-related quality-of-life.¹⁵ Discovering marker profiles linked with UC pouchitis patients is a precursor to unraveling the etiologic mechanisms underlying IBD pathogenesis and has significant clinical implications.¹⁶ In the future, it is likely

that genetic markers will be implemented in an integrated molecular diagnostic and prognostic approach to managing patients – this multi-faceted approach will help to improve clinical management as well as to predict susceptibility to IBD, in general, and phenotypic subsets, such as pouchitis, in particular.^{17, 18}

The concept of an integrated classification system involving clinical, serological and genetic parameters is strongly advocated due to the discovery of a series of genetic and serological markers associated with disease susceptibility and phenotype in IBD.¹⁹ Currently, the classification of IBD is based largely on clinical parameters, whereas molecular and serological markers largely remain in the research arena.¹⁹ The introduction of a widely acceptable clinical sub-classification would allow detailed correlations among serotype, genotype and clinical phenotype to be examined and confirmed in independent groups of patients and provide a vital foundation for future work.¹⁹ Screening tests for IBD would be more accurate and non-invasive. Functional genomics can be used to assess genetic risk associated with postoperative outcome, permitting better prognostication and targeting of therapy (i.e. prophylactic therapy),²⁰ and reducing morbidity and mortality of genetically susceptible patients who undergo surgery.¹¹ Overall, genetic analysis of disease has become an important biomedical research goal.¹² To date, the single greatest known risk factor for IBD remains a positive family history.²¹ In addition, epidemiological data from twin and familial studies highlights the key role that genetic factors play in the onset and course of IBD.

Together, the ileal pouch and pouchitis represent a unique *in vivo* opportunity to study mucosal adaptation and inflammation.²² Recently, molecular data relating to pouchitis has significantly improved and these data have provided important insight into intracellular and extracellular events that underpin mucosal adaptation and inflammation. Advances in classification, risk factor evaluation and prevention have meant that a better understanding of serology as well as its relationship to our current understanding of pouchitis, is both timely and warranted.²² Therefore, this study will aim to evaluate the contribution of several biologically

relevant candidate genes and serological markers to phenotypic manifestations of IBD. Specifically, the study will analyze the incidence and features of pouchitis and CD-like phenotype in a large cohort of UC patients, and determine if a significant association exists between specific biomarkers and susceptibility to chronic/relapsing pouchitis or CD-like phenotype of the pouch.

1.2. BACKGROUND

Even though it is unclear when the entity of IBD first appeared, there are descriptions of patients demonstrating features consistent with UC from as early as 1859.²³ By 1932, Dr. Burrill Crohn and colleagues coined the term “terminal ileitis” or “regional ileitis” which later became known as Crohn’s disease.²⁴ Currently, the causes of IBD remain unknown but strong epidemiological data and clinical experience suggests that the etiologic basis of complex human disease is a result of the interplay between genetic and environmental factors.^{20, 25} In addition to the genetic and immunologic factors which have proven to be major determinants of disease, environmental factors and impaired defense mechanisms have emerged as significantly important players in disease onset and progression.^{12, 26, 27} In healthy individuals, the coordinated action of the innate and the acquired immune systems enable epithelial defense lines to withstand microbial insults. Hence, an individual with a mechanistic defect may develop an overgrowth of normal colonic flora, with increased aerobic bacteria and appearance of atypical bacteria and fungi.²⁸ Correspondingly, the current hypothesis is that intestinal inflammation is triggered by exposure to an environmental/microbial insult in the setting of an inherited genetic susceptibility; subsequently, this results in perpetuation of a chronic inflammatory process due to a failure to down regulate the inflammatory response.¹ Hence, an important causative role has been assigned to dysregulation of the innate immune system in the enteric mucosa associated with the abnormal inflammatory response to intestinal microflora.²⁹

At present, the clinical application of genetic testing is limited; however, genetic research has improved our understanding of the clinical heterogeneity and the complex interactions

between genetic and environmental risk factors in IBD.¹⁸ Genetic susceptibility for IBD pathogenesis has been supported by family studies,^{25, 30, 31} twin studies³²⁻³⁴ and ethnic differences.³⁵ Recent advances in scientific know-how, involving the integration of human genetics, high-throughput technology, functional genomics, molecular biology and computational methods, has allowed for better understanding of the contribution of genetic factors to disease susceptibility, pathogenesis and behaviour.^{20, 21, 36} As well, it has been suggested that serologic markers may be used as an adjunctive non-invasive test for supporting a diagnosis of IBD, and for helping to stratify disease phenotype in patients with UC.^{37, 38} Today, there exists conflicting data on the utility of serological markers in identifying phenotypic subsets of IBD with accuracy and in differentiating between patients with UC and isolated colonic CD.³⁹⁻⁴² There is concern regarding the specificity, sensitivity and stability of markers over time which has impeded the integration of these biomarkers into widespread clinical use; this highlights the need for novel IBD biomarkers and for further investigation of the existing markers in large, independent populations and in prospective study designs.⁴²

Immunogenotyping and immunophenotyping have important roles in the study of IBD and pouchitis, by achieving two goals: firstly, to investigate the etiology and pathogenesis of the disease, and secondly, to evaluate diagnostic and prognostic factors.¹⁰ Currently, results from immunogenotypic and immunophenotypic studies are not consistent. Factors contributing to difficulties in conducting and interpreting bench and clinical studies in pouchitis include the lack of unified diagnostic criteria and standard classification.¹⁰

1.2.1. Epidemiology

The accumulation of epidemiological evidence for a genetic component in IBD has led to a variety of approaches aimed at the identification of susceptibility genes.⁴³ Studies which demonstrate familial aggregation and ethnic variation imply a significant intrinsic (i.e., genetic) factor, while, marked differences in incidence and prevalence in distinct populations under various stimuli underscore extrinsic (i.e. environmental) factors as contributory variables.²¹

Incidence and Prevalence of IBD

The incidence rate, which is the number of newly diagnosed cases within a specified time period, for UC appears to be relatively stable and ranges from 1 to 10 per 100,000 per year.^{44, 45} Greater incidence rates have been reported in Europe and Scandinavia, ranging from 12 to 15 per 100,000 per year, which can be partly explained by differing diagnostic criteria applied between countries. For example, there are significantly fewer studies from North America which include UC proctitis for a definitive diagnosis compared to their European counterparts; this may somewhat explain the discrepancy in incidence rates found. The worldwide incidence rates for CD are lower than that of UC, ranging from 1 to 7 per 100,000 per year; however, the occurrence of CD has increased over the last several decades. Similar to UC, higher rates for CD appear to be found in northern regions such as in England, Sweden and the Netherlands.^{32, 46, 47} The highest incidence rate of CD ever reported at 14.6 per 100,000 per year, is from a population-based study from Manitoba, Canada.⁴⁵ Regardless of differences in study characteristics, there appears to be a north-south gradient as well as geographic “hotspots” where high incidence rates of IBD are found.

Prevalence rates, which are the total number of cases of disease within in a population at a specific time, are more difficult to determine, varying from 35 to 170 per 100,000 for UC, and from 3 to 200 per 100,000 for CD. UC and CD patterns show similar geographic and ethnic distribution⁴⁸ with low rates reported amongst Asians and Middle Eastern Arabs and higher rates found in Scandinavia, Northern USA, Manitoba, and in Middle Eastern Jews.^{45, 49-51} The impact

of environmental variables in initiating IBD has gained support based on changing geographic and socioeconomic trends, particularly demonstrated in Asian populations where IBD was considered uncommon.⁵²

Many risk factors have also been shown to affect susceptibility to IBD in epidemiological studies; however, data are often conflicting and inconclusive.⁵³ The hypothesis that different etiologic agents contribute to disease is consistent with the bimodal distribution of age of onset for both UC and CD with the initial peak between the ages of 15 and 25 and a second peak between ages 50 and 80.⁵⁴ Polito *et al.* contend that perhaps the early age of onset cases represent those with greater genetic influence whereas the late onset cases may be influenced more by environmental factors.⁵⁵

Environmental Influences and IBD

Geographical variation in the incidence and prevalence of IBD suggests the existence of a north-south gradient, such that these rates decrease as one moves towards the geographic equator.⁵⁶ Regions with temperate climates (e.g., Canada, Scandinavia, Northern Europe, Australia) seem to have higher incidence and prevalence rates of IBD compared to tropical areas (Mediterranean basin, Southern Europe, Middle East, Africa).⁵⁶ Raw data shows a four to five times greater incidence of UC and CD in northern areas compared to southern areas globally;^{30, 46, 54, 57-64} however, direct comparisons can be misleading because IBD is less prevalent in the developing world.⁶⁵ In addition, higher incidences of both CD and UC have been identified in urban populations compared to rural populations in North American and European areas.^{54, 58, 65-68} Western countries have seen a rise in IBD, which is more marked in urban areas, underscoring the extrinsic, non-genetic factors which influence disease heterogeneity.⁶⁹

Ethnicity and Race

Data found on ethnicity and race has shed light on various possible explanations of IBD etiology and confirms the theory that relevant risk factors are both genetic and environmental. In terms of ethnicity and the risk for IBD, one of the most studied groups is that of the Jewish

population. There is a 2- to 4- fold increase in both incidence and prevalence rates in Jews compared to non-Jews in most reports – this effect seems to be independent of country of birth or of residence.^{70, 71} Furthermore, the higher rate of IBD among Jews versus non-Jews in the same geographic region highlights the notion that certain individuals have a genetic predisposition for disease. On the other hand, environmental factors appear to modify an individual's inherited predisposition for disease in meaningful ways; this is demonstrated by the fact that rates of IBD among Jews vary significantly from country to country.⁷² Furthermore, the incidence and prevalence rates of IBD which have been reported to be higher in North American, Northern European and Scandinavian Caucasians as compared to other racial groups, lends support to environmental factors initiating disease. However, it is difficult to assess the true prevalence of IBD in places like South America, Africa and Asia due to the scarcity of data and high rates of enteric infection.⁷³

Potential Risk Factors

A variety of potential risk factors which include dietary factors,⁷⁴ infectious agents,^{54, 75, 76} smoking,^{77, 78} oral contraceptive pills, non-steroidal anti-inflammatory drugs (NSAIDs) and vaccinations have been linked to IBD by numerous epidemiological studies; however, these studies have seldom been consistently replicated. The strongest and most consistent data on risk factors associated with IBD has been reported on smoking behaviour; interestingly, it is paradoxical in its effects on CD and UC. Nonsmokers appear to have a 3 times greater risk of having UC, whereas smokers appear to have a 2 to 3 times greater risk of having CD.⁷⁸ In addition, current, and to a smaller extent, former smokers have been consistently shown to have an increased risk for CD.^{77, 78}

1.2.2. Description of Inflammatory Bowel Disease

CD and UC are perceived as distinct conditions, albeit part of a clinical spectrum which includes intermediate variants, such as IBDU.¹² It is critical to have a proper identification and classification for IBD patients so as to allow for appropriate clinical assessment and care, as well as to allow for proper execution of research studies. The diagnostic criteria associated with CD and UC are based on endoscopic, radiological, and histological examinations. At the time of diagnosis, an important differential requires discriminating chronic IBD from infectious etiologies, where most gastrointestinal pathogens are acute and self-limited.⁷⁹ IBD displays chronic inflammation, confirmed by history, and involves the presence of symptoms for several weeks as well as evidence of pathologic changes, including the presence of intestinal crypt branching.⁷⁹ Differential diagnosis is difficult in a minority of cases due to overlapping symptomatology and nonclassic histologic and radiographic findings. It is medically important to differentiate UC and CD as these diseases have different prognoses and treatments.

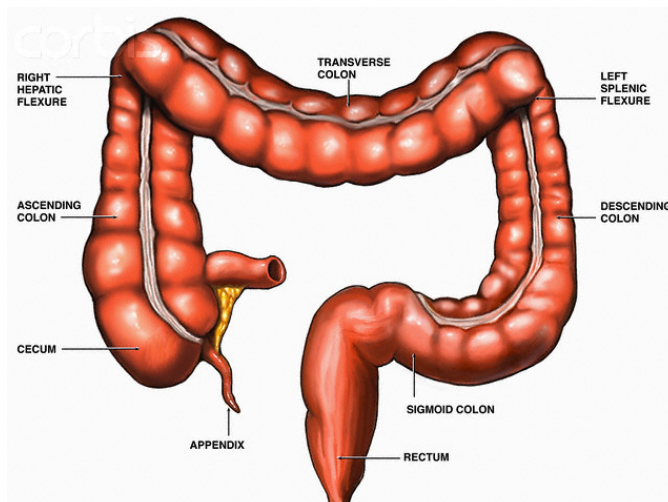
1.2.3. Ulcerative Colitis and Crohn's Disease

UC and CD are two distinct but often overlapping entities that are the result of interrelated genetic and environmental factors.¹² The interrelationship between UC and CD remains unknown and is under intensive investigation. In general, IBD is characterized by chronic, relapsing and unremitting intestinal inflammation, in addition to a number of extraintestinal manifestations (EIMs) affecting the eyes, joints and skin.²⁰ The hallmark of these disorders involve chronic, typically intermittent, intestinal inflammation resulting in diarrhea, abdominal pain, rectal bleeding, and in children, growth retardation.⁷⁹

Ulcerative colitis is characterized by chronic, noninfectious inflammation of the colorectal mucosa and can include proctitis, left-side colitis (or distal colitis), and extensive colitis (or pancolitis), involving the transverse colon to the cecum. The large intestine measures approximately 1.5 meters in length. The Montreal Classification uses a three-tiered classification

system for UC which is useful in differentiating patients by prognosis and by medical therapy (refer to Figure I – 1). The extent of colorectal inflammation in UC can be defined at an endoscopic, radiologic or histological level.¹⁹ The three subgroups of UC are defined by disease extent and include: ulcerative proctitis (E1) in which involvement is limited to the rectum (i.e. inflammation is distal rectosigmoid junction); left-sided UC (E2), in which involvement is limited to the portion of the colorectum distal to the splenic flexure; and extensive UC (E3), in which involvement extends proximal to the splenic flexure.¹⁹ One drawback of the extent based classification scheme is that disease extent is unstable over time, underscoring the dynamic nature of IBD. It has been recognized for years that the issue of classification of disease behaviour over time bears significant importance with respect to clinical management and may also have an impact on future genetic studies.^{3, 19} There exists continued debate over the importance of the potential bimodal distribution in age of onset of UC. To date, introducing age of onset as a separate subgroup in UC has not been validated.³ Treatment administered to each patient depends on their disease severity. Medical treatments include 5-aminosalicyclates (5-ASA), corticosteroids, immunosuppressive and biological agents. Patients, who have severe disease refractory to medical therapy, may be candidates for proctocolectomy.

Figure I – 1: Schematic Depiction of the Large Intestine



Crohn's disease is characterized by non-continuous, transmural, and sometimes granulomatous inflammation that may affect any part of the gastrointestinal tract, with the potential for systemic and extra-intestinal manifestations. For CD, it has been shown that disease behaviour is dynamic over time and has a tendency to progress from one category to another during prolonged follow-up.^{19, 80} Although the Montreal Classification has not been widely used in clinical practice, researchers often return to it to assist in categorization of patient populations for study purposes. Although this classification scheme creates an artificial distinction between disease subtypes, it is beneficial for its relative ease of use and simplicity.¹⁹ The Montreal Classification of CD considers age of onset (A), disease location (L) and disease behaviour (B) as the predominant phenotypic elements.³ Age of onset is classified into three groups: A1, diagnosis at 16 years or younger, A2, and A3, diagnosis at 17-40 years and >40 years, respectively.³ The major divisions of disease site are ileal (L1), colonic (L2), ileocolonic (L3) and upper GI (L4). Disease behaviour has three subtypes: B1 is non-stricturing, non-penetrating, B2 is stricturing, and B3 is penetrating. A "p" is added to B1-B3 when concomitant perianal disease is present.³ Due to change or progress of disease behaviour over the course of disease, some aspect of time course is required.³ An early age at diagnosis of CD has been shown to be more frequently associated with specific phenotypes and genotypes.³ Treatment relies on key factors such as location and severity of disease, complications and response to previous therapy. Medical treatments include 5-aminosalicylic acids (5-ASA), corticosteroids, immunosuppressives (e.g. azathioprine, methotrexate), and biological agents, such as anti-tumour necrosis factor (TNF) alpha monoclonal antibodies (Remicade).⁸¹ In addition, antibiotics such as ciprofloxacin and metronidazole may be used to treat fistulizing and colonic CD. For CD patients, surgical therapy may also be employed where disease is medically refractory or where complications have developed.

Crohn's disease can usually be distinguished from UC by the distribution, location and depth of involvement of intestinal inflammation.⁷⁹ CD is characterized by patchy transmural inflammation (involving all layers of the bowel wall) affecting any part of the gastrointestinal tract (GI) (skip lesions may occur). In UC, inflammation extends continuously from the rectum to a variable extent in the colon and is confined to the superficial layers of the colonic mucosa and submucosa.²⁰ UC patients generally present with symptoms which include passage of bloody stools or gross blood, rectal urgency and tenesmus; on the other hand, CD patients display systemic manifestations such as fatigue, malaise and weight loss. At physical examination, a diagnosis of CD is strongly supported by fistulous penetration of adjacent structures, and by perianal expression, such as external anal tags, abscess or fistulous openings.

Even though UC and CD share several epidemiological and clinical features, they differ significantly in their course, response to therapy and other clinical aspects. The differences between CD and UC highlight the heterogeneous nature of IBD; however, the similarities between these two subsets of disease make a strong case against classifying IBD into these two independent entities.²⁰

1.2.4. Indeterminate Colitis and Inflammatory Bowel Disease – Unclassified (IBDU)

Despite the clinical categorization of IBD into well known subtypes of CD and UC, many patients do not fit neatly into a particular category. In up to 10% of patients, it can be difficult to distinguish between CD and UC, even after full pathological examination; as a result, a diagnosis of inflammatory bowel disease- unclassified (IBDU) is made.^{8, 20} This is exemplified by the fact that only a small percentage of patients will have pathology revealing the presence of granulomas, which is a hallmark of CD.²⁰ Hence, IBDU is considered a clinical overlap syndrome where colonic inflammation lacks definitive features of CD or UC.⁸

The term indeterminate colitis was coined by Ashley Price in 1978, and this name was based on examination of a surgical specimen which displayed overlapping features of both CD

and UC.⁸²⁻⁸³ Hence, Price suggested that a diagnosis of IC be made following colectomy in patients who displayed features which were not sufficient to decipher between either UC or CD, but were adequate to allow a diagnosis of IBD affecting the colon.⁸² It was believed that the majority of patients who received a temporary diagnosis of IBDU would prove to have either CD or UC during follow-up.^{84, 85} In subsequent years, the concept that IBDU was in fact a distinct disease, instead of a temporary diagnosis, emerged due to the development of an integrated diagnostic system based on clinical features in addition to endoscopy with biopsies.¹⁹ As a result, clinicians have broadened the definition of IBDU over time to include the following parameters: patients with IBD affecting the colon confirmed by clinical and endoscopic evaluation but in whom other clinical parameters and histology do not clearly allow a distinct diagnosis of either UC or CD.³ The Montreal Working Party recommends that the term, indeterminate colitis, be used for those individuals in whom colectomy has been performed and after full examination, pathologists are not able to make a definitive diagnosis of UC or CD.³ On the other hand, the term, IBDU is reserved for patients in whom there is evidence from clinical and endoscopic evaluations for chronic IBD of the colon, devoid of small bowel involvement, and no histological or additional evidence to support either UC or CD.³ Infection must be ruled out before the term IBDU is applied.³ Deciphering between the chronic colitis phenotypes is important because studies have shown that the clinical course and prognosis of patients with IBDU is worse than that of UC. As well, studies have shown that postoperative outcome is worse in IBDU patients because they have a greater risk of developing chronic pouchitis compared to patients with a pre-operative diagnosis of UC.⁸⁶⁻⁹¹ These findings have been replicated in some but not all follow-up studies; consequently, it remains unclear whether IPAA with total colectomy should be recommended in IBDU patients.⁹¹⁻⁹³

Although laboratory testing has become a more objective means to test IBD, results are not always consistent and do not distinguish CD from UC.⁹⁴ More recently, the use of serologic markers has begun to be utilized as a means to differentiate CD from UC, and IBD from other

colitides. A positive test for perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) is found in up to 79% of UC patients compared to as few as 13% of CD patients.⁹⁵ Alternatively, CD is often associated with a positive assay for the anti-*Saccharomyces cerevisiae* antibody (ASCA). When serological testing for pANCA and ASCA are combined, there is reasonable sensitivity and specificity in distinguishing CD from UC. To date, clinical utility of serological testing remains questionable and requires further refinement.⁹⁶⁻⁹⁹

Overall, the fact that a number of IBD-affected individuals appear to manifest an overlap syndrome with features characterized by both CD and UC suggests that at least some susceptibility genes are shared between UC and CD. The current literature supports specific genetic markers associated with disease phenotype. For example, three nucleotide-binding oligomerization domain containing 2/caspase-activation recruitment domain 15 (NOD2/CARD150) mutations that are established genetic risk factors for CD are: Cins1007fs, G908R and R702W. Nonetheless, at present, there is insufficient evidence to recommend applicability of any individual marker in a classification scheme.¹⁹

Collectively, this data suggests that individuals with any type of IBD are vulnerable to intestinal inflammation and the exact location and pattern of inflammation is mediated partly by underlying genetic defect(s) and partly by the microbial environment found in that intestinal location. Further refinement of serological testing may provide insight into IBD classification; for example, IBDU patients may represent a unique clinical entity i.e. seronegative IBD patients.¹⁰⁰

1.2.5. Familial Adenomatous Polyposis (FAP)

Familial adenomatous polyposis (FAP) is an autosomal-dominant, inherited disorder of the adenomatous polyposis coli (APC) gene on chromosome 5 with complete penetrance, characterized by the progressive formation of hundreds, even thousands, of colorectal adenomas.¹⁰¹ Offspring of affected patients have a 50% chance of developing the disease. If left

untreated, most patients will have adenomas develop by age 10, and all affected individuals will eventually develop colorectal carcinoma, often by the age of 40. IPAA eradicates the risk of colorectal cancer in patients with FAP. A proctocolectomy extends survival by approximately 30 years, but this is still 10 years less than for the general population.¹⁰¹ In FAP patients, every epithelial cell of the colon and rectum carries the APC mutation, which could lead to the adenoma-carcinoma sequence; therefore, prophylactic surgery should theoretically remove the entire diseased mucosa.¹⁰² IPAA achieves the goal of removing cancer risk in the colonic mucosa and has become firmly established as the standard operative procedure and method of choice for classic FAP.¹⁰² IPAA can be performed with low mortality (0.5-1%), carries an acceptable risk of non-life-threatening complications (10-25%), and accomplishes good functional results over the long-term, with excellent patient satisfaction (over 95%).^{101, 102}

1.2.6. Etiology and Pathogenesis of IBD

The mechanisms involved in IBD pathogenesis are beginning to be identified; nonetheless, there still exists a considerable lack of knowledge as to what the background variables, triggers and modulators are.⁵³ The etiologic basis of IBD can be divided into two major categories. The first scenario is one in which a specific causative factor such as a microbial infection or a chemical agent, when exposed to the lumen, causes an inappropriate and persistent inflammatory response targeted at the offending agent. The second is that there is an abnormal host response to an ubiquitous agent which may also be microbial or chemical in nature.⁴ This abnormal, overly aggressive immune response may be a result of a disruption in the mucosal barrier (i.e. permeability defect), and/or due to a dysregulated immune response (i.e. an imbalance between pro- and anti-inflammatory molecules or an array of other mechanisms). Additionally, it appears that the location of intestinal inflammation may be a genetically mediated trait which has been supported by recent genetic studies; for example, there seems to be an association between NOD2 variants and ileal inflammation in CD patients.¹ Also, it has been shown that expression

of certain IBD susceptibility genes (e.g. interleukin 23 receptor (IL23R)) is found throughout the gastrointestinal tract, raising the question of why inflammation occurs only in particular areas?⁷ A plausible explanation is that the local endogenous microbial flora interacts with the aberrant gene expression in the same area. The role of endogenous intestinal microflora in initiating or perpetuating IBD has gained attention over the years, supported by observations that the distal ileum and colon are sites with the highest bacterial concentrations in the GI tract¹⁰³ and that the intestinal tract has increased permeability in IBD patients compared to healthy controls. Accordingly, endogenous bacteria have a portal of entry to the enteric immune system through the compromised intestinal barrier.¹⁰⁴ Techniques aimed at altering the bacterial content of the lumen, such as bowel rest, bypass or treatment with antibiotics and probiotics, seem to diminish the inflammatory process in IBD.^{35, 105, 106} In addition to their surface antigens, endogenous intestinal bacteria have a range of substances that may provoke an inflammatory response which include *n*-formylmethionyl-leucylphenylalanine (FMLP),¹⁰⁷ lipopolysaccharide (LPS),¹⁰⁸ and peptidoglycan-polysaccharide (PG-PS).¹⁰⁹

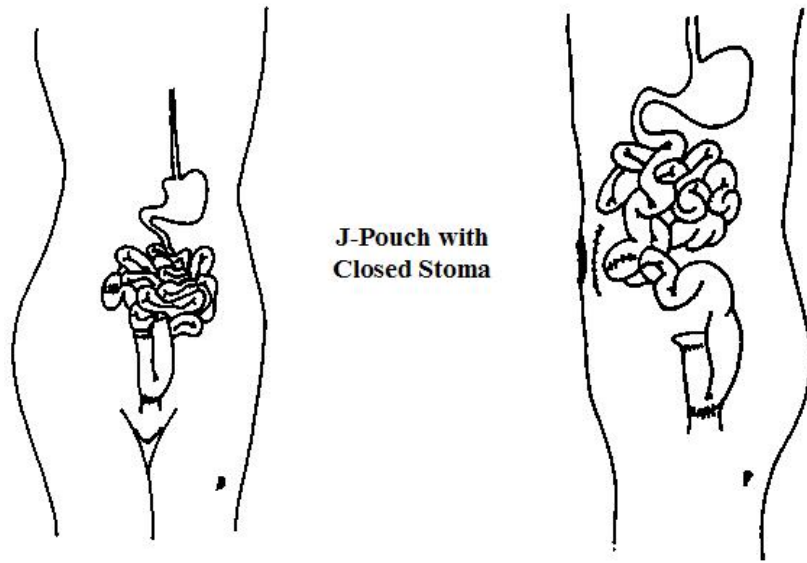
Exploring a range of hypotheses is important because all or none of these possibilities may be operative. Currently, the groundwork for research utilizes the accepted idea that an individual with a susceptible background, who is exposed to a triggering factor will develop disease.⁴ Improving our understanding of this interplay will enable important advances in our understanding of mechanisms of intestinal inflammation.

1.3. SURGERY AND POST-OPERATIVE OUTCOME

1.3.1. Ileal Pouch-Anal Anastomosis (IPAA)

So far, many advances have been made in the medical management of UC patients; however, up to 30% of those affected will eventually require colectomy for medically refractory disease, development of dysplasia or malignancy. By 1982, the pelvic pouch procedure was pioneered, and quickly became the procedure of choice for UC patients requiring colectomy.¹³ IPAA not only provides symptomatic relief but significantly reduces the risk of developing neoplasia in the UC population.¹¹⁰ This procedure involves removal of all diseased large bowel mucosa, construction of an ileal reservoir from the terminal ileum, and anastomosis of the newly formed ileal pouch to the anal canal in a sphincter-sparing manner.¹¹¹ IPAA allows the pouch to function as a reservoir with both gastrointestinal continuity and fecal continence preserved.¹¹² In order to allow time for anastomotic healing, IPAA is usually performed with a temporary loop ileostomy, followed by closure 3-6 months after initial surgery.¹⁰ Today, RPC with IPAA is the most widely accepted form of surgical treatment for both UC and FAP patients. Even though the majority of UC patients with RPC are candidates for IPAA, the main contraindications for re-anastomosis include absent or decreased sphincter muscle tone, pelvic floor dysfunction, and a pre-IPAA diagnosis of CD.¹¹³ In many UC patients, IPAA improves quality-of-life and preserves transanal passage of stool by obviating the need for an external ileostomy.¹³ Although the UC patient with IPAA is supposed to be rendered free of disease, adverse outcomes may develop postoperatively, including pouchitis or ileitis, CD of the pouch, irritable pouch syndrome (IPS) and cuffitis, greatly affecting outcome and patient health-related quality-of-life.¹⁰ For this reason, the IPAA patient represents an ideal human model to study this poorly characterized phenomenon, where the acquisition of *de novo* ileal inflammation occurs after definitive treatment for isolated colonic inflammation.

Figure I – 2 : Schematic Depiction of J Shaped Pouch With Closed Stoma After IPAA

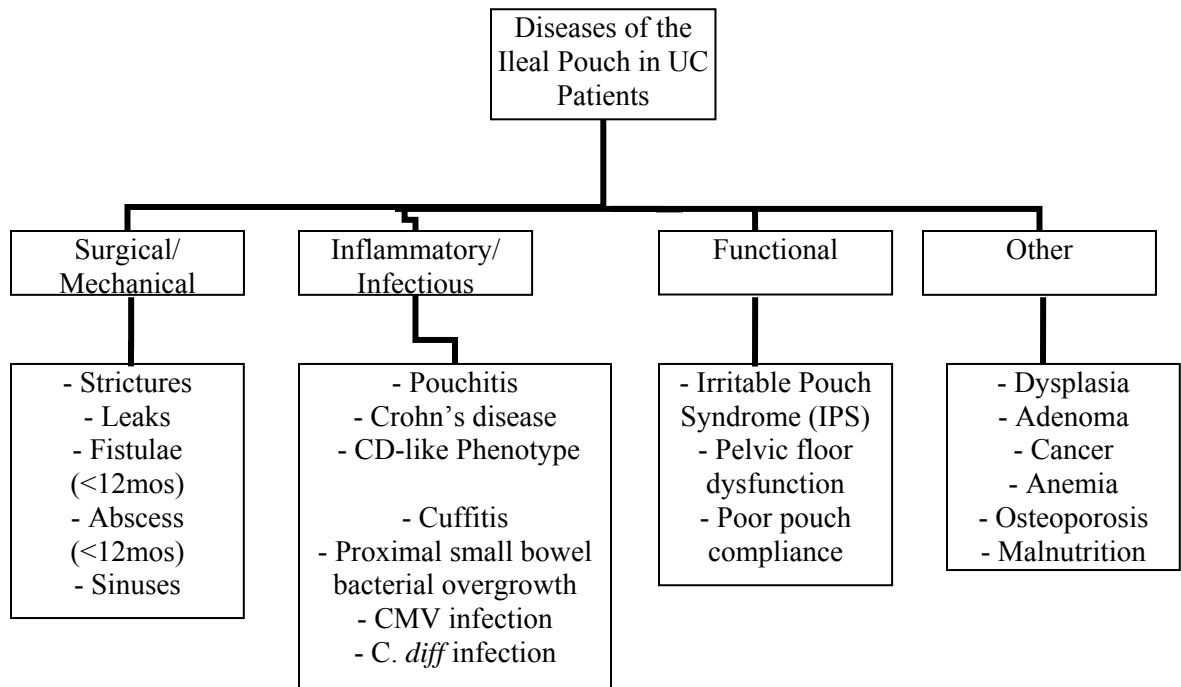


1.3.2. Diseases of the Pouch

The benefits of IPAA are partially offset by a high morbidity rate.^{22, 114} Undoubtedly, IPAA significantly improves quality-of-life for UC patients; however, it is reported that up to 59% of patients will develop an inflammatory disease of the ileal pouch. There are various manifestations of disease post-IPAA which can be classified into: surgery-related/mechanical complications; inflammatory or infectious disorders; functional disorders; dysplasia or neoplasia; and systemic or metabolic disorders (refer to Figure I -2).¹¹³ Often times patients present with a combination of problems, while in other cases, the disease status changes over time; as a result, diagnosis and management of these diseases is a challenge. While the etiology and pathogenesis of these conditions are not entirely understood, most evidence points toward an abnormal mucosal immune response (innate and adaptive) as well as altered microflora in the pouch which leads to acute and/or chronic inflammation.¹¹⁵⁻¹¹⁷ In order to determine frequency of pouch dysfunction, Evans *et al.* looked at the incidence of pouch dysfunction syndromes in UC or IC patients who had a J shaped pouch with IPAA (J-IPAA). Their results showed that a significant number of patients experienced some form of pouch dysfunction, including pouchitis, the most

common long-term adverse sequela after IPAA, CD of the pouch, IPS and cuffitis, which was the least frequent. Most pouchitis was chronic and required treatment with continuous antibiotic or immunosuppressive agents. The authors reported that despite the fairly regular occurrence of pouch dysfunction syndromes, they are for the most part treatable.^{13, 15, 114, 118}

Figure I – 3: Classification of Diseases of IPAA in Patients with UC¹¹³



Epidemiology studies have revealed that 5% to 10% of patients undergoing IPAA with a diagnosis of UC at the time of surgery are subsequently diagnosed with CD post-IPAA. In a recent study conducted by Melmed *et al.*, they prospectively examined predictors of CD post-IPAA by assaying serum for the IBD-associated antibodies ASCA (IgG and IgA), pANCA, anti-outer membrane porin C (OmpC), and anti-CBir1 flagellin. Melmed and colleagues defined CD of the pouch based on confirmed inflammation in the small bowel mucosa proximal to the ileal pouch and/or formation of a pouch fistula or other perianal complication more than 3 months

after ileostomy closure. They reported that UC patients who had a family history of CD or who are ASCA-IgA seropositive before surgery were more likely to be diagnosed with CD post-IPAA.¹¹⁹ These observations underscore the variability in clinical and endoscopic characteristics of inflammation arising after IPAA and the different ways phenotype can be categorized. First, there are a group of individuals who do not develop inflammation in the pouch, which eventually takes on the appearance of colonic mucosa; these individuals have a normal looking pouch on endoscopy. Secondly, there is a cohort of individuals who develop inflammation in the pouch, which looks similar to that seen in UC. Third, a small cohort develops an inflammatory process in the afferent ileal limb (pre-pouch ileum) with or without concurrent inflammation in the pouch. These patients have a pouch that endoscopically has an appearance similar to CD, namely discrete serpiginous ulceration, without the use of non-steroidal anti-inflammatory drugs (NSAIDs). While this latter group is thought to have been misdiagnosed pre-IPAA, critical review of the colectomy specimen frequently confirms the initial diagnosis of UC or occasionally IBDU. This CD-like phenotype has been reported to be associated with UC patients who have a family history of CD and/or who are ASCA seropositive prior to surgery.¹²⁰ Overall, we hypothesize that IPAA inflammation is characterized by various clinical phenotypes which are associated with different serological and genetic events and elucidation of these events will improve our understanding of why patients with IBD develop distinct patterns of intestinal inflammation.

1.4. DESCRIPTION OF POUCH OUTCOME

1.4.1. History and Definition of Pouchitis

Pouchitis is defined as nonspecific, acute inflammatory process in the ileal reservoir and is the most common long-term adverse sequela after pouch surgery for UC.^{13, 15, 110, 114, 121} In 1977, pouchitis was initially defined as inflammation of the Kock continent ileal reservoir. In 1978, this definition was changed to involve the IPAA, which subsequently gained widespread international acceptance.¹²² Although pouchitis remains somewhat ill-defined, it is an inflammatory process that develops *de novo* in the small intestine in spite of previous surgically treated UC. FAP patients who undergo IPAA rarely experience pouchitis, and it has been suggested that pouchitis may represent a disease process similar to UC.^{123, 124} Pouchitis remains the most common complication associated with IPAA, and the morbidity of this disease has prompted investigation that thus far has failed to identify an etiology.²² Hence, this clinical phenomenon makes IPAA an ideal human model to study new onset ileal inflammation in individuals thought to be rendered free of disease with surgical removal of the colon.

There is variability in the literature on pouchitis frequency largely due to the fact that it remains a poorly described entity. Pouchitis has been reported in 3-14% of patients with FAP and 7-59% of those with UC.¹³ The link between genetic components and pouchitis pathogenesis is illustrated by the high incidence of pouch inflammation in UC patients, up to 59% compared to a low occurrence in FAP patients. Hence, any variation in the development of inflammation between UC and FAP patients must be a product of inherited susceptibility factors or differences in microbial flora mediated by differences in host genetics.

1.4.2. Pathogenesis and Etiology of Pouchitis

While the overall incidence of pouchitis is low, extensive research continues at both clinical and experimental levels in attempts to unravel its etiology.²² Though the causes of pouchitis remain largely unknown, the immunopathogenesis of pouchitis appears to involve the complex interplay of genetic, immune, microbial and toxic mediators.⁹⁴ Accordingly, Shen and colleagues hypothesize that the altered immunity in a genetically predisposed host together with the irregular interaction between increased microbial load in the ileal pouch, are involved in the etiology and pathogenesis of pouchitis.¹⁰ Solid evidence to support this theory is that pouchitis develops subsequent to closure of the protective ileostomy which results in re-diversion of the fecal stream towards the pouch. In this regard, as time passes the ileum will undergo a degree of adaptation, with recreation of the physiological conditions found in the native colon, i.e. colonization. It has been shown that the presence of feces within the ileal pouch is associated with mucosal adaptive changes such as chronic inflammation and villous blunting.¹²⁵ These mucosal changes are accompanied by increases in total anaerobe counts and colonization by additional anaerobe species.¹²⁶ An important alteration involves an overgrowth of commensal bacteria with an anaerobic composition comparable to the colon.¹¹⁴ Accordingly, the prevailing theory holds that pouchitis is triggered by an overgrowth of commensal bacteria.^{114, 117, 126} Thus, bacterial involvement, including fecal stasis and bacterial overgrowth, is assumed to be a key player in the pathogenesis of pouchitis.¹²⁷ Moreover, the bacterial composition in the ileal pouch seems to be affected by whether a UC patient has a genetic predisposition to pouchitis. Although levels of anaerobes (*Bifidobacterium*, *Lactobacilli*, *Bacteroides*, and *C. perfringens*), *Enterococci* and *Coliform* in pouches in UC patients are similar to that of FAP patients, sulfate-producing bacteria are almost exclusively detected in pouches in UC patients.¹²⁶ There is a decrease in the total number of anaerobes, with an increase in *C. perfringens* and hemolytic strains of *E. coli*, and a decrease in total aerobes.¹¹⁵

Antibiotic therapy decreases both total aerobic and anaerobic bacterial concentrations, in addition to selectively inhibiting *Bacteroides*, *Bactobacillus*, *Enterococci*, *Bifidobacterium*; subsequently, there is resolution of pouch inflammation.¹¹⁶ The two main antibiotics used to treat pouchitis include ciprofloxacin and metronidazole. Ciprofloxacin therapy results in the eradication of *C. perfringens* and all *Coliforms*, including hemolytic strains of *E. coli*, whereas metronidazole therapy reduces anaerobic flora.¹¹⁵ A causative bacterial species remains to be identified.

Aisenberg *et al.* imply that altered bacterial flora is indeed important, demonstrated by the response of pouchitis to oral antibiotics,¹²⁸ the “microbial imbalance” in the pouches of these patients,¹²⁹ and the prevention of relapse by the administration of oral probiotics.¹¹⁶ The majority of patients with pouchitis respond to a 2-week course of treatment with a single antibiotic; however, some patients may have refractory disease, requiring long-term anti-inflammatory agents or immunomodulators.¹¹³ Pouchitis is a heterogeneous disease, and likely represents a disease spectrum ranging from an acute, antibiotic-responsive entity to a chronic, antibiotic-refractory disorder. Hence, Shen and colleagues point out that while acute antibiotic-responsive pouchitis likely stems from an infectious etiology, chronic antibiotic-refractory pouchitis seems to result from a chronic inflammatory mechanism, similar to IBD.¹⁰ Possibly, the latter pouchitis group and IBD patients share common pathogenesis pathways.¹¹³

A recent study conducted by Spehlmann *et al.* compared the bacterial microbiota in the inflamed and non-inflamed pouch mucosa to the non-inflamed afferent loop mucosa. There was a significantly lower microbial diversity in patients with pouchitis compared to non-pouchitis controls.¹³⁰ The bacterial microbiota seems to remain stable along the digestive tract, confirmed by the fact that the diversity of the afferent loop mucosa did not significantly differ from that of the pouch mucosa.¹³⁰ In addition, clone libraries revealed that non-inflamed status was associated with higher diversity of bacterial species, including members of the normal, anaerobic enteric microbiota.¹³⁰

Other factors, such as immune dysregulation, may also contribute to the etiology and pathogenesis of the disease process, particularly in patients who develop chronic antibiotic-refractory pouchitis or those with a CD-like phenotype of the pouch. For instance, immune-mediated diseases or autoimmune disorders (AD) have been found to be more prevalent in IBD patients compared to individuals without IBD, underscoring the possibility that AD and IBD share etiologic factors.^{131, 132} As a result, immune-mediated diseases may be associated with chronic inflammatory diseases such as pouchitis or CD of the pouch. Moreover, the extraintestinal manifestations (EIMs) associated with UC may coincide with pouchitis, suggestive of similar immunologic effector mechanisms which are active at both systemic and local levels.²²

In summary, the etiopathophysiology of pouchitis has yet to be clearly elucidated, but appears to involve the complex interplay of genetic, immune and microbial variables. The fact that pouchitis almost always occurs in patients with underlying UC, and is almost never found in patients with FAP, provides strong evidence for an infectious etiology in genetically susceptible patients with IBD.^{114, 133} Patients who have a genetic susceptibility to intestinal inflammation, that is those with IBD and not FAP, may develop an altered bacterial composition in their ileal pouches. Accordingly, the prevailing theory suggests that pouchitis is caused by an overgrowth of commensal bacteria¹¹⁴ in patients who have a genetic predisposition to pouchitis. The cellular and immunological changes observed in pouchitis involve analogous mechanisms to those found in other inflammatory processes of the GI tract. The notion that pouchitis is reminiscent of underlying UC is supported by the similarities in mucosal inflammation between UC and pouchitis; however, pouchitis seems to present as a less threatening form of mucosal inflammation than that in UC patients.¹³

1.4.3. Risk Factors Associated with Pouchitis

There is no sound consensus in the literature as to which risk factors definitely increase a patient's probability of developing pouchitis; however, purported risk factors include backwash ileitis,⁷⁶ extensive UC,^{15, 76} extraintestinal manifestations, in particular primary sclerosing cholangitis,¹³⁴⁻¹³⁶ interleukin-1 receptor antagonist gene polymorphisms,^{137, 138} the presence of pANCA antibodies,^{139, 140} being a nonsmoker,¹⁵ and the use of NSAIDs.¹⁴¹ There is a discrepancy in agreement on risk factors associated with pouchitis, which could be due to any or all of the following; duration and intensity of follow-up after IPAA;¹⁴² diagnostic criteria of pouchitis used; stratification of pouchitis – acute versus chronic pouchitis or a combination of both;¹⁴³ inclusion or exclusion of CD of the pouch¹¹³ or cuffitis; and number of patients studied. Further research in assessing risk factors associated with the various clinical phenotypes in pouch patients may shed light on disease pathogenesis.¹⁰

1.4.4. Diagnosis and Classification

Symptoms characteristic of pouchitis include increased stool frequency and urgency, liquid consistency of stool, abdominal cramps, blood-stained diarrhea, malaise and anal bleeding; however, symptoms alone do not reliably diagnose pouchitis and may be seen in diseases of IPAA other than pouchitis.^{13, 113} The lack of consistent criteria for making a diagnosis has caused controversy regarding clinical features indicative of pouchitis.¹⁴⁴ A strictly clinical definition is based on symptom criteria and response to antibiotics; however, symptoms of pouchitis are not specific and can also be seen in pouch ischemia, rectal cuff inflammation, anastomotic stricture, CD, or IPS.¹⁴⁴ Hence, endoscopic and histologic evidence of ileal inflammation are needed as symptoms alone do not reliably diagnose this pattern of ileitis.^{13, 113} There is limited literature describing how to best treat symptomatic pouchitis patients with IPAA; nonetheless, deciphering pouchitis from cuffitis, CD of the pouch and IPS is essential because treatment and prognoses differ.¹⁵ The incidence of CD in the pouch in patients with UC who undergo surgery is unknown.

CD of the pouch can be present with a range of clinical phenotypes which include inflammatory, fibrostenotic and fistulizing disease, each of which may have a different natural history, etiology and pathogenesis.¹⁰ Shen *et al.* found that each clinical phenotype was associated with different demographic and clinical factors. CD of the pouch seldom develops, leaving only a small number of patients available for meaningful statistical analysis; as a result, it is a difficult task to identify pre- and post-operative risk factors for CD in pouches. In previous studies, Shen and colleagues found that the most significant risk factor for the development of CD of the pouch is a preoperative diagnosis of IC.¹⁰ Recently, Shen *et al.* determined that the presence of family history of CD was strongly associated with the development of CD of the pouch in UC/IC patients who underwent RPC.¹⁴⁵ The key to accurate diagnosis of pouchitis requires endoscopic evaluation together with symptom assessment and histological evaluation.¹¹³ Endoscopic findings include inflammation of the pouch which can be patchy or diffuse with edema, contact bleeding, mucosal hemorrhage, granularity, nodularity, friability, exudates and ulceration.¹³ Usually, endoscopy together with histological evaluation can be used to help distinguish pouchitis from other inflammatory disease or functional disorders of the pouch.¹⁴⁴

Currently, there is no universally accepted diagnostic criteria for pouchitis, but semi-objective evaluations to diagnose IPAA patients with pouchitis have been proposed using composite scores such as the Pouchitis Triad,¹⁴⁶ Heidelberg Pouchitis Activity Score,¹⁴⁷ and Pouchitis Disease Activity Index (PDAI).¹⁴⁸ The most commonly used diagnostic instrument is the PDAI. The PDAI scoring system provides simple, objective, sensitive and quantitative criteria for pouchitis classification, and consists of the following parameters: clinical symptoms, endoscopic findings, and histology.¹⁴⁹ A total PDAI score ≥ 7 points indicates a diagnosis of pouchitis.¹⁴⁸ One drawback is that histologic evaluation has limited value in the quantification of mucosal inflammation; nonetheless, it is useful for the assessment of characteristic features of certain diseases of the pouch, such as granulomas in CD of the pouch, cytomegalovirus (CMV)

infection and pyloric gland metaplasia in chronic pouchitis, ischemic changes, and mucosal prolapse.¹¹³

In addition, there is no validated and universally accepted classification system for pouchitis, but it is important to accurately classify disease before initiating therapy. Two approaches frequently used by clinicians to diagnose pouchitis in symptomatic patients include: a diagnostic-therapeutic trial with antibiotics and pouch endoscopy with or without biopsy.¹¹⁴

Sandborn *et al.* outline the different ways that pouchitis can be categorized: idiopathic *versus* secondary, based on etiology; remission *versus* active, based on disease activity; acute *versus* chronic, based on symptom duration with a cut-off of 4 weeks; infrequent episodes *versus* relapsing *versus* continuous course, based on disease course; and responsive *versus* refractory, based on response to medical therapy.¹⁵⁰ Shen and colleagues have come up with another useful

classification scheme which is based specifically on patient response to antibiotic therapy.^{15, 151}

Antibiotic-responsive pouchitis is a condition in which patients have infrequent episodes (<4 episodes per year) responding to a 2-week course of a single antibiotic; antibiotic-dependent pouchitis a condition with frequent episodes (≥ 4 episodes per year) of pouchitis or with persistent symptoms requiring long-term, continuous antibiotic or probiotic therapy; antibiotic-refractory pouchitis as a condition in which patients fail to respond to a 2-4 week course of a single antibiotic (metronidazole or ciprofloxacin), require therapy over 4 weeks with 2 antibiotics, 5-aminosalicylate, corticosteroid or immunomodulator therapy.¹¹³ This type of classification

system can be quite helpful in the clinical milieu due to the lack of standardized diagnostic criteria for pouchitis. For example, there is a different prognosis for antibiotic-responsive pouchitis compared to antibiotic-refractory pouchitis - the latter diagnosis is a common cause of pouch failure, leading to a pouch resection. For instance, one study found that of 100 consecutive UC patients who underwent IPAA, 5 patients developed chronic, antibiotic-refractory pouchitis, of which 2 ended up with pouch failure as well as pouch resection.¹⁵⁰ An alternative approach

which shows promise for future clinical diagnosis of pouch inflammation is the measurement of fecal lactoferrin which has shown high sensitivity and specificity.¹⁵²

Overall, the diagnosis of pouchitis is based on specific clinical, endoscopic and pathologic features and is typically categorized as either acute (duration less than 4 weeks), chronic (duration greater than 4 weeks), or relapsing (recurrent episodes of pouchitis).⁹⁴ Proper diagnosis and classification is essential for the future study of pouchitis pathogenesis.¹⁰

Due to the lack of universally accepted criteria to diagnose pouchitis, clinical trials utilize different classification systems making outcome comparison difficult.^{114, 144, 153, 154} Both ciprofloxacin and metronidazole have been shown to significantly lower PDAI symptom, endoscopic, and histologic inflammation scores.¹⁴⁴ Recurrence of pouchitis is common, 61% of patients with acute pouchitis develop at least one relapse,¹³⁵ and 5-19% of patients develop refractory or rapidly relapsing symptoms that require frequent and/or prolonged antibiotic therapy.¹⁵⁵ These refractory patients do not respond to full dose, single-agent antibiotic therapy. Some possible causes included NSAID use¹⁵⁶, concurrent *C. difficile*¹⁵⁷ or CMV infection¹⁵⁸, celiac disease, cuffitis and/or CD.¹⁵¹

1.4.5. Crohn's Disease and Crohn's Disease - like Phenotype of the Pouch

There is sparse data in the literature describing the etiology and pathogenesis of post-IPAA CD. Although the incidence of CD or a Crohn's-like phenotype of the pouch in patients who undergo surgery with an initial diagnosis of UC is unknown, reported cumulative frequencies range from 2.7% to 13%.^{143, 159-161} This range depends on preoperative and postoperative diagnostic criteria for IBD subsets, UC or CD, duration and intensity of postoperative follow-up and inclusion of indeterminate colitis as a denominator.¹¹³ It is important to emphasize that a CD-like phenotype post-IPAA may not be the same as classic CD, nor does it suggest that the patient had a misdiagnosis of CD pre-colectomy. The manifestation of a CD-like phenotype post-IPAA has become an area of interest due to the fact that patients had a confirmed

diagnosis of UC pre-colectomy and only later on did they begin to manifest characteristics in line with a CD phenotype.¹⁶²

It is difficult to identify pre- and postoperative risk factors for CD of the pouch because of the small number of patients available for meaningful statistical analysis.¹⁶³ For example, of 231 patients with a preoperative diagnosis of UC, 0.4% developed postoperative CD,¹⁶⁴ whereas of 115 patients with a preoperative diagnosis of IC, 4.3% developed postoperative CD which was confirmed by pathology.¹⁶⁴ In addition, active smokers have 4.77 times the odds (95% CI: 1.39, 16.25) of having CD compared to CD patients who self-reported being a nonsmoker.¹⁵ It appears that each of the clinical phenotypes of CD of the pouch is associated with different risk factors.¹⁵

Patients with a diagnosis of UC scheduled to undergo a proctocolectomy followed by IPAA should be informed of possible development of CD postoperatively. Inadvertently, CD has been found in colectomy specimens of patients with a preoperative diagnosis of UC.^{159, 160, 163, 164} In this case, the patients may have been given a diagnosis of IC because severe or toxic colitis prevented a firm pathological diagnosis of UC or CD.¹⁶³ When IPAA is performed in selective patients with a preoperative diagnosis of Crohn's colitis without perianal and small intestinal diseases, CD of the pouch can occur after IPAA.¹⁴ As well, weeks or years after IPAA, *de novo* CD of the pouch may develop with no evidence of CD found when proctocolectomy specimens are reassessed.¹⁶⁵

Similar to clinical symptoms of pouchitis and cuffitis, symptoms of CD of the pouch consist of abdominal pain, diarrhea and pelvic discomfort. CD of the pouch should be considered if a patient is an active smoker and presents with weight loss, nausea, vomiting, fever, malnutrition, iron deficiency, anemia, osteoporosis, and/or perianal fistulae.¹⁴¹ Patients with CD may also present with obstructive symptoms. Features suggestive of a CD-like phenotype consist of perianal disease and fistulae outside the pouch-anal anastomosis, including pouch-vaginal, pouch-vesticular, and pouch-cutaneous fistulae. Because perianal fistulae or abscesses may result from pouch surgery itself or CD, postsurgical complications such as abscess, anastomotic leak or

sepsis need to be ruled out. In order to determine if the complication is indicative of CD, factors to be considered include time and location of development of the abscess or fistulae, concurrent small bowel or afferent limb diseases, and absence or presence of characteristic histologic features, such as granulomas. In general, if fistulae or abscesses develop more than 12 months post-IPAA in the area outside pouch-anal anastomosis a diagnosis of CD should be considered. As well, a diagnosis of CD should be considered if there are granulomas on histology, ulcerative lesions in the afferent limb proximal to the pouch¹⁶⁶ and ulcerative strictures at the pouch inlet, afferent limb or mid-pouch in the absence of current NSAID use.¹¹³

Diagnosing CD of the pouch can be quite challenging, especially when trying to distinguish it from chronic refractory pouchitis.¹¹³ The fact that smoking has the opposite effect for UC versus CD, smoking status may provide a clue for the differential diagnosis of pouchitis compared to CD.¹⁵ Overall, to confirm a diagnosis of CD, it is useful to assess whether there is small bowel involvement by endoscopy or small bowel contrast radiography.¹¹³ Currently, the use of capsule endoscopy, or serologic markers in IPAA patients in a diagnostic capacity warrants further evaluation.¹¹³

Treating patients with CD of the pouch, similar to patients with pouchitis, is difficult and requires long-term maintenance therapy.¹¹³ In certain cases, patients are able to keep their pouch with proper medical, surgical and endoscopic treatment; nonetheless, there is limited data on safe and effective therapies.¹¹³ CD of the pouch can lead to fistulae, pouch failure and septic complications.¹⁶³ Today, patients who present with strictures or sepsis may be treated with anti-tumor necrosis factor therapy (anti-TNF), such as infliximab. In a case series of 26 patients with CD of the pouch who received infliximab infusion, 62% had a complete response and 23% had a partial response. After a median follow-up of 22 months, 67% of the patients had a functional pouch, whereas 33% of the patients had lost their pouch.¹⁶⁷ Treatment for mainly mucosal inflammation includes topical or oral 5-ASA, corticosteroids or oral antibiotics. Immunomodulators may be useful for patients with fistulae or strictures.¹¹³

Depending on the duration and intensity of follow-up, and use of medical or endoscopic therapy in IPAA patients, pouch failure, resulting in pouch resection, ranges from 25% to 100%.^{143, 159, 160, 163} These studies were performed before immunomodulators and anti-TNF therapy were used routinely to treat this patient group. Overall, most patients with CD of the pouch appear to be able to retain their pouches with medical and endoscopic therapy, despite the limited duration of follow-up in most studies.^{163, 167}

1.4.6. Irritable Pouch Syndrome (IPS) and Cuffitis

Irritable Pouch Syndrome

Currently, IPS is described as a functional disorder in patients with IPAA.¹⁶⁸ Patients with IPS report significantly lower quality-of-life compared to individuals with a healthy pouch. One study showed that out of 61 consecutive symptomatic UC patients with IPAA (excluding surgically-related complications and CD of the pouch), 51% had pouchitis, 7% had cuffitis and 43% had IPS based on a combined assessment of symptoms, endoscopy and histology.¹⁶⁸ Additional studies have demonstrated that IPS patients have visceral hypersensitivity of the small intestine as well as the colon and have gastrointestinal dysmotility.¹⁶⁹

IPS is a diagnosis of exclusion and may present as IPS alone or IPS coexisting with inflammatory disease of the ileal pouch.¹⁷⁰ Diagnosis of IPS is based on the presence of symptoms of increased bowel frequency with change in stool consistency, abdominal cramping or pain and pelvic or perianal discomfort in the absence of endoscopic and histologic inflammation. Diagnostic criteria for IPS remains to be standardized.¹¹³ There are no established treatment regimes for the management of IPS – treatment is empiric. Pharmaceutical therapy includes antidiarrheal agents, antispasmodic agents and tricyclic antidepressants.¹¹³ It appears that the etiology and pathogenesis of IPS is likely multifactorial.

Cuffitis

Patients with cuff inflammation on endoscopy and histology may present with symptoms similar to pouchitis, CD of the pouch and irritable pouch syndrome, while others may be

asymptomatic all together. Cuffitis can be considered a form of UC where bleeding, ranges from blood on tissue paper to blood clots, and is significantly more common in patients with cuffitis than in patients with pouchitis or CD of the pouch.¹⁴¹ There exist similarities in endoscopic and histologic features of mucosal inflammation in cuffitis and pouchitis, such as ulceration, erythema, friability, nodularity and neutrophil infiltration.¹⁷¹ To date, cuffitis remains an under-recognized disease, with limited data available in the literature. Two anastomotic techniques are used to construct IPAA: one is a hand-sewn anastomosis with mucosectomy of the anal transitional zone mucosa (also known as rectal columnar cuff mucosa); and the other method is a stapled anastomosis at the level of the anorectal ring without mucosectomy.¹⁷² A hand-sewn anastomosis takes longer, removes the rectal columnar mucosa as completely as possible and has a relatively higher risk for postoperative functional problems, (i.e. incontinence and seepage). On the other hand, a stapled IPAA is a simpler procedure with a reduced likelihood of resulting functional or septic complications.¹⁷²

Commonly used treatment for cuffitis include topical 5-ASA, corticosteroid agents or topical mesalamine. Generally, oral antibiotics are not effective in cuffitis patients. The use of topical 5-ASA appears to be effective in patients with coexisting pouchitis and cuffitis.¹⁷¹

An important question to ask is whether pouchitis, particularly antibiotic-refractory pouchitis, and cuffitis have common etiology and pathogenesis?¹¹³

1.5. SEROLOGY OF INFLAMMATORY BOWEL DISEASE

1.5.1. Summary of Serological Markers in IBD

Already in 1959, investigators reported antibodies to colon extract in UC patients.¹⁷³ Since then, several antibodies have been reported in IBD. The most well studied are pANCA¹⁷⁴ and gASCA.¹⁷⁵ Other antibodies reported in CD patients include: anti-OmpC,¹⁷⁶ anti-I2¹⁷⁷ and anti-CBir1.¹⁷⁸ To date, serological markers alone have only a modest accuracy in detecting IBD, and deciphering between CD and UC. However, serological markers may contribute to disease stratification. In patients with CD, a more aggressive disease course, defined by fibrostenotic and internal perforating disease and the need for small bowel surgery was associated with higher serological levels of ASCA.¹⁷⁹ In accordance with this finding, Mow *et al.* reported a correlation between the presence and amount of antibody production (anti-I2, OmpC and ASCA) and a complicated disease course.²⁸

The use of antibody responses to microbial antigens may facilitate measurement of immune responses to either exogenous or endogenous microbes, traditionally difficult to measure. A variety of clinical outcomes in IBD and their association with specific antibody responses has been described. For example, IgA and IgG antibodies to ASCA are noted to be more prevalent in patients with CD. Sensitivities and specificities for CD range largely between 39-72% and 82-89%, respectively. ASCA has been associated with small bowel inflammation, severe disease and disease requiring surgical intervention. Recently, it has been suggested to be a risk factor for CD development in IPAA patients with a pre-operative diagnosis of UC.¹⁸⁰ ASCA is an antibody marker that may be a marker of immunoreactivity against enteric microbial products. The antigen that is used to detect this serum antibody in IBD patients is derived from a yeast.² Serum antibodies that are reactive against enteric microbes signify a loss of immunologic tolerance to the flora that normally resides in the intestine and otherwise coexists at peace with our immune system. For example, pANCA stain as an undefined protein and is thought to originate from the nuclear or the nuclear periphery of neutrophils. Sensitivity and specificity for

UC ranges between 50-67% and 75-94%, respectively.¹⁸¹ Currently, pANCA has been associated with a “UC-like” behaviour pattern in CD (colonic CD) and the development of ileal pouch inflammation in patients with IPAA. Anti-CBir1 has primarily been identified in patients with CD and has been found to be more prevalent in patients with internal perforating disease and small bowel disease.^{178, 182}

1.5.2. Anti-Glycan Antibodies

More recently, the presence of anti-glycan antibodies in IBD was evaluated based on the existence of Crohn’s disease-specific antibodies against sugars such as ASCA.¹⁸³ Glycans (polysaccharides) are predominant surface components that can be found on micro-organisms, immune cells, erythrocytes, and tissue matrices.³⁸ Glycan is a generic term describing molecules with glycosidic bonds, including sugar (mono-saccharides, oligosaccharides, polysaccharides or carbohydrates). Each individual human, as apart of the adaptive immune response, has circulating antibodies towards a large range of non-self glycan structures existing on bacterial, fungal, and parasite cells.¹⁸³ In addition to antibodies against mannan (IgG anti-covalently attached anti-Saccharomyces cerevisiae antibodies or gASCA; and anti-mannobioside (Man(α 1,3)Man(α)) carbohydrate IgG antibody or AMCA), additional antibodies to laminaribioside (anti-laminaribioside (Glc(β 1,3)Glc(β)) carbohydrate IgG antibodies or ALCA) and chitobioside (anti-chitobioside (GlcNAc(β 1,4)GlcNAc(β)) carbohydrate IgA antibodies or ACCA), have been shown to have discriminative capability between CD and UC.¹⁸⁴ Laminaribioside is the building block of laminarin and can be found in food (oats), algae, as well as in cell walls of saprophytic and pathogenic fungi and yeast. Chitobioside is a component of chitin, an important constituent of the insect cuticle and cell walls of infectious pathogens such as yeast and bacteria.¹⁸⁴

Several interpretations of the relation between these antibodies and disease pathogenesis have been put forth. One theory is that they represent true autoantibodies; however, evidence suggest that they may recognize components of the enteric flora.² In fact, these autoantibodies

may result from “molecular mimicry”, where a microbial antigen is structurally similar to a self-antigen. Alternatively, these antibodies may represent a global loss of immunologic tolerance to the enteric flora.¹⁸⁵ This theory is supported by the existence of immunoreactivity to enteric microbes in humans and animal models of IBD. Nonetheless, this immunoreactivity is specific, such that antibody and T cell immune responses are not demonstrated to every enteric microbial antigen.¹⁸⁶ Furthermore, serum antibodies may eventually be used to identify a specific organism that is directly pathogenic in IBD. For example, serum antibodies to bacterial species like *Mycobacterium avium* ssp *paratuberculosis* expressed in CD have been hypothesized as evidence for an infectious etiology in CD.¹⁸⁷ However, many such antibodies have been detected in mice and humans, making this scenario highly unlikely.²

Recently, a number of groups have investigated the novel anti-carbohydrate markers: anti-laminaribioside carbohydrate antibodies (ALCA), anti-chitobioside carbohydrate antibodies (ACCA), and anti-mannobioside carbohydrate antibodies (AMCA), and two IgA cell wall polysaccharide antibodies anti-laminarin (anti-L) and anti-chitin (anti-C).¹⁸⁸ Evidence from preliminary results supports the potential role of these markers in the development of aggressive ileal inflammation in CD.¹⁸⁸ The anti-glycan antibodies recognize carbohydrate epitopes – these antigens are found in the cell wall of pathogenic bacteria and fungi.¹⁸³ Two new anti-glycan markers, anti-L and anti-C are important markers to study in order to evaluate their clinical utility. In a recent study anti-L improved differentiation between CD and UC and may be useful in differentiating between isolated colonic CD versus UC.¹⁸⁸ Future studies with larger samples need to be performed in order to substantiate these findings. If anti-L proves to help differentiate between CD and UC, it may have a role in re-classifying patients with an indeterminate phenotype as well as assisting in treatment regimens tailored to this specific cohort of patients. Of the 24 patients (60.0%) seronegative for gASCA IgG, 12.5% were anti-L positive and 4.2% were anti-C positive. This suggests that these novel markers may bind to different epitopes.¹⁸⁸

1.5.3. Serological Contributions to Pouchitis

There are a limited number of previous studies which have investigated the association between serological markers and the development of ileitis in pelvic pouch patients – these studies have for the most part been inconsistent and poorly replicated. For instance, although many studies have demonstrated an association between pANCA and pouchitis¹⁸⁹⁻¹⁹¹, others have not identified such a correlation.^{178, 192} Most of these studies had small sample sizes, and an unclear definition of pouchitis, both factors of which could help explain why there is a discrepancy in results reported. One study looked at the prevalence of ASCA positivity in pelvic pouch patients and found that 15% of patients were ASCA positive. However, this study did not identify an association between pouchitis and ASCA positivity.¹¹ Currently, there exists a lack of data evaluating the association of serological markers with ileitis of the pouch; in particular, there is a paucity of information on the novel markers ALCA, AMCA, ACCA and the pelvic pouch. For this reason, we propose that serological markers may reflect an immune response to different populations of microbial flora in the intestinal lumen of IPAA patients and hypothesize that unique serological associations will be present in individuals with ileal inflammation of the pelvic pouch as compared to those with a normal ileal pouch. Specifically, the markers ASCA, ALCA, AMCA, ACCA, anti-L and anti-C will be compared between the three different groupings of pelvic pouch patients to determine the association between marker positivity and pouch outcome.

The potential roles for IBD serology in the clinical atmosphere are manifold¹⁹³ and include its use to identify IBD in patients with an unclear diagnosis; to aid in assessing the need for endoscopy in young patients who are suspected of having IBD; to assist in differentiating between UC and CD limited to the colon; to help to improve the accuracy of diagnosis prior to surgery (eg. colectomy, IPAA); and to facilitate the identification of patients at risk for aggressive disease behaviour. Initially, serological markers were designed to help distinguish between different types of IBD, particularly in the case of an elective colectomy for IC patients.⁹⁶ Positive serological testing, for ASCA and more recently OmpC, has proven to be helpful in classifying

CD patients; nevertheless, only ½ to ⅔ of Crohn's patients' are seropositive to one of these markers.^{193, 194} Several studies have demonstrated an association between the presence of anti-neutrophil cytoplasmic antibodies with perinuclear staining (pANCA) and UC.^{11, 195-197} The antibody is found in 48-84% of UC patients but in less than 25% of CD patients and other colitides.¹²⁵ In fact, CD patients positive for pANCA generally demonstrate a UC-like phenotype.^{179, 193}

Many authors have looked at whether the presence of pANCA is associated with or predictive of pouchitis, with some individuals reporting evidence in favour of this hypothesis,¹⁸⁹⁻¹⁹¹ while others finding data opposing this idea.¹⁹² Aisenberg *et al.* looked at whether the UC-associated pANCA or the CD-associated ASCA were predictive of pouchitis. The authors noted that the prevalence of UC-specific pANCA prevalence was 54% in the overall patient population, which is consistent with previous observations,⁴⁰ and did not find a correlation between pANCA prevalence and the development or severity of pouchitis.¹¹ Additional series have reported similar results,¹⁹⁸ whereas other studies have suggested that pANCA may be a marker for pouchitis.¹⁸⁹ In contrast, Fleshner *et al.*, showed that pre-colectomy high-titer (>100 EU/ml by ELISA) UC-specific pANCA predicts the development of chronic pouchitis post-surgery; however, they did not measure postoperative titres.¹³⁹ Results of this study raises the question, do pANCA titer levels correlate with severity and disease course of pouchitis?¹⁰ The discrepancy may be attributed to the difference in demographics. In Aisenberg's study the number of patients with left-sided colitis was predominant (88%) as compared to other published series, such as Fleshner's study where 23% of patients had left-sided colitis; hence, extent of UC prior to surgery may be related to frequency of pouchitis.^{11, 139} From the Aisenberg study, the lack of high-titer pANCA in their patient population may reflect the fact that the patients' sera was obtained many years postoperatively, and pANCA levels may fall over the long term after colectomy.^{11, 139} Similar findings from additional series report a decrease in pANCA titers in the majority of patients post-proctocolectomy; this may be due to a reduction of inflammation or available

antigenic material, which modifies the immune disturbance related to UC.^{199, 200} Further studies are required to determine whether pANCA can be used to determine the disease activity of UC in relation to therapeutic measures other than proctocolectomy.¹⁹⁹ Overall, the divergent results reported on these studies may reflect differences among study populations, laboratory assays, or lengths of follow-up.¹¹

Understanding association between CD-associated markers and pelvic pouch outcome is an increasing important area to study. Overall, approximately 39-61% of CD patients test positive for ASCA,^{175, 201} compared to 5-15% of UC patients; on the otherhand, pANCA is present in 50-73% of UC patients^{202, 203} and 4-24% of CD patients.^{174, 202} Aisenberg *et al.* studied the prevalence of ASCA in their patient population, and found that approximately 15% were ASCA positive and that there was no association between ASCA and pouchitis. This finding is in line with data from previous studies that discourage the hypothesis that pouchitis may signify occult CD or that ASCA is simply a marker of ileal inflammation.¹¹

Hui *et al.* looked at whether preoperative responses to microbial antigens (oligomannan anti-Saccharomyces cerevisiae, outer membrane porin C or Escherichia coli, and an antigen (I2) from Pseudomonas fluorescens) are associated with CD in IC patients after IPAA. They found that patients who have a positive reactivity profile prior to IPAA have a significantly higher incidence of continuous pouch inflammation post-IPAA than those with a negative profile. Anti-flagellin (or CBir1), a newer serological marker, appears to enable discrimination between UC and CD patients. Accordingly, individuals who are CBir1 and pANCA positive have CD, whereas CBir1 negative but pANCA positive individuals are more likely to be UC¹⁹³. In addition, increasing evidence suggests that the presence of multiple or high titers of certain antimicrobial antibodies in CD patients predicts more severe disease. These phenotypes include a greater probability of developing fistulizing and penetrating disease, fibrostenosing small bowel CD, more relapses, and earlier and more frequent need for surgery.¹⁹³ Serological marker results can help to predict patients with a severe disease phenotype near the beginning of the clinical course

so that clinicians can elect to employ immunomodulatory drugs earlier. Complication prevention strategies have yielded promising results in rheumatological disorders, such as in prevention of joint destruction.¹⁹³

Enzyme-Linked Immunosorbent Assay (ELISA) Technique

Indirect solid phase enzyme-linked immunosorbent assay (ELISA) is a test used to determine whether a specific antibody is present in a serum sample. First, the appropriate antigen is absorbed to the walls of a microtiter plate. Then serum is added to the walls of the microtiter plate in order to determine if the antibodies are indeed present in the serum sample. Rinsing removes any antibodies that do not specifically attach to the antigens absorbed to the well. If antibodies attach to the antigen, they can be detected by adding an antibody-enzyme conjugate to the wells. The antibodies in the antibody-enzyme conjugate are reporter antibodies that will bind to the antibody-antigen complex. The sample is rinsed again to remove unbound antibodies. Finally, substrate for the enzyme is added and a colour change will indicate that the sample contains antibody that reacted against the original antigen.¹⁸⁴

1.6. GENETICS OF INFLAMMATORY BOWEL DISEASE

1.6.1. Summary of Genetic Markers in IBD

CD and UC are likely to share certain susceptibility genes as both forms of IBD can coexist in single families with a frequency greater than that expected by chance alone; nonetheless, CD and UC have distinct clinical features which suggests that disease-specific genes also exist.¹⁸ The mode of inheritance is unknown, but there is little support for a simple Mendelian inheritance pattern in either subset of IBD.²⁰⁴

Evidence that genetic susceptibility contributes to the etiology of IBD has been demonstrated in several epidemiological studies. Familial aggregation reflects both shared genetic and environmental factors.⁷⁹ Epidemiologic studies have elucidated that genetic variants which represent IBD likely include genes that increases susceptibility to IBD, in general, (e.g. IL23R associations), and variants that augment risk for particular phenotypic subsets (e.g. NOD2/CARD15 in CD).¹⁵⁶

Familial Clustering

Familial clustering of IBD illustrates that disease pathogenesis is in part due to genetic susceptibility. A positive family history of IBD is the best established risk factor for the development of disease. Although precise estimates vary, consistent findings have been reported in the studies that have been performed.⁸ For instance, in two population-based studies, approximately 5-10% of affected individuals report a positive family history.^{205, 206} In general, between 6%-32% of patients with IBD have an affected relative.²⁰⁷ Another study demonstrated that 75% of multiply-affected families with IBD are concordant for disease type, either all affected patients have UC or all have CD); while 25% are mixed, having one member with CD and another with UC.²⁰⁸ These data are consistent with a heterogeneous model of disease susceptibility where certain alleles are unique to CD or UC whereas some variants are common to both disorders.⁷⁹

Relative Risk

The degree to which genetic factors contribute to disease pathogenesis can be studied quantitatively by looking at the risk in first-degree relatives of individuals affected by disease. Both cohort and case-control studies have been conducted looking at the relative risk for IBD among first-degree relatives.

Orholm and colleagues found that first degree relatives of both CD and UC patients had approximately a 14- and 10-fold increased risk of developing the same disorder, respectively, in a cohort study.³⁰ On the other hand, Meucci *et al.* found lower risks for relatives of CD and UC individuals¹⁹⁰ (λ_r) = 6.6 and 3.4, respectively) in a multi-centered study from northern Italy. In the latter experiment, the authors found that the risk to siblings was higher in CD ($\lambda_s=15$) than in UC ($\lambda_s=6.0$).¹⁹⁰ In case-control studies, similar relative risk estimates, 14- to 15-fold increased risk, have been reported.³¹ Furthermore, in a British study, overall relative risk to sibs was greater than 24.7.²⁵ Although an exact relative risk for IBD among first-degree relatives is not known, there appears to be a significant genetic susceptibility to disease.

Twin Concordance

Further support for the contribution of genetic factors in IBD is provided by twin concordance studies.^{32, 34} Reported concordance rates for monozygotic (MZ) and dizygotic (DZ) twins with UC range from 6-19% and 0-5% respectively. A landmark study from the Swedish Twin Registry performed by Tysk *et al.* showed a genetic component shared between twins with concordance for CD in MZ twins equal to 58.3% compared to 6.3% for UC³². In a British study, Thompson *et al.* found that the concordance rates in MZ for CD and UC were 20% and 16%, respectively. In general, twin concordance rates in CD patients ranges from 33%-50% in MZ twins compared to a 0%-10% risk in DZ twins. Reported concordance rates for MZ and DZ twins with UC range from 6-19% and 0-5% respectively.³²⁻³⁴ These results suggest that genetic susceptibility contributes to the pathogenesis of CD and UC, with a stronger effect in CD; hence, these results point to a relatively greater role for nongenetic factors in UC disease pathogenesis.³⁴

⁷⁹ In addition, these findings indicate that IBD is not inherited as a Mendelian trait, but rather has a complex genetic basis with many contribution genes.²⁶ In this regard, the role of being an ex-smoker at the time of diagnosis is of importance in UC pathogenesis, where studies suggest a causal relationship between disease onset (particularly late onset) and prior cigarette smoking.²⁰⁹ Alternatively, a well-established association exist between NOD2 polymorphisms and CD; this association illustrates the role of single gene variants in disease pathogenesis for common, complex multigenic disorders.⁷⁹

Genetic Model of Disease Heterogeneity

The prevailing model for IBD is that the intestinal flora drives an unmitigated intestinal immune response and inflammation in the genetically susceptible host.²¹⁰ Currently, it remains unclear how genetic factors interact with the environment in disease pathogenesis.^{8, 211} The genetic model that best fits is one in which CD and UC are related polygenic diseases that may share some susceptibility loci but differ at others²¹² The variations in disease phenotype likely represent the effects of allelic variation of these genes, and the interaction between these allelic variations and environmental factors.⁸

1.6.2. Gene Identification Techniques in IBD

Two general approaches for gene identification in multi-factorial diseases include the positional cloning approach, based on genome-wide linkage studies, and the candidate gene approach, based on association studies.^{211, 213-220}

Linkage Analysis

Linkage studies analyze the co-segregation of the disease with a marker within families and require that the whole genome be scanned. Linkage studies look at families with more than one affected member with the disease of interest, as well as genotype markers throughout the genome, in order to identify genomic regions shared between affected relative pairs.⁷⁹ A linkage or lod score (logarithmic ratio of odds) is often used to quantify the extent of increased sharing

throughout the genome.⁷⁹ The study of complex diseases has required access to large numbers of multiply affected families (typically sibling pairs), semi-automated technology for genotyping, and particularly the evolution of techniques for analysis.⁸ Proceeding from the initial observation of linkage through replication to gene identification remains a difficult task for investigators.⁸

Genome scans have been undertaken in IBD, resulting in the identification of a number of susceptibility regions on chromosomes 1, 3, 4, 5, 6, 7, 10, 12, 14, 16, 19 and X.^{211, 213-220} Regions on chromosomes 16, 12, 6, 14, 5, 1, 1 and 3 have been renamed IBD1 to IBD9, respectively, based on their initial date of reporting. Independent investigations have confirmed that these loci from the various chromosomal regions as containing IBD susceptibility genes (refer to Table I – 1).⁴³ Authors have found a definitive association within the IBD1 and IBD5 loci on chromosomes 16q12 and 5q31, respectively. One of the initial definitive disease gene associations made via genetic linkage was that of NOD2, which involves disease mutations within the IBD1 locus.²²¹⁻²²³ Later studies show functional polymorphisms in the organic cation transporters OCTN1 (SLC22A4) and OCTN2 (SLC22A5) implicated in the IBD5 linkage region.^{224, 225} It remains a significant challenge to definitively identify disease genes within suggested genetic linkage regions in multigenic complex disorders. Hence, major disadvantages of genetic linkage are the large genomic regions, which contain scores of potential disease genes, and the problem of obtaining false-positives.⁷⁹ However, genetic linkage is non-biased and comprehensive which is an advantage of this approach.⁷⁹

Table I – 1: IBD linkage regions⁷⁹

Linkage Region	Chromosome	Gene Association	Disease
IBD1	16q12	NOD2	CD
IBD2	12		UC
IBD3	6p	HLA	IBD, UC
IBD4	14q11-12		CD
IBD5	5q31	OCTN1, OCTN2	CD, UC
IBD7	1p36		
IBD8	16p		
IBD9	3p26		

Candidate Gene Approach

Candidate gene analysis is another, often complementary approach, where genetic variation within the candidate gene is typed and compared between cases and controls.^{43, 79} Candidate gene studies attempt to determine the importance of specific genes in disease pathogenesis and necessitate understanding of disease pathophysiology. In the past, this method was done based on putative gene functions, such as immune regulation or maintenance of epithelial integrity, but it now also includes positional candidate genes which lie in areas of linkage defined by genome screening.²⁰ For example, in IBD the immunopathology of the disease led to examination of genes involved in the regulation of the immune system, cell-cell interactions and maintenance of mucosal integrity.⁸ Hence, a candidate gene approach investigates a specific gene with a known or potential interest for the studied disease. This method can be used to examine the significance of genes to disease susceptibility and severity which is based on the frequencies of functional single nucleotide polymorphisms (SNPs).²²⁶ Several candidate genes have been subject to analysis in IBD, including genes of the human leukocyte antigen (HLA) system, and genes involved in the regulation of cytokine production, mucin synthesis and other aspects of epithelial barrier function.⁸ Carrier trait analysis can be used to study combination of SNPs and to explore the implication of various SNPs in disease susceptibility and severity as a result of their synergistic action.²²⁶ The allelic frequencies, in case-control studies, or the transmission of a SNP towards affected offspring, in trios, are studied and differences between patients and controls, or an over transmission towards affected children, may implicate this particular gene in the pathogenesis of the disease under investigation.⁴³ In addition, candidate gene selection can be based on animal models of disease, (e.g. targeted disruption of the gene of interest precipitates an IBD-like phenotype); location within a linkage region; association in other related diseases; and/or recognition of important pathways possibly relevant to disease.⁷⁹

It is important to consider several factors when interpreting these studies.²⁰ Meaningful analysis relies on large populations which are ethnically matched. Adequate correction must be made for multiple comparisons, if multiple polymorphisms of genes are being tested. If this is not possible, the results cannot be regarded as positive unless replicated in a second independent dataset.²⁰ On the other hand, all polymorphisms must be tested so that the gene can confidently be excluded as a candidate. Thus, a negative study of one polymorphism within a gene does not mean that the gene is not involved if other polymorphisms are subsequently discovered or have not been tested.²⁰ Overall, the candidate gene approach has not been particularly fruitful in IBD.²²⁷

1.6.3. Genome-Wide Association Studies

A recent approach being applied to multigenic disorders is by genome-wide association (GWA) studies, a method that has markedly evolved from the low-resolution linkage analyses based on multiply affected families.²²⁷ This approach uses the basic premise that most IBD risk alleles are relatively common in the population.^{228, 229} Hypothesis-free methods of genome scanning provide a much more powerful by providing an unbiased survey of the entire genome for IBD-associated loci, and have the potential to provide novel insights regarding disease mechanisms/pathways.²²⁷ Hence, if multiple functional polymorphisms are simultaneously required for disease development, each of these risk alleles must be relatively common in the control population, allowing for the expected population prevalence to be reached. Subsequently, several hundred thousand well-selected SNPs would be required to sample much of the common genetic variation in the human genome since many of the several million common SNPs are in linkage disequilibrium with each other. Currently, genetic technologies can test high numbers of SNPs in high throughput-machinery. By testing 1000 cases and controls, each, there would most probably be sufficient power to detect a significant fraction of contributing risk alleles.⁷⁹ There are two ways to test association with disease: directly, by investigating the disease-causing mutation; or indirectly, by testing genetic variants that act as surrogates for the mutation by way

of linkage disequilibrium.²²⁷ It has become apparent that the latter is more powerful and that the majority of variants associated with complex disease are non-coding, likely regulatory polymorphisms.²²⁷ Nonetheless, there exists several obstacles impeding completion and interpretation of GWA studies which include the need for extensive replication, the reality that there exists pathophysiologic heterogeneity, and the lack of analytic frameworks for testing for locus-locus and gene-environment interactions.⁷⁹

1.6.4. Single Nucleotide Polymorphisms (SNPs)

The most abundant genetic variation in humans is SNPs which account for >90% of all sequence polymorphisms²³⁰ – other variations include insertions, deletions and short tandem repeats. A SNP is a DNA sequence variation occurring when a single nucleotide A, T, C, or G, in the genome differs between members of the species.⁷⁹ Inter-individual genetic differences likely result from SNP variation, where the rare allele frequency is $\geq 1\%$. Almost all SNPs have only two alleles. The high propensity of SNPs enables very high resolution genotyping, essentially revolutionizing human molecular genetics by providing a dense panel of genetic markers distributed across the entire genome.²³¹ SNPs are relatively frequent within populations, and are considered relatively common in the population of interest if they have an allelic frequency of >5%. SNP microarrays detect mutations or polymorphisms in a gene sequence which can be used to test an individual for any disease expression pattern in order to determine whether the individual is susceptible to that disease or not.²³² Even though most SNPs do not necessarily affect gene function, they can be used to study population dynamics and evolution, to investigate the basis of complex phenotypes and to develop diagnostic assays.²³¹ It is known that some SNPs do indeed cause dramatic functional effects (e.g. introduction of a stop codon) on protein function; however, not all SNPs having functional effects are necessarily coding region SNPs. Many functional polymorphisms are thought to exert their effects through modulation of gene expression.²³³ Hence, SNPs may fall within coding sequences of genes, non-coding

regions of genes, or in the intergenic regions between genes. Due to the degeneracy of the genetic code, SNPs within a coding sequence will not necessarily change the amino acid sequence of the protein that is produced. A SNP is termed synonymous if both forms lead to the same polypeptide sequence (also referred to as a silent mutation).²³⁴ A SNP is termed nonsynonymous if a different polypeptide sequence is produced. In this case, a nonsynonymous change may be either missense or nonsense: a missense change results in a different amino acid, whereas a nonsense change results in a premature stop codon. In addition, SNPs that are not in protein-coding regions may still affect transcription factor binding, gene splicing, or the sequence of the non-coding RNA.²³⁵

The arena of common human SNPs is largely definable (estimated at 7 million in number), and significant attention is being directed toward defining and characterizing which subset of those SNPs have direct effects on gene function and expression.²³⁶ SNPs are evolutionarily conserved, and have been proposed as a marker for use in quantitative trait loci (QTL) analysis and association studies in place of microsatellite markers.²⁶ Important information about the genetic background of individuals can be gleaned from genome scans, and have been used to study linkage disequilibrium in human populations and perform association mapping and linkage studies of common complex diseases.²³³ There exist several variations in the nomenclature for an individual SNPs which can be confusing.²³⁷

It is important to take into consideration that there exists significant linkage disequilibrium between SNPs in humans (i.e. significant correlation in transmission between nearby SNPs, such that they “travel together”), demonstrating nonrandom association between distinct genetic variants. The fact that closely spaced SNPs are in linkage disequilibrium with each other can complicate definitive disease gene associations.²³⁶ SNP genotyping can be used to identify genetic regions associated with a broad variety of indications, allowing researchers to target particular areas of interest and begin to reveal relevant genes associated with a disease. The term haplotype refers to a set of SNPs on a single chromatid that are statistically associated.

It is believed that these associations, and the identification of other alleles of a haplotype block, can unambiguously identify all other polymorphic sites in its region. This type of information is invaluable when investigating the genetics involved in common diseases.¹⁴⁴

Within a given population, SNPs can be assigned a minor allele frequency (MAF) which is the lowest allele frequency at a locus that is observed. Put simply, the MAF is the lower allele frequency of the two allele frequencies for any given SNP. Due to the variations between human populations, SNP allele frequencies differ from one ethnic or geographic group such that a SNP may be common in one group but rare in another.²³⁸

In summary, studying SNP profiles or haplotypes associated with a disease trait has the potential to reveal relevant genes associated with a disease. Association studies can detect differences in SNP patterns between two groups (affected and unaffected individuals), thereby indicating potential SNP patterns most likely associated with disease-causing genes, warranting further study of specific genetic regions. Furthermore, understanding the role of genetic factors in disease will also allow researchers to better evaluate the role that non-genetic factors (i.e. behaviour, diet, lifestyle, and physical activity) have on disease.

1.6.5. SNP Genotyping

Genotyping provides a measurement of genetic variation between members of a species, and serves as an excellent tool for research. Deviations in the structure, function and behaviour of genes results from genetic differences between normal individuals. Important validation strategies include analysis of the structure of target genes and identification of sequence variants such as SNPs in promoter and exon regions. Polymorphic studies provide information that can be used in target validation, where highly polymorphic targets are abandoned.²³⁹ Using SNP analysis to study genetic differences within populations has the potential to highlight a number of biological mechanisms implicated in disease status, and could lead to the discovery of targets for

therapeutic intervention.²⁴⁰ High-resolution SNP arrays have been used to demonstrate clinically relevant allelic imbalances in human bladder tumors²⁴¹ and age-related macular degeneration.²⁴² homogeneous, single-step assay used in determination of mutation status of DNA.²³¹

1.7. GENETIC CONTRIBUTION TO INFLAMMATORY BOWEL DISEASE AND POUCHITIS

Rapid progress has been made in identifying susceptibility genes for IBD, which includes a panel of candidate genes confirmed to confer susceptibility to inflammation and IBD.²⁴³ Large scale, genome-wide association (GWA) studies have enabled the identification of genes shown to be associated with IBD as well as to play an important role in etiology of IBD. This panel includes the three common NOD2/CARD15 mutations associated with CD (R702W, G908R, 3020insC)²²¹ as well as known polymorphisms within NOD1/CARD4,²⁴⁴ IBD5 (SLC22A4, SLC22A5),^{245, 246} HLA Class II (DRB1*0103),²⁴⁷ TNF-alpha promoter polymorphisms,^{214, 248, 249} toll-like receptor 2 and 4,²¹⁴ TGF- β 1,²⁵⁰ interleukin 6 (IL-6),²⁵¹⁻²⁵³ interleukin 10 (IL-10)²⁵⁴ and interleukin-12/interleukin 23 receptor (IL-12/IL23R).^{7, 156} Other genes include autophagy related 16-like 1 (ATG16L1), immunity related GTPase family M (IRGM), protein tyrosine phosphatase non-receptor type 2 (PTPN2) and tumor necrosis factor superfamily member 15 (TNFSF15). Most of these genes were discovered in studies containing primarily CD-affected individuals; however, UC –associated genes from GWA studies have implicated the MHC region, interleukin 1 receptor antagonist (IL1RA), ATP-binding cassette, subfamily B (MDR/TAP), member 1 (ABCB1), protein tyrosine phosphatase, receptor type, S (PTPRS), butyrophilin-like 2 (BTNL2), cyclin-Y (CCNY) and the IBD2 region on chromosome 12.^{43, 248, 255-258} Other studies have shown evidence for gene associations with CD and UC (e.g. IBD5, IL23R).⁷⁹ Several Crohn's disease-associated loci, including macrophage stimulating 1 (MST1) on chromosome 3p21, NK2 transcription factor related locus 3 (NKX2-3) transcription factor gene and IL-23 pathways associations have also been shown to contribute to UC susceptibility, thus, are generic IBD loci. On the other hand, CD-specific genes included NOD2 and autophagy genes ATG16L1, as well as

IRGM, which relate to microbial processing and handling of bacterial antigens.^{227, 259} Recently, a genome-wide candidate gene experiment investigating 10,886 nonsynonymous SNPs in 1,470 British controls and 936 UC cases identified extracellular matrix protein 1 (ECM1) on 1q21.2 as a unknown UC susceptibility locus; nonetheless, a systematic, genome-wide analysis of UC has not been reported thus far.^{259, 260} It is this overlap in genetic associations that further strengthens the prevailing view that IBD is a spectrum of clinical disorders whereby phenotype is determined by a combination of both genetic and environmental factors. Hence, these preliminary findings lead us to the hypothesis that individuals who develop ileitis post-IPAA have both common IBD susceptibility genes as well as genes specific to either UC or CD.

More specifically, there exists a small number of studies which examine the role of genetic variants in the setting of pelvic pouch ileal inflammation and the papers which have been published are generally underpowered (<200 total patients). A few reports have found an association of an interleukin-1 receptor antagonist (IL-1RA) gene polymorphism (rs2232353) with ileitis of the pouch.^{137, 138} In another, smaller study, a NOD2 variant was reportedly associated with ileal pouch inflammation.²⁶¹ Another study identified a haplotype of the CD14 -260T and TLR9 -1237C alleles which may identify a subgroup of IPAA patients who are at risk of developing pouchitis.²²⁶ Scientific evidence indicates that colonic forms of IBD share pathogenic mechanisms which are distinct from small bowel inflammation indicating the need to reassess classifications of IBD.²²⁷

1.7.1. The Role of the Microbial Environment

Several reports have demonstrated that luminal bacteria play an important role in the development of pouchitis.¹²⁹ Lammers *et al.* investigated SNPs in genes involved in bacterial recognition and the susceptibility to pouchitis and/or pouchitis severity. They explained that luminal bacteria play an important role in the development of pouchitis, supported by various reports on bacterial overgrowth and dysbiosis in pouchitis,¹²⁹ along with the proven efficacy of

antibiotic and probiotic therapy when administered to pouchitis patients.^{116, 262} The authors concurred that given the role of luminal bacteria in driving the inflammatory response in pouchitis, recognition and functional characterization of polymorphisms in innate immunity genes may shed light on genetically determined susceptibility to pouchitis and/or chronic relapsing pouchitis.¹⁰ Lammers and colleagues explored whether SNPs in innate immunity genes played a part in the susceptibility to pouchitis and/or severity of pouchitis by looking at candidate genes of CD14, TLR4, TLR9, NOD2/CARD15, and IRAKM for their involvement in bacterial recognition and intracellular signaling pathways.²²⁶ They showed that these SNPs did not predispose individuals to pouchitis post-IPAA; however, they found that the combined carriership of the CD14 -260T and TLR9 -1237C alleles in the chronic relapsing pouchitis group may identify a subgroup of IPAA patients with a risk of developing chronic or refractory pouchitis.²²⁶ Exploring combined carriership of specific alleles is important because SNPs in different genes may work synergistically and contribute a small to moderate relative risk of developing disease.²²⁶

In another study conducted by Kiehne *et al.* defensin expression in ileoanal pouches of individuals with UC and FAP was analyzed. Pouch patients were stratified into various groups; one group consisted of those with ileostoma- protected pouches after surgery, and the other groups consisted of pouch patients (after closure of their ileostomy) without pouchitis. To determine whether defensins play a protective role against the development of pouchitis, expression data of antimicrobial peptides were related with expression of cytokines and the pouch groups.²⁶³ The mammalian innate immune system is comprised of both α - and β - defensins which are small cationic peptides with high activity against a variety of microbials, encoded by genes of which some are regulated in response to challenge with bacterial antigens.²⁶³ Current understanding of the role of defensins suggests that they play a significant role in intestinal microbial homeostasis and in the primary defence against enteral and systemic infection.²⁶⁴ Kiehne *et al.* found results consistent with this idea, reporting that decreased defensin expression

and high expression of cytokines correlated with the development of pouchitis in UC patients. On the other hand, the low incidence of pouchitis in FAP pouches correlated with increased expression of hBD-1 β -defensin in association with low cytokine levels.²⁶³ In general, defensin expression is much higher in pouches compared to normal ileum, and both α - and β - defensins are expressed in higher levels in UC pouches than in FAP pouches.²⁶³ In addition, there is a strong and sustained expression of cytokines in UC pouch patients while there is a weaker and more transient cytokine expression in FAP pouch individuals.²⁶³

1.8. SUMMARY

A thorough understanding of pouchitis etiology and pathogenesis is a requisite factor for proper diagnosis and classification and will lead to strategies for improved treatment as well as prevention of this disease.¹⁰ Despite the known benefits of surgery, IPAA patients who develop inflammatory and noninflammatory diseases after surgery often present with nonspecific symptoms that compromise their quality-of-life. Proper clinical management of disease depends on both accurate classification of pouchitis and the differential diagnosis of pouch disorders.¹¹³ To date, pouch endoscopy remains a key procedure to help diagnose pouchitis accurately.¹¹³ Notwithstanding, it is important to track patients postoperatively in order to establish whether serologic and genetic tests correlate with pouch-specific complications; more-specifically, post-operative follow-up will help determine if chronic, antibiotic refractory pouchitis patients and/or patients who display a CD-like phenotype, will prove to have CD.²

As a result, this study aims to comprehensively evaluate recently-discovered, confirmed genetic variants plus other published genetic associations in individuals with prior UC who do or do not develop ileal inflammation after IPAA. These data will provide new information in our understanding of those genes critical to the onset of different phenotypes of ileal inflammation in humans and their relationship to the microbial environment. Identification and functional

characterization of polymorphisms in specific genes may provide insight into a possible genetically determined susceptibility to pouchitis and/or a CD-like phenotype.²²⁶

CHAPTER II

AIMS, OBJECTIVES, HYPOTHESIS, SIGNIFICANCE AND JUSTIFICATION

2.1. AIM

The main aim of this study is to evaluate the contribution of biologically relevant candidate genes and serological makers to phenotypic manifestations of IBD patients following pelvic pouch surgery.

2.2. OBJECTIVES

1. Evaluate the clinical factors associated with post-operative outcome in UC patients with IPAA.
2. Study the genetic and serologic markers that are associated with the development of pouchitis and CD-like phenotype after the pelvic pouch procedure.
3. Determine the frequency and characteristics of pouchitis and other pouch complications in long-term follow-up in large patient cohort.

2.3. HYPOTHESIS

We hypothesize that new onset ileal inflammation in the IPAA patient is mediated by important interactions between underlying genetic defect(s) and the local microbial environment. We propose a detailed evaluation of clinical, serological and genetic features of the IPAA model with the principle objective of identifying the critical determinants of this new onset ileal inflammation.

1. Individuals with definitive UC who develop chronic relapsing pouchitis in the ileal pouch will have a divergent genetic and serological profile compared to those with definitive UC who have a normal ileal pouch.
2. Individuals with definitive UC who develop CD-like features or pre-pouch ileitis or fistulizing disease will have a genetic and serological profile similar to individuals with CD.
3. There are clinical variables that are predictive of pouchitis and/or a CD-like phenotype after pelvic pouch surgery for UC.

2.4. SIGNIFICANCE

Ileal inflammation arising in the setting of confirmed UC and an ileal pelvic pouch represents the best *human* model for studying the factors which lead to new onset ileal inflammation in IBD. These patients are not initially predisposed to ileal inflammation and rarely develop it; however, after IPAA, the environment in the ileum changes to become more similar to the colon. The high incidence of pouchitis post-IPAA in UC patients suggest that IBD genetic susceptibility factors are still at play, in addition to microbial determinants or other genes that dictate when or where ileal inflammation occur. The critical serological and genetic factors which lead to pouchitis in the ileal pouch are most probably key mechanistic factors that lead to ileal inflammation, in general. Discovery of these variables is likely to lead to significant improvement in the overall understanding of the etiology of IBD. Furthermore, these findings should result in

an improved ability to predict those individuals at risk of ileitis of the pouch so that predictive information can be utilized as part of the clinical decision making process for patients eligible for IPAA.

2.5. JUSTIFICATION

The identification of alleles and serological markers associated with pouchitis will assist in attempts to identify those at risk for specific clinical features, reduce clinical and genetic heterogeneity in IBD assessment and provide information for further research into mechanisms (i.e. inflammatory pathways) involved in such phenotypes. Careful documentation of disease diagnosis and phenotypic manifestations is essential in aiding the discovery of important susceptibility genes in IBD. Hence, improved knowledge of genetic and serologic factors contributing to disease pathogenesis, prognosis, and disease courses will allow effective application of human genetics and serology to clinical practice in the future.⁷⁹ More specifically, functional genomics can be used to assess genetic risk associated with postoperative outcome, permitting better prognostication and targeting of therapy (i.e. prophylactic therapy),²⁰ and reducing morbidity and mortality of genetically susceptible patients who undergo surgery.¹¹ In addition, serological testing

CHAPTER III

METHODS

3.1.1. Study Design

An association study utilizing retrospective data.

3.2.1 Subject Selection and Recruitment

Eligible patients were recruited from the Mount Sinai Hospital (MSH) IBD Centre, specifically the MSH Pelvic Pouch Database. This database was started in 1985 and contains detailed information on more than 1900 patients who have undergone an ileal pelvic pouch procedure at MSH. Utilizing demographic information stored in this database, potentially eligible patients were identified as those having a confirmed pre-operative diagnosis of UC or IBDU and who had pelvic pouch surgery with closure of the protective ileostomy at least 1 year prior to study enrollment. As well, patients with confirmed FAP who had a colectomy and IPAA ≥ 1 year prior to study initiation were recruited from the Mount Sinai Hospital Familial GI Group. Then, these patients were contacted by an initial mail-out which provided information explaining the study and requested participation of patients. A consent form that allowed the individual to be contacted, and for study procedures to be explained, was enclosed in the envelope package. This study was approved by the Research Ethics Board of Mount Sinai Hospital and Toronto Academic Health Sciences Network (TAHSN) prior to study initiation.

IBD patients, FAP patients and healthy controls were recruited at MSH (see Table III – 1 below). It was expected that the study population would contain equal proportions of males and females to reflect current IBD demographics. Individuals who are not affected with IBD or who had familial adenomatous polyposis (FAP) were asked to participate in the study to serve as controls. Individuals with FAP were recruited from the MSH Familial GI Cancer Registry (FGICR) which contains information on the 105 subjects (as per October 2007) who received an IPAA for FAP. The method of recruitment and elements required of participating individuals were identical to the UC recruitment. FAP patients are closely followed and attend MSH every

6-12 months as part of their standard of care. Healthy Controls were recruited by asking consented patients if they had any friends or a spouse who would be willing to participate. Twenty FAP patients and three control patients (spouses unrelated to anyone with a diagnosis of IBD) were recruited. Due to insufficient numbers recruited, FAP patients and healthy controls were excluded.

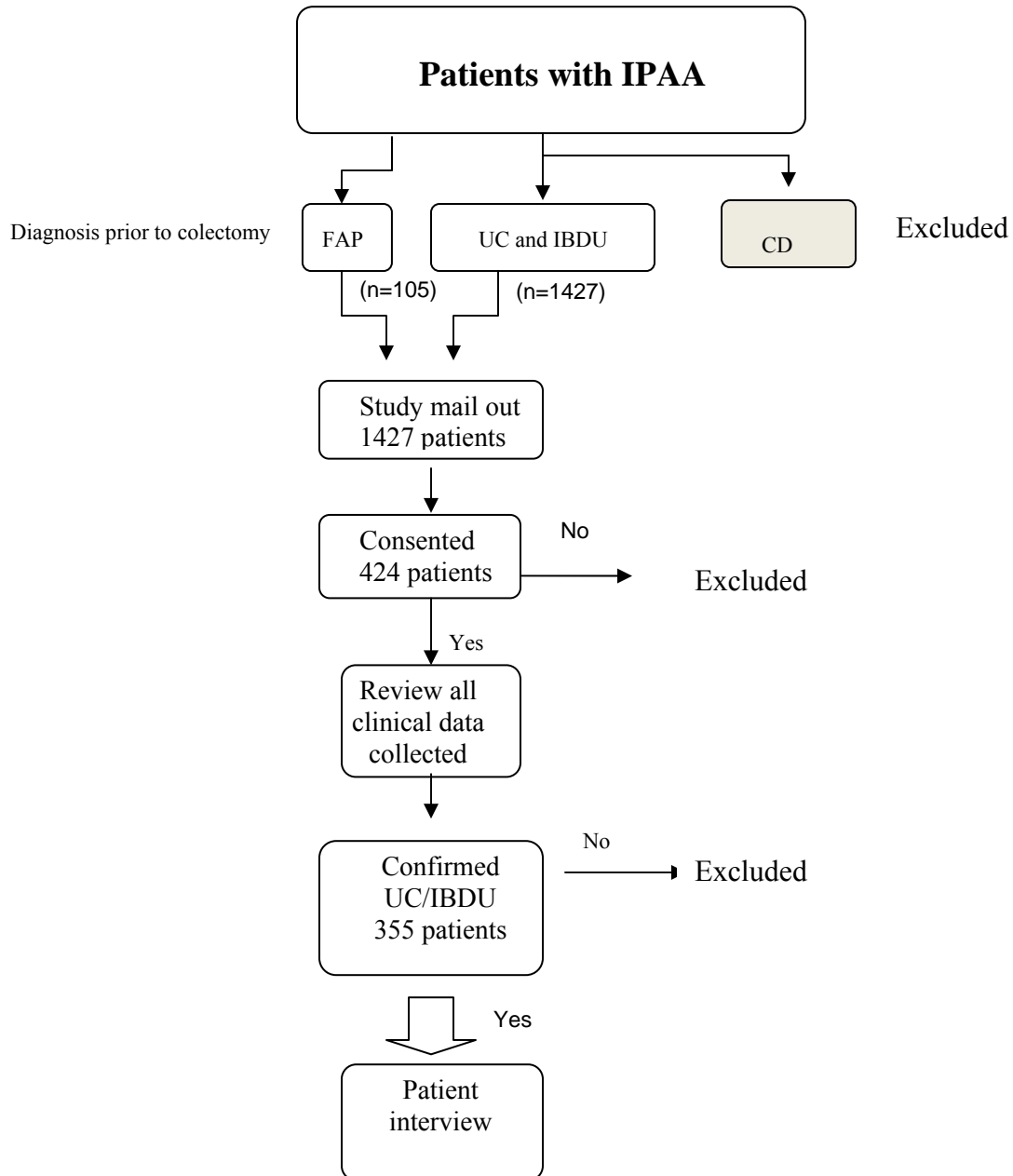
Table III - 1: Breakdown of Patients in Databases and Request for Study Participation Sent to Following Pouch Patients

Patient Category	Patient Subcategory	Number of Patients in IBD Genetics Database	Number of Patients in Surgical Database
UC	No pouch	400	263
	Pouch	82	1425
	Pouch with pouchitis	38	125
CD	No pouch	700	285
	Pouch	11	39
	Pouch with pouchitis	4	16
IC	No pouch	40	5
	Pouch	9	37
	Pouch with pouchitis	5	8
FAP	Pouch	-	105
	Pouch with pouchitis	-	1

Patients who provided written consent to partake in the study were enrolled as study subjects. The study participants were required to provide clinical data by answering a questionnaire as well as DNA and serum via a blood sample. Each participant signed a consent form, and was interviewed with a data form completed by DV.²¹⁸ Diagnosis was confirmed by documents supplied by the patient's family doctor, gastroenterologist, colorectal surgeon, or hospital records department.²¹⁸ Patients' pathology report from the time of colectomy had to be made available in order to confirm pre-operative diagnosis of UC or IBDU. Patients with confirmed or possible CD prior to colectomy were excluded. Each patient was assigned a unique identification number to allow for anonymity. Prior to performing the final statistical analysis, all records were updated and reviewed to capture any changes in diagnosis and to clarify cases where subjects were originally diagnosed as having suspected IBD or IBDU.²¹⁸ The questionnaire was completed in person with the subject at the Dr. Zane Cohen Digestive Disease Clinical Research

Centre (DDCRC) or by telephone interview. Blood samples were collected by an available research coordinator at the DDCRC, a certified laboratory technician from an MDS laboratory, or by a certified lab technician at a lab using kits with containers for collection of blood for DNA/serum extraction sent and collected by courier (as per the protocol for the ongoing IBD genetics studies.) See Figure III – 1 for schematic depiction of study recruitment design.

Figure III - 1: Study Recruitment Diagram



3.2.2. Clinical Data Collection

Once the study subjects provided their signed consent form, their clinical data was reviewed. Historical data were obtained from chart review and telephone interviews. Charts were by DV with the help of a physician. DV administered a telephone questionnaire to each patient. Each patient was questioned regarding general clinical and demographic data, the history of their UC prior to surgery, and their gastrointestinal health since surgery. Each patient was asked whether or not they had received the diagnosis of “pouchitis” from their gastroenterologist and/or colorectal surgeon, experienced the characteristic symptoms of pouchitis, or received oral antibiotic treatment at any point since surgery. Patients who had been given a diagnosis of pouchitis were asked to estimate how often they had experienced attacks: 1-3 attacks/year or >3 attacks/year. These patients went on to answer additional questions in the latter part of the questionnaire regarding specific symptoms during attacks. Specifically, they were asked whether during an attack they experienced cramping or pelvic pain, loss of control of bowel movements, blood in the bowel movement or fevers.¹¹ Patients were asked to estimate their usual baseline stool frequency and their stool frequency when unwell. Additionally, patients were asked about their treatment for pouchitis and details outlining the effectiveness of treatment.

In order to ensure reproducibility and control for interobserver variation, DV performed all the interviews with the subjects.

A pre-colectomy diagnosis of UC or IBDU and FAP was confirmed via review and full data collection was obtained from a combination of chart review and patient interview. Information was collected on prior reports of symptoms of inflammatory disease activity and prior endoscopic, histologic and/or imaging studies in order to document the presence or absence of ileal inflammation in the pouch or pre-pouch ileum or for evidence of perianal/fistulizing disease greater than 12 months after pouch surgery and ileostomy closure. Symptom data obtained included information regarding increased bowel frequency, incontinence, bleeding, abdominal cramping and presence of fevers during bowel unrest.

3.3. PATIENT GROUPING

424 patients completed the written consent, 69 patients were excluded because pre-colectomy pathology was not confirmed as UC (i.e. pathology revealed CD or a non-IBD entity) or due to insufficient clinical data after IPAA. Diagnosis of pouch-associated disorder was based on recently proposed classification schemes.²⁶⁵ Elements of the Pouchitis Disease Activity Index (PDAI) were used to assist in gathering pertinent information for the questionnaire to enable final patient categorization.¹⁵¹ Patients who stated that they had never experienced pouchitis, and who showed no evidence in their medical record to suggest pouchitis were classified as “no pouchitis.” Patients who stated they had pouchitis, in whom the telephone interview corroborated the diagnosis of pouchitis, and in whom chart review did not reveal a different etiology for their symptoms, were classified as “pouchitis.”¹¹ Patients who had only surgical complications, anismus, or cuffitis were classified in the “no pouchitis” group.

Based on chart evidence, including clinical, endoscopic and/or histological information, and patient recollection, patients were divided into three groups; no/limited acute pouchitis, chronic relapsing pouchitis restricted to the pelvic pouch, and CD-like phenotype of the pouch, including ileitis in the pre-pouch ileum or fistulizing/perianal disease occurring >12 months after surgery (refer to Section 3.3.3.).

In order to simplify the disease categories, the no pouchitis and limited acute pouchitis groups were further classified as no pouchitis and the antibiotic-dependent and antibiotic-refractory pouchitis were termed chronic pouchitis and these patients were classified as having pouchitis.²⁶⁵

3.3.1. Group 1 – No/Acute Limited Pouchitis

From the acute pouchitis group, patients who experienced infrequent episodes, < 4 episodes/yr, of pouchitis with each episode responding to a 2-week course of antibiotics (single or double), were deemed antibiotic-responsive. These patients would have experienced resolution of pouchitis between attacks due to medical treatment and were therefore considered to have acute pouchitis. The majority of patients were treated with ciprofloxacin and/or metronidazole.²⁶⁵ As a result, patients who fit the criteria of having, at most, acute episodes of pouchitis were classified in Group 1.

Patients who demonstrated endoscopic and histologic inflammation of the rectal columnal cuff and/or experienced relief of symptoms when treated with rectal enemas and/or suppositories were defined as having cuffitis. In cases of concurrent inflammation of the pouch and the cuff, the patient was diagnosed with pouchitis or cuffitis depending on whether the inflammation was found predominately in the pouch or cuff.¹⁵ Hence, patients with cuffitis without pouchitis were placed into Group 1. Patients who experienced surgically-associated complications, particularly within 12 months post-IPAA, were defined as having adverse outcomes related to surgical techniques, including pouch leaks and sinuses; subsequently, these patients were classified in the Group 1 unless they had features of chronic relapsing pouchitis or a CD-like phenotype in which case they would be classified in Group 2 or Group 3, respectively. Patients who complained of symptoms predominately related to dyschezia were defined as having anismus. Anismus may be due to spastic pelvic floor syndrome and can cause constipation. Anismus patients were classified in the Group 1. Finally, patients who had symptoms such as abdominal pain, diarrhea with no inflammation of the afferent limb, pouch or cuff on endoscopy, and pelvic discomfort were considered to have IPS. Patients experienced symptoms repeatedly, but were considered to have healthy pouches with no proven endoscopic and/or histological inflammation, and fewer than 4 episodes of antibiotic-responsive pouchitis per year.¹⁶² Accordingly, these patients were categorized as Group 1.

3.3.2. Group 2 – Chronic Relapsing Pouchitis

Patients who experienced ≥ 4 episodes per year of pouchitis or persistent symptoms and required long-term, continuous antibiotic therapy to maintain remission were labeled as having antibiotic-dependent pouchitis. These patients were considered the relapsing pouchitis group. Patients who failed to respond to a 4-week course of antibiotics and who required prolonged therapy of ≥ 4 weeks consisting of ≥ 2 antibiotics for 3 months or longer, oral or topical 5-ASA, corticosteroid therapy or oral immunomodulator therapy were labeled as the antibiotic- or medication-refractory pouchitis. This cohort of patients was considered to have chronic pouchitis and often had a very poor quality-of-life. Hence, the pouchitis group included patients who reported either having relapsing or chronic pouchitis (refer to Table III – 2 and Table III - 3) and required repeated antibiotic therapy, suffered recurrent episodes of pouchitis and/or had need for additional medications in conjunction to their antibiotics. In this group, patients who self-reported having at least 4 episodes of pouchitis per year, including those who had persistent symptoms, despite medical therapy, were placed in Group 2. Chart review confirmed or did not suggest a different diagnosis. In this group, patients were antibiotic/medication dependent or antibiotic/medication refractory.

3.3.3. Group 3 - CD-like Phenotype

Patients were diagnosed with CD-like phenotype of the pouch if there were non-surgery related fistulae, granulomas on histology or inflammation and ulceration in the afferent limb or in the small bowel on endoscopy in the absence of non-steroidal anti-inflammatory drug use.¹⁵ Patients who developed post-surgical complication(s) (i.e. fistula(e), abscesse(s)) more than 12 months after ileostomy closure or who experienced recurrent post-surgical complications that persisted for more than a year after reconstructive pelvic pouch surgery with ileostomy closure were classified as having a CD-like phenotype of the pouch. In the case of patients who underwent reconstructive pelvic pouch surgery at MSH and developed fistulae 6 months after ileostomy closure with persistent fistulae and potential perianal abscesses, were classified in Group 3. For example, a patient who had a pre-operative diagnosis of UC and developed a pouch vaginal fistula after reconstructive pouch surgery, demonstrated complications that may indicate a true diagnosis of CD; as a result, this patient was classified as having a CD-like phenotype.²⁶⁶ Clinical, endoscopic, histologic and/or radiographic evaluations were used to confirm a diagnosis of CD-like phenotype of the pouch.

Table III – 2: Diagnosis, Classification and Treatment

Diagnosis	Classification	Management
<i>Post-operative Grouping</i>	<i>Based on response to Antibiotics</i>	<i>Medical Treatment</i>
No Pouchitis	No Antibiotics	
Acute Limited Pouchitis	Antibiotic-responsive	Abs
Relapsing Pouchitis	Antibiotic-dependent	Abs
Chronic Pouchitis	Antibiotic-refractory	Prolonged combined Abs, 5-ASA, steroids
CD-like Phenotype	Antibiotic-refractory	Prolonged combined Abs, 5-ASA, steroids, immunomodulators, biologics

113

Table III - 3: Classification of Patient Population

Grouping	Episodes	Medications	Entities	Grouping
Group 1	-	No medications	No pouchitis, anismus, cuffitis, IPS	No/Acute Limited pouchitis
	1-3 attacks/yr	No need for continuous medication -resolution between attacks	Acute/limited pouchitis	
Group 2	≥4 attacks/yr	Constant medication	Relapsing pouchitis	Chronic Relapsing Pouchitis
	≥4 attacks/yr	persistent symptoms despite constant medication	Chronic pouchitis	
Group 3	-	constant medication	CD-like phenotype	CD-like phenotype

3.3.4. Inclusion and Exclusion Criteria

The inclusion criteria were: 1) underlying UC or IBDU and 2) restorative proctocolectomy with IPAA performed at Mount Sinai Hospital or an outside institution with 1 year follow-up information available. Patients were excluded if they had a proctocolectomy and IPAA performed for CD, FAP or other non-IBD conditions.

3.4.1. Study Variables and Definitions

Demographic and clinical variables were defined as indicated below.¹⁵

Table III – 4 Study Variables and Definitions

Variable	Definition
Inflammatory Bowel Disease – Unclassified	Clinical and endoscopic evaluations confirm chronic IBD; no small bowel involvement; no histological evidence to favour CD of UC; non-colectomy clinical diagnosis when a distinction between UC and CD cannot be made ¹⁹
Indeterminate colitis	Histopathological diagnosis on proctocolectomy specimens which defies clear distinction between UC and CD ¹⁴⁵
Family history of IBD	First or second degree relative with CD, UC or IC
Current smoker	Consumption of >7 cigarettes per week for at least 6 months prior to data entry ¹⁴⁵
Duration of IBD	Time interval from IBD diagnosis to time of proctocolectomy
Pre-operative diagnosis	UC or IBDU
Extensive ulcerative colitis	Endoscopic, macroscopic and microscopic disease extending proximal to splenic flexure
Toxic megacolon	Patients with continuous bloody diarrhea, fever, abdominal distention, tachycardia, and anemia who required immediate surgical intervention
Duration of pouch	Time interval between ileostomy closure and time of study initiation or pouch defunctioning.
EIMS	Axial arthritis, sacroiliitis, large joint inflammation, small joint inflammation, non specific inflammation, osteoporosis/osteopenia, erythema nodosum, pyoderma gangrenosum, oral ulcers, eye inflammation (conjunctivitis, uveitis, iritis, episcleritis), primary sclerosing cholangitis (PSC), renal stones. ¹⁴⁵
Use of biologic agents	Any use of infliximab or adalimumab for inflammatory conditions of the pouch (including CD of the pouch or chronic pouchitis) or concurrent autoimmune disorders. ¹⁴⁵
Autoimmune Diseases	Psoriasis, asthma, type 1 diabetes, rheumatoid arthritis, autoimmune thyroid disease (including Grave's disease and Hashimoto's thyroiditis, autoimmune hemolytic anemia, celiac disease, systemic lupus erythematosus, vitiligo, pernicious anemia, and multiple sclerosis. ¹⁵
Non-specific Joint Inflammation	Patient reported redness, swelling, heat, pain or decreased/loss of function of joints/muscles

Pre-colectomy Perianal Diseases	Fissure-in-ano, perianal abscesses, fistula-in-ano, rectovaginal fistula, significant hemorrhoids/skin tags. ²⁶⁷
Pouch failure	Defined as the need for permanent diversion of IPAA with or without pouch excision. ¹⁵
Pouch excision	Surgical removal of the pelvic pouch.

3.5. LABORATORY DETAILS

3.5.1. Biospecimen Collection and Storage

All enrolled patients were asked to provide a venous blood sample – 3 tubes of blood were drawn for DNA and serum extraction. DNA extraction was performed utilizing the Gentra Puregene kit (Gentra System, Minneapolis, MN). Optical density at 260 and 280nm was measured to determine quality and concentration of DNA obtained. Average expected yield of DNA from 3ml whole blood was 50-150µg. DNA samples were stored at 4°C. Whole blood samples were centrifuged and the serum extracted and stored at -70°C until needed. Average expected yield of serum was 3-4ml.

3.5.2. Serum Assays

Serum samples were shipped to Lod, Israel for analysis of the following antibodies (Glycominds, Ltd.): ASCA, ALCA, ACCA, AMCA, anti-L and Anti-C.

3.5.3. Serotyping

The serum antibodies represent a comprehensive panel of antibodies that reflect immune responses to the microbial flora in order to evaluate serological similarities and differences between groups. Extracted serum was thawed and tested using 250µl aliquots. Antibodies against glycans were assessed using a GlycoChip (Glycominds Ltd., Lod, Israel) and appropriate ELISAs.³⁸ Antibody titers were determined by means of a fixed enzyme-linked immunosorbent assay (ELISA) with reference values. To be considered positive, each marker titer level had to exceed the normal reference range. The cut-off values for positivity in ELISA units/ml was as follows: gASCA >50; ACCA >90; ALCA >60; AMCA >100; ASCA-A >50; L-units >60; and C-units >90 determined by Glycominds Ltd.¹⁸⁸

3.5.4. DNA Genotyping

The subjects were genotyped using a 768 single nucleotide polymorphism (SNP) panel of the most current and relevant IBD-associated SNPs to evaluate the genetic similarities and differences between one another. Genotyping was performed using Illumina GoldenGate custom SNP assay on Illumina BeadStation500G (San Diego, CA) at The Centre for Applied Genomics in Toronto. Analysis was performed to test 768 SNPs for each study subject. The SNPs which were selected include 768 SNPs with known associations to CD and UC or relevance to intestinal inflammation from current GWA studies and other candidate gene studies. The SNP list was chosen at the time of genotyping as the field is evolving rapidly and SNPs that have been reported in single GWA studies and have been replicated in independent studies were of particular interest.SNPs.

3.6. STATISTICAL ANALYSIS

The statistical analysis for the study was supervised by Dr. Wei Xu.

3.6.1. Serum Analysis

Summary statistics were provided as sample means and ranges for continuous (non-parametrically distributed variables); and frequencies or proportions for categorical variables. Logistic regression analyses were applied to determine the effect of combining markers. Pearson's chi-square test was applied to detect the differential rates of seropositivity of the antibodies between groups.

3.6.2. Genetic Analysis

The data was cleaned for potential genotype and phenotype errors. The descriptive statistics were performed including distributions of gender and age and summaries of genotyped polymorphisms and their linkage disequilibrium patterns using HAPLOVIEW.²⁶⁸ PLINK was applied to test for Hardy-Weinberg Equilibrium for each SNP based on Pearson's chi-square and Fisher's Exact tests.²⁶⁹ The impact of single SNP on the outcome was analyzed using a logistic regression model.²⁷⁰ Of the 768 SNPs genotyped, 25 SNPs were included in the analysis based on an *a priori* hypothesis of their potential role in this setting.

CHAPTER IV

RESULTS

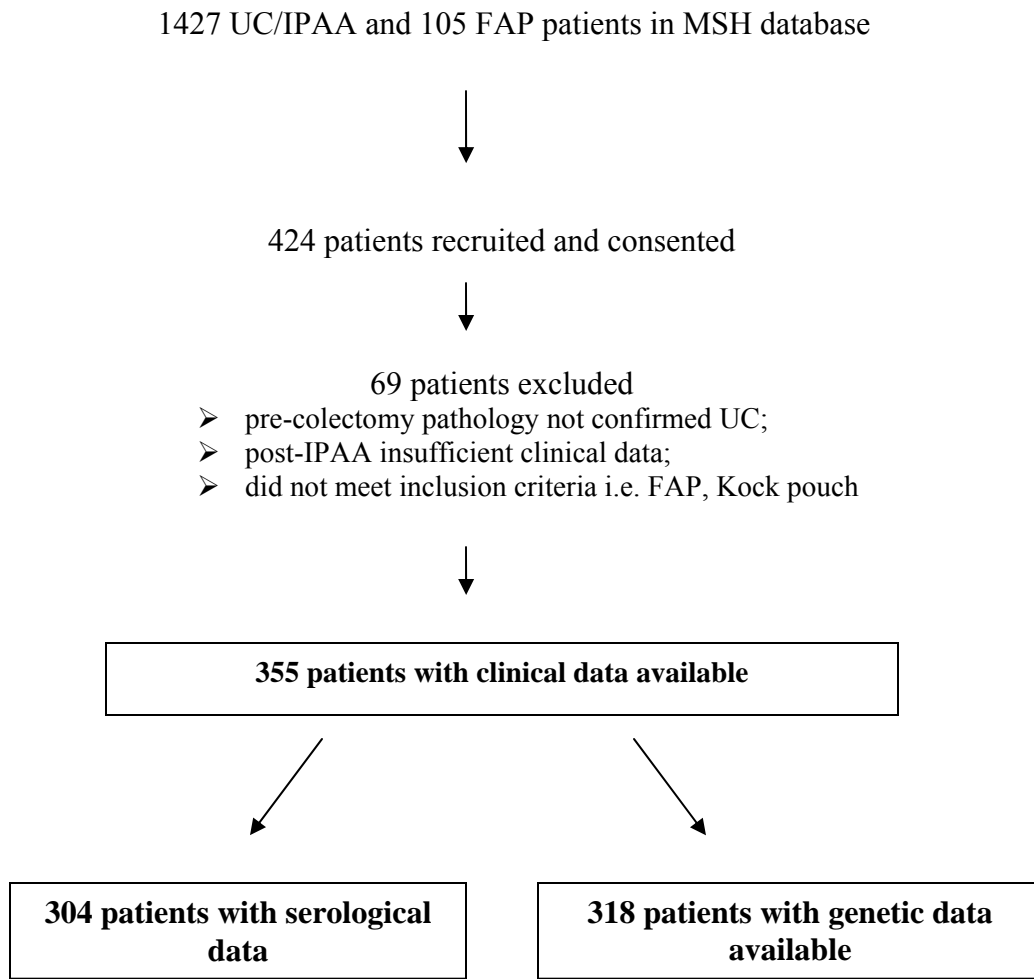
4.1. PATIENT CHARACTERISTICS

4.1.1. Patient Characteristics and Pouchitis Prevalence

From the approximately 1678 patients in the Mount Sinai Hospital IPAA registry, 1400 patients were contacted by mail-in letter. Four hundred and twenty four patients consented to participate in the study. Of these, three hundred and seventy three charts were available for chart review from the patient's colorectal surgeon and/or gastroenterologist. One or both physician(s) charts were reviewed depending on whether complete information could be obtained from the medical charts for the purposes of the study. Of the 424, three hundred and forty-eight were contactable by telephone and underwent an interview based on a standard questionnaire. After review of each patient, sixty five patients were excluded from the total due inclusion/exclusion criteria. For example, patient had a pre-op diagnosis of FAP or CD, or patient did not have sufficient follow-up information etc. From the 355 patients remaining with clinical data available, 304 had available serum and 320 had available DNA for analysis (refer to Figure IV – 1). Of the samples sent for DNA testing, 2 samples were not successfully analyzed such that 318 samples had available DNA data.

Multiple contacts were attempted by mail and by phone for all patients who had consented. For those patients who did not undergo questions from the questionnaire, complete chart review was required in order to come to a decision of whether or not the patient could be classified and therefore used in the study.

Figure IV – 1: Patient Recruitment and Final Study Inclusion



4.1.2. Reasons for Exclusions

There were 69 who were excluded from the study for various reasons listed in Table IV - I. From the total number of patients recruited, nine patients were excluded because a pre-operative diagnosis of CD was confirmed after thorough chart review. Twenty FAP patients were recruited, in addition to one patient with hyperplastic polyposis, to serve as a control group. The reason for final exclusion of the FAP patients, as well as the unaffected, unrelated healthy controls, was due to the insufficient numbers recruited (20 FAP and 3 healthy controls, respectively).

Table IV – 1: Patients Excluded From Study

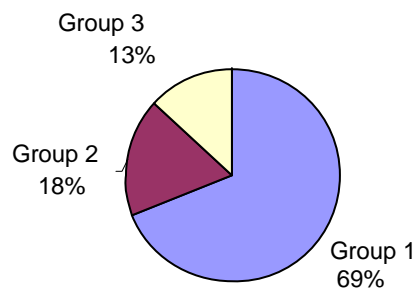
Reason for Exclusion	Number of Patients
Patient with pre-op diagnosis of toxic megacolon without IBD	1
Patient with a diagnosis of hyperplastic polyposis	1
Never had a functioning pouch	1
Pouch Failure <1 year post-op	1
Moved away	2
Recent pelvic pouch surgery	2
Ileostomy with no report of closure	2
Kock pouch	2
Permanent ileostomy <1 yr post-op	3
Pre-op diagnosis of CD	9
Not Enough Information to Classify	24
FAP	20
TOTAL	69

4.2. CLINICAL RESULTS

4.2.1. Overview of Patient Characteristics and Post-IPAA Grouping

Demographic and clinical characteristics of the 355 patients are summarized in Table IV - 2. Of the 355 patients, 246 patients (69%) had no/limited acute pouchitis, 64 patients (18%) had chronic/relapsing pouchitis and 45 patients (13%) had a CD-like phenotype of the pouch (refer to Figure IV – 2). From patients in Group 1, 163 (66%) patients had no pouchitis, 2 (1%) patients had anismus, 2 patients had cuffitis and 78 (32%) patients had acute pouchitis. The demographic characteristics of the patients grouped by no/limited acute pouchitis, chronic relapsing pouchitis and CD-like phenotype are listed in Table IV - 2.¹¹ The patients in the three groups were similar with regards to age, gender, age of onset of IBD, duration of disease prior to proctocolectomy, extent of disease prior to colectomy as well as reported family history of IBD, blood type, history of dysplasia, history of colorectal cancer (CRC), family history of CRC (refer to Table IV-2, and Table IV-4). The mean age of the patient cohort at study initiation (2007/9) was 30 years old ($p=0.79$ among groups); mean age at IBD diagnosis was 37 years of age; mean age at pouch surgery was 47 years of age (range 6-63); and mean duration of disease prior to proctocolectomy was 6.5 years ($p=0.08$ among groups).

Figure IV – 2: Classification of 355 IPAA Patients According to Occurrence of Post-Surgical Outcome During Follow-up.



Of the 355 patients included in the sample, 347 (98%) had a pre-op diagnosis of UC and 8 (2%) had a pre-operative diagnosis of IBDU. Pre-colectomy diagnosis was based on clinical, endoscopic, histologic, and radiological findings. Reasons for colectomy included inability to tolerate medication, refractory inflammatory diarrhea, toxic megacolon, profuse bleeding, and poor quality-of-life.

Overall, race distribution in the patient cohort was as follows: 83% of the patients were Caucasian, 10% were of Jewish Ashkenazi descent, 5 % were of Asian descent and the final 1% was comprised of other races such as African American, and North American Indian (refer to Figure IV – 3). There was a modest difference in racial distribution between groups ($p=0.053$) (refer to Figure IV – 4).

Figure IV – 3: Classification of 355 IPAA Patients According to Racial Distribution

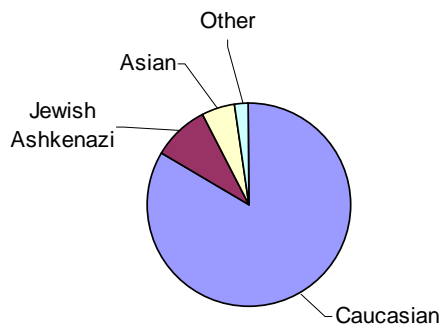


Figure IV – 4: Histogram Depicting Modest Association between Post-IPAA Grouping and Race

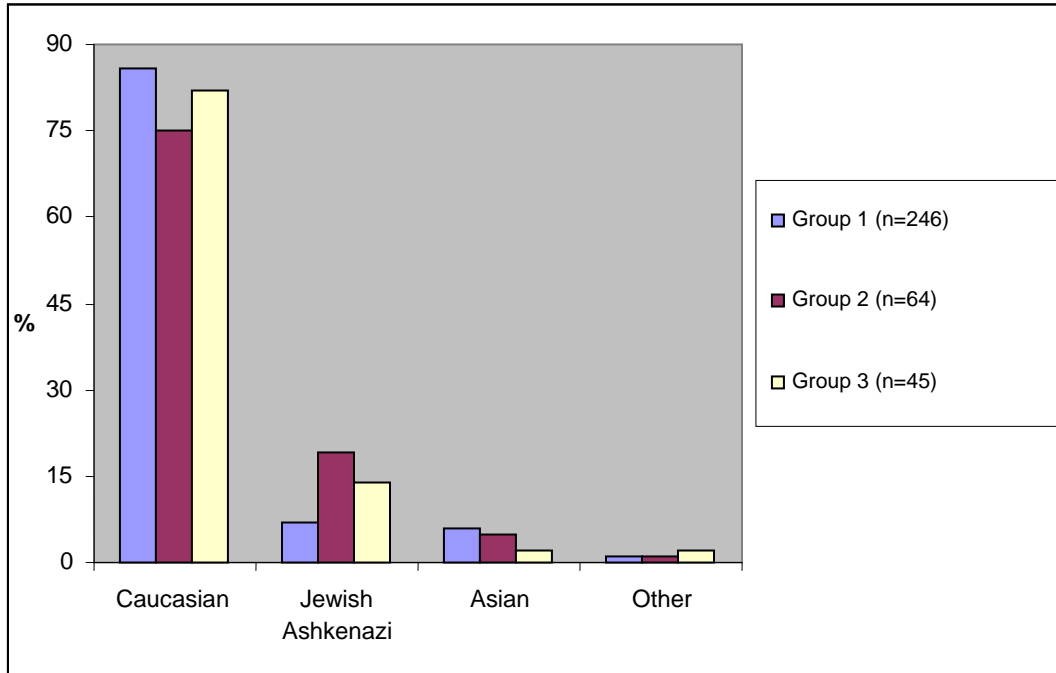


Table IV - 2: Demographic Characteristics of Patients by Post-IPAA Group

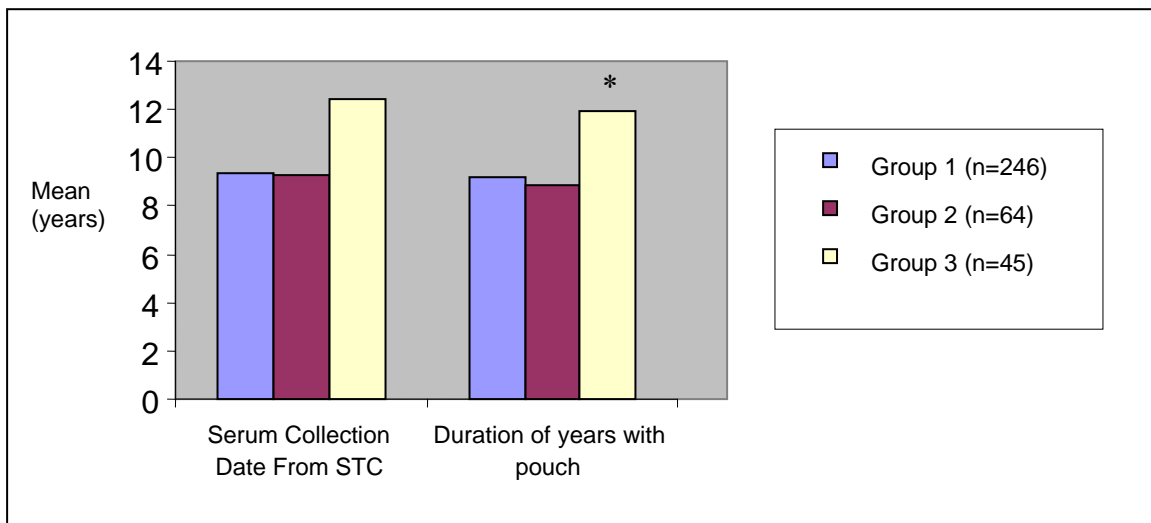
		Categorization			<i>p</i>	All Patients n=355
		Group 1 n=246	Group 2 n=64	Group 3 n=45		
Pre-op Diagnosis	UC	243 (99)	61 (95)	43 (96)	0.14	347 (98)
	IBDU	3 (1)	3 (5)	2 (4)		8 (2)
Age (yrs) at study start (2007)	Mean	31	29	28	0.23	30
	Range	6-62	9-56	2-54		2-62
Age (yr) at Diagnosis	Mean	38	36	34	0.19	37
	Range	6-63	14-59	8-55		6-63
Age (yr) at pouch surgery	Mean	47	46	47	0.58	47
	Range	17-77	21-73	20-67		17-77
Sex	Male/Female – no. (%)	125/121 (51/49)	28/36 (44/56)	18/27 (40/60)	0.30	171/184 (48/52)
Duration of Disease	Mean years (SD)	6.4 (6.8)	7.2 (6.1)	5.7 (7.6)	0.08	6.5 (6.8)
Race (%)	Caucasian	210(86.1)	48(75.0)	35(81.4)	0.053	293(83.5)
	Jewish Ashkenazi	18(7.4)	12(18.8)	6(14.0)		36(10.3)
	Asian	15(6.1)	3(4.7)	1(2.3)		19(5.4)
	Other	1(0.4)	1(1.6)	1(2.3)		3(0.8)
Blood Type	A	43	15	8	0.80	66
	B	15	3	1		19
	AB	5	1	2		8
	O	36	11	8		55
Dysplasia	Yes/No	7/220	3/55	2/39	0.68	12/314
Family Hx IBD	Yes/No	90/152	28/35	13/30	0.32	131/217
Family Hx CD vs UC	CD/UC (%)	11/25 (31/69)	1/6 (14/86)	2/2 (50/50)	0.47	14/33 (30/70)
Fam Hx CRC	Yes/No	47/164	16/38	8/29	0.50	71/231
CRC	Yes/No	8/232	3/61	1/40	0.81	12/333

Table IV - 2: Demographic Characteristics of Patients by Post-IPAA Group

		Categorization				
		Group 1 (n=246)	Group 2 (n=64)	Group 3 (n=45)	<i>p</i>	All Patients (n=355)
EIMs Yes/No (%)	Axial Arthritis/ Ankylosing spondylitis	12/230 (4.9)	3/61 (4.7)	8/36 (18.2)	0.004	23/327 (6/6)
	Sacroilitis	7/235 (2.9)	4/60 (6.3)	2/42 (4.6)	0.43	13/337 (3.7)
	Large joint	60/182 (24.8)	16/40 (28.6)	15/29 (34.1)	0.42	91/259 (26)
	Small joint	13/229 (5.4)	6/58 (9.4)	1/43 (2.3)	0.27	20/330 (5.7)
	Non specific joint inflammation	23/219 (9.5)	13/51 (20.3)	4/40 (9.1)	0.05	40/310 (11.4)
	Osteopenia/ osteoporosis	55/187 (22.7)	10/54 (15.6)	13/31 (29.6)	0.22	78/272 (22.3)
	Erythema nodosum	5/237 (2.1)	4/60 (6.3)	2/42 (4.6)	0.20	11/339 (3.1)
	Pyoderma gangrenoum	6/236 (2.5)	1/63 (1.6)	2/42 (4.6)	0.62	9/341 (2.6)
	Oral ulcers	31/211 (12.8)	13/51 (20.3)	9/35 (20.5)	0.19	53/297 (15.1)
	Eye inflammation	35/207 (14.5)	13/51 (20.3)	9/35 (20.5)	0.62	57/293 (16.3)
	Primary Sclerosing Cholangitis	4/238 (1.7)	3/61 (4.7)	2/42 (4.6)	0.27	9/341 (2.6)
	Renal stones	33/209 (13.6)	9/55 (14.1)	4/40 (9.1)	0.69	46/304 (13.1)
Serum Collect	Mean years (SD)	9.4 (7.3)	9.3 (6.7)	12.4 (8.5)	0.08	9.7 (7.4)
Duration of pouch	Mean years (SD)	9.2 (6.6)	8.9 (6.0)	11.9 (6.1)	0.01	9.5 (6.5)
Complication Onset	Mean years (SD)	2.9 (2.9)	1.8 (3.0)	2.6 (4.4)	0.04	2.4 (3.3)
Pouch failure with permanent ileostomy	Yes/No (%)	5/187 (3)	4/50 (8)	12/38 (32)	<0.0001	21/275 (8)

There was an average of 9.7 years post-op between serum collection and colectomy for the patient cohort ($p=0.08$). The mean duration of years from proctocolectomy to study enrolment was 8.9 years for the chronic/relapsing pouchitis and no/acute limited pouchitis patients and 11.9 years for the CD-like patients ($p=0.01$). Chronic/relapsing pouchitis patients experienced pouch complications commencing 1.8 years post-IPAA which significantly differed from the other two groups ($p=0.04$). CD-like patients were more likely to experience pouch failure with permanent ileostomy (32%) and pouch excision (26%) compared to chronic/relapsing pouchitis (8%; 6%) and no/acute limited pouchitis (3%; 1%) patients ($p<0.0001$, $p<0.0001$, respectively), which is depicted in Table IV - 2.

Figure IV – 5: Histogram of Serum Collection Date From STC and Duration of Years with Pelvic Pouch between Groups



* - statistically significant difference between groups ($p=0.01$)

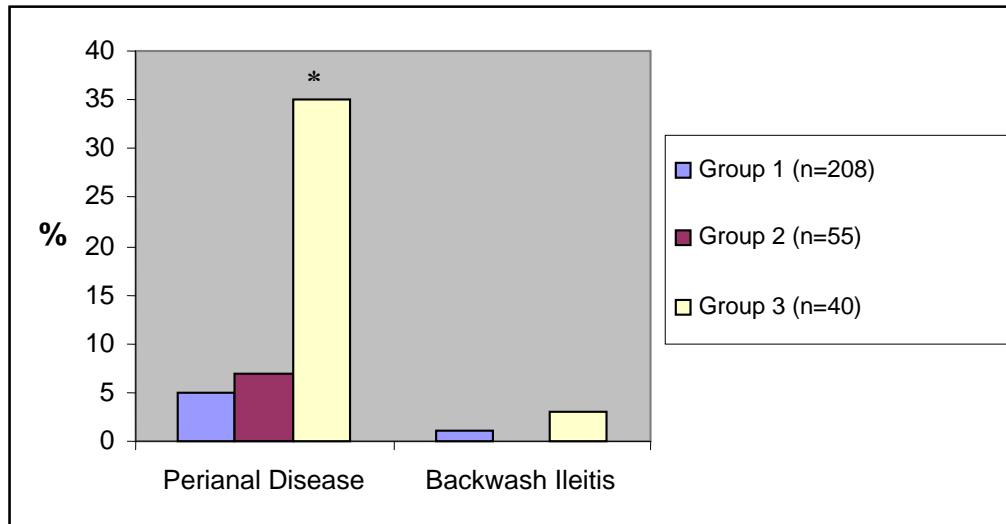
4.2.2. Extent of Disease Pre-Colectomy

Severity of colitis was determined prior to surgery according to the Montreal Classification: 4% had at minimum ulcerative proctitis (rectum) (which included patients whose full disease extent was not known), 7% had left sided colitis (rectum to descending colon), and 89% had extensive UC (pancolitis), with involvement extending proximal to the splenic flexure.^{3, 19} There was no reported difference in the extent of colitis pre-subtotal colectomy (STC) between groups ($p=0.81$).¹¹ There was no difference in the prevalence of backwash ileitis prior to colectomy ($p=51$); however, 35% of CD-like patients had perianal disease pre-STC compared to 6.5% and 5% of pouchitis and no pouchitis patients, respectively ($p<0.0001$), illustrated in Figure IV – 6. It is important to note the definition used in this study for pre-colectomy perianal disease, which includes hemorrhoids and skin tags; this differs from more recent definitions which exclude hemorrhoids and fissures (refer to Table III – 4 for details on definition).

Table IV – 3: Extent of Disease Pre-Colectomy of Patients by Post-IPAA Group

	Categorization			<i>p</i>	All Patients (n=355)
	Group 1 (n=246)	Group 2 (n=64)	Group 3 (n=45)		
Extent of Colitis					
E1 (%)	8 (3)	2 (3)	3 (7)	0.81	13 (4)
E2 (%)	20 (8)	4 (6)	3 (7)		27 (8)
E3 (%)	218 (89)	58 (91)	39 (87)		315 (89)
Perianal Disease Yes/No (%)	11/226 (4.6)	4/58 (6.5)	15/28 (34.8)	<0.0001	30/312 (8.7)
Backwash ileitis Yes/No (%)	3/224 (1.3)	0/57 (0)	1/39 (2.5)	0.51	4/320 (1.2)

Figure IV – 6: Pre-Colectomy Perianal Disease and Backwash Ileitis Prevalence between Post-IPAA Groups



* - statistically significant difference between groups ($p < 0.0001$) with a higher proportion of patients in Group 3 manifesting perianal disease

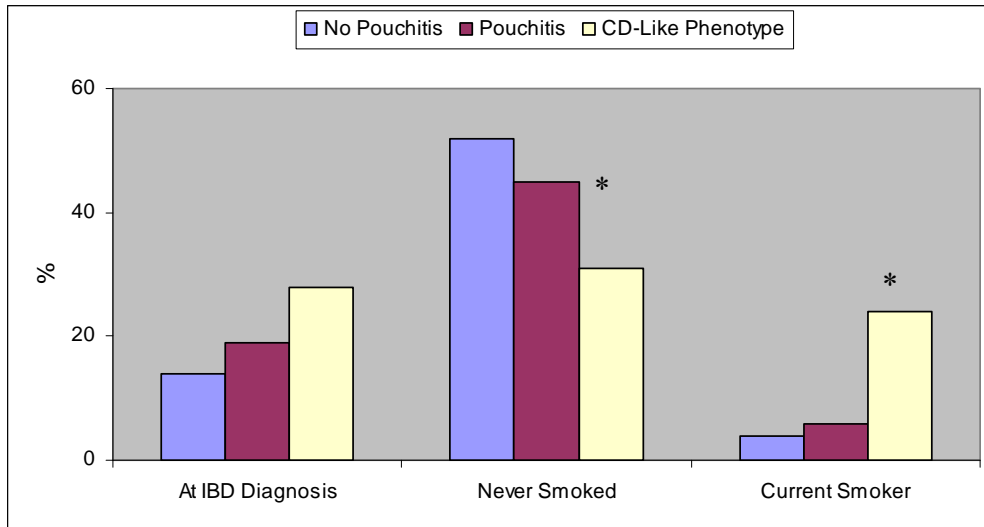
4.2.3. Association between Smoking behaviour and CD-like phenotype Group

Smoking data were collected by questionnaire. Among smokers, there was a significant difference between the CD-like phenotype group and the no/limited acute pouchitis and chronic/relapsing pouchitis groups, respectively. Of the CD-like patients, 24.4% were current smokers compared to 6.3% and 4.2% of the chronic/relapsing pouchitis and no/limited acute pouchitis patients, respectively ($p < 0.0001$). Similarly, a higher proportion of CD-like patients were likely to have ever smoked, 69%, compared to 55% and 48% of pouchitis and no pouchitis patients ($p = 0.04$). Smokers at IBD diagnosis showed a trend towards developing a CD-like phenotype post-IPAA ($p = 0.07$).

Table IV – 4: Association between Smoking Behaviour and Post-IPAA Group

		Categorization			<i>p</i>	All Patients n=355
		Group 1 n=246	Group 2 n=64	Group 3 n=45		
Smoking Status	At IBD Diagnosis	33/206 (13.8)	12/52 (18.8)	11/28 (28.2)	0.07	56/286 (16.4)
	Never Smoked	125/114 (52.3)	29/35 (45.3)	12/27 (30.7)	0.04	166/176 (49.5)
Yes/No (%)	Current Smoker	10/229 (4.2)	4/60 (6.3)	10/31 (24.4)	<0.0001	24/320 (6.9)

Figure IV – 7: Histogram of Smoking Behaviour and Post-IPAA Group

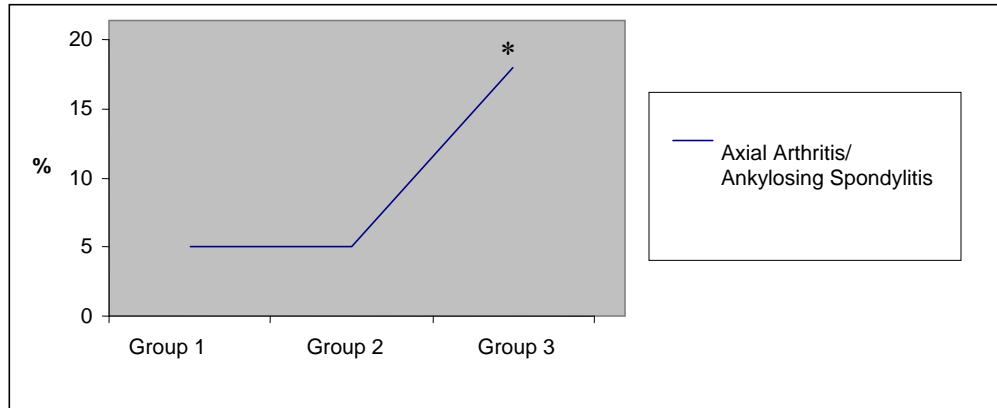


* - statistically significant difference between groups (p=0.04; p<0.0001, respectively)

4.2.4. Extra-intestinal Manifestations

Of the 355 patients with available information, twenty patients (7%) had axial arthritis/ankylosing spondylitis (AS). CD-like patients (Group 3) had significantly higher prevalence of axial arthritis/AS, 18%, compared to 5% of the chronic/relapsing pouchitis patients (Group 2) and 5% of the no/limited acute pouchitis patients (p=0.01). A significantly higher proportion of pouchitis patients (20.3%) reported having experienced non-specific joint inflammation compared to the CD-like and no pouchitis patients (p=0.05). There were no significant differences between groups for the other EIMs investigated (refer to Table IV - 2).

Figure IV – 8: Prevalence of Axial Arthritis/Ankylosing Spondylitis between Groups



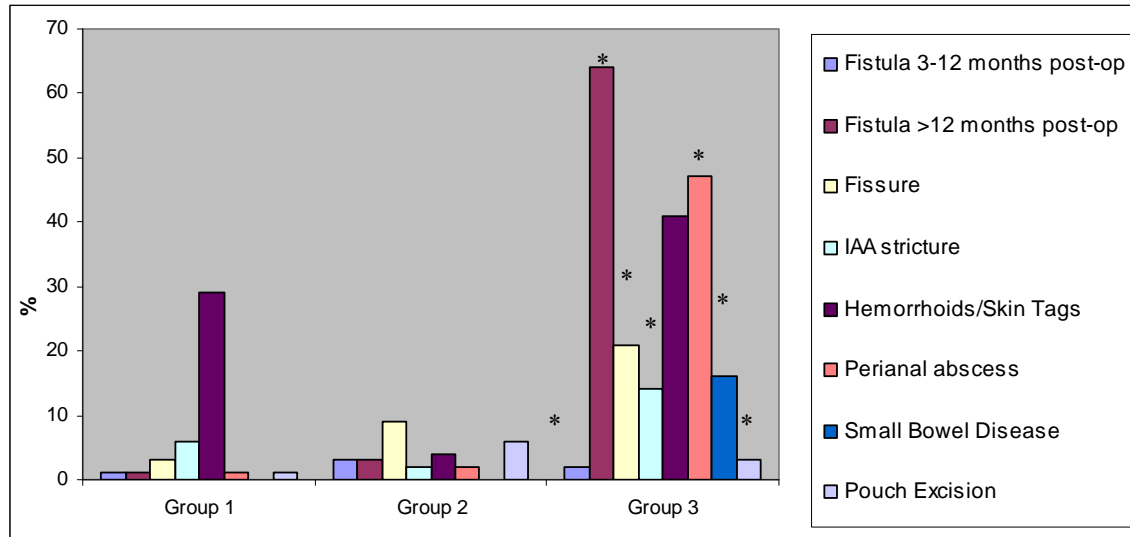
4.2.5. IPAA Surgery, Early and Late Postoperative complications

Surgical characteristics and post-operative complications are summarized in Table IV - 5. 80% of patients had a stapled pouch without mucosectomy and 58% of patients had a J-pouch. Early post-operative complications (within 3 months of STC or IPAA) included minor anatomic leaks requiring antibiotics with or without drainage.³⁸ Fistula occurring between 3-12 months post-IPAA was seen in 3% of patients, and was significantly more prevalent in CD-like patients (17%) compared to the other two groups ($p < 0.0001$). Fistula occurring more than 12 months post-IPAA was reported in 10% of the patient cohort, and was significantly more frequent in CD-like patients, 64%, compared to the other two groups ($p < 0.0001$). Twenty-one patients (6.5%) had a fissure, twenty patients (6%) had an IAA stricture, one hundred and six patients (32%) had hemorrhoids or skin tags, nineteen patients (6%) had a perianal abscess and twenty-eight patients (8%) had small bowel disease, all variables occurring at least 1 year after IPAA. There was an association between the presence of perianal abscess, small bowel disease and pouch excision with the CD-like group ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$, respectively). Refer to Table IV - 5 and Figure IV – 9 for details.

Table IV - 5: Surgical Characteristics and Post-operative Complications

	Categorization			<i>p</i>	All Patients Yes/No (%)
	Group 1 Yes/No (%)	Group 2 Yes/No (%)	Group 3 Yes/No (%)		
Type of anastomosis					
Stapled without mucosectomy	156/39 (80)	43/9 (17.3)	30/8 (79)	0.90	229/56 (80.4)
Handsewn with mucosectomy	39/156 (20)	9/44 (17)	8/30 (21.1)	0.87	56/230 (19.6)
Type of pouch					
J-pouch	139 (56.5)	37 (57.8)	30 (66.7)	0.19	206 (58)
S-pouch	21 (8.5)	2 (3.1)	4 (8.9)		27 (7.6)
U-pouch	2 (1)	0 (0)	2 (4.4)		4 (1.2)
Others	84 (34)	25 (39.1)	9 (20)		118 (33.2)
Post-operative complications - Yes/No					
Fistula 3-12months post-op	1/221 (0.4)	2/56 (3.4)	7/34 (17.1)	<0.0001	10/311 (3.1)
Fistula >12 months post-op	1/220 (0.4)	2/56 (3.4)	29/16 (64.4)	<0.0001	32/292 (10)
Fissure	7/215 (3.2)	5/52 (8.8)	9/35 (20.5)	<0.0001	21/302 (6.5)
IAA stricture	13/209 (5.9)	1/56 (1.8)	6/38 (13.6)	0.05	20/303 (6.2)
Hemorrhoids/Skin Tags	65/158 (29.1)	23/34 (40.4)	18/26 (40.9)	0.13	106/218 (32.7)
Perianal abscess	3/219 (1.4)	1/56 (1.8)	14/30 (31.8)	<0.0001	19/304 (5.9)
Small Bowel Disease	0/210 (0)	0/47 (0)	7/36 (16.3)	<0.0001	28/293 (2)
Pouch Excision	3/238 (1.2)	4/60 (6.3)	11/32 (25.6)	<0.0001	18/330 (5.2)

Figure IV – 9: Post-Operative Complications between Groups



* - refers to statistically significant difference between groups when comparing Groups 1, 2 and 3. There was a higher proportion of individuals in Group 3 who manifested fistulae (3-12, >12 months post-op), fissure, IAA stricture, perianal abscesses, small bowel disease and pouch excision.

4.2.6. Functional Outcome post-IPAA and Medications

Functional outcome was assessed in patients who underwent IPAA with at least one year of follow up available and is summarized in Table IV - 6. For urgency and cramping, 40% of patients reported none, 16% reported occasional and 44% reported usual symptoms. The chronic relapsing pouchitis patients were the most likely to report the usual occurrence of urgency and cramping, 67.2%, compared to the CD-like and no/limited acute pouchitis patients, 47.1% and 37%, respectively (p=0.001). For fecal incontinence, 59% had none, 21% had occasional and 20% had usual symptoms. However, the usual occurrence of fecal incontinence was associated with Group 1 (41.2%) compared to group 2 (24.1%) and group 3 (15%) (p=0.0002). Forty six patients (15%) reported having had a fever associated with pelvic pouch flares. Both the CD-like patients and chronic relapsing pouchitis groups had similar frequency of fevers associated with pelvic pouch complications (29%) compared to the no/limited acute pouchitis patients (9%) (p<0.0001). Two hundred and thirty six patients (73%) reported an increase in stool frequency compared to their baseline due to pouch irritation and flares. The chronic relapsing pouchitis

patients reported a significantly higher prevalence of increased stool frequency, 89%, compared to the no/limited acute pouchitis and CD-like patients (p=0.003).

The chronic/relapsing pouchitis group had the highest frequency of antibiotic use, where 90% had been treated with ciprofloxacin and 97% with flagyl (metronidazole) compared to the CD-like patients (79%; 86%) and the no/limited acute pouchitis patients (35%; 41%) (p<0.0001). In addition, the chronic/relapsing pouchitis patients were more likely to take probiotics, 25%, compared to the CD-like (2.5%) and no/limited acute pouchitis groups (5%) (p<0.0001). Of the total patient cohort, 49% of patients have taken or continued to take Imodium to feel well (p=0.70). The highest proportion of CD-like patients were treated with remicade, 45%, compared to patients in group 2 (8%) and group 1 (0.5) (p<0.0001).

Table IV - 6: Functional Outcome and Medications of Groups Post-IPAA and Patient Cohort

	Categorization			p	All Patients
	Group 1	Group 2	Group 3		
Urgency/Cramping (%)					
None	98 (45.8)	12 (20.7)	12 (35.3)	0.001	122 (39.9)
Occasional	37 (17.3)	7 (12.1)	6 (17.6)		50 (16.3)
Usual	79 (36.9)	39 (67.2)	16 (47.1)		134 (43.8)
Fecal Incontinence (%)					
None	142 (66.7)	27 (46.6)	10 (30.3)	0.0002	180 (59.0)
Occasional	39 (18.3)	17 (29.3)	9 (27.3)		65 (21.3)
Usual	32 (15.0)	14 (24.1)	14 (42.4)		60 (19.7)
Fever Yes/No (%)	19/195 (8.9)	17/42 (28.8)	10/24 (29.4)	<0.0001	46/261 (15.0)
Increase in Stool Frequency from baseline Yes/No (%)	158/65 (70.9)	55/5 (91.7)	23/11 (67.6)	0.003	236/81 (74.4)
Medications Used Post-IPAA Yes/No (%)					
Ciprofloxacin	83/154 (35.0)	56/7 (88.9)	34/9 (79.1)	<0.0001	173/170 (50.4)
Flagyl	96/141 (40.5)	62/2 (96.9)	38/6 (86.4)	<0.0001	196/145 (57.5)
Imodium/lomotil/ loperamide	113/115 (49.6)	32/29 (52.5)	18/23 (43.9)	0.70	163/167 (49.4)
Vancomycin	2/229 (1)	4/57 (6.6)	0/41 (0)	0.008	6/327 (1.8)
Probiotics	11/213 (4.9)	15/46 (24.6)	1/39 (2.5)	<0.0001	27/298 (8.3)
Remicade	1/241 (<1)	5/58 (7.9)	18/22 (45.0)	<0.0001	24/321 (7.0)

4.3. SEROLOGICAL RESULTS

Of the 304 patients with serological data available, overall prevalence of CD-associated IgG ASCA in the study population was 21%. 40% of CD-like phenotype patients were gASCA positive which was significantly different than the no/limited acute pouchitis and chronic/relapsing pouchitis patients (p=0.006). From Table IV – 7, it appears that the patients with chronic/relapsing pouchitis had a serological profile that was similar to patients with no/limited acute pouchitis. Of the 24 patients (60.0%) seronegative for gASCA IgG, 12.5% were anti-L positive and 4.2% were anti-C positive. The overall positive prevalence of anti-L units in the study population was 5%. The CD-like phenotype group had a higher rate of anti-L positive status, 12.5%, compared to the no/limited acute pouchitis and chronic/relapsing pouchitis groups (4.3% and 1.8%, accordingly; p=0.04). The seropositive prevalence of ASCA-A, AMCA, anti-C, ALCA and ACCA was not significantly different between groups (p=0.27, p=0.40, p=0.58, p=0.63, p=0.68, accordingly). See Table IV – 7 for serological results.

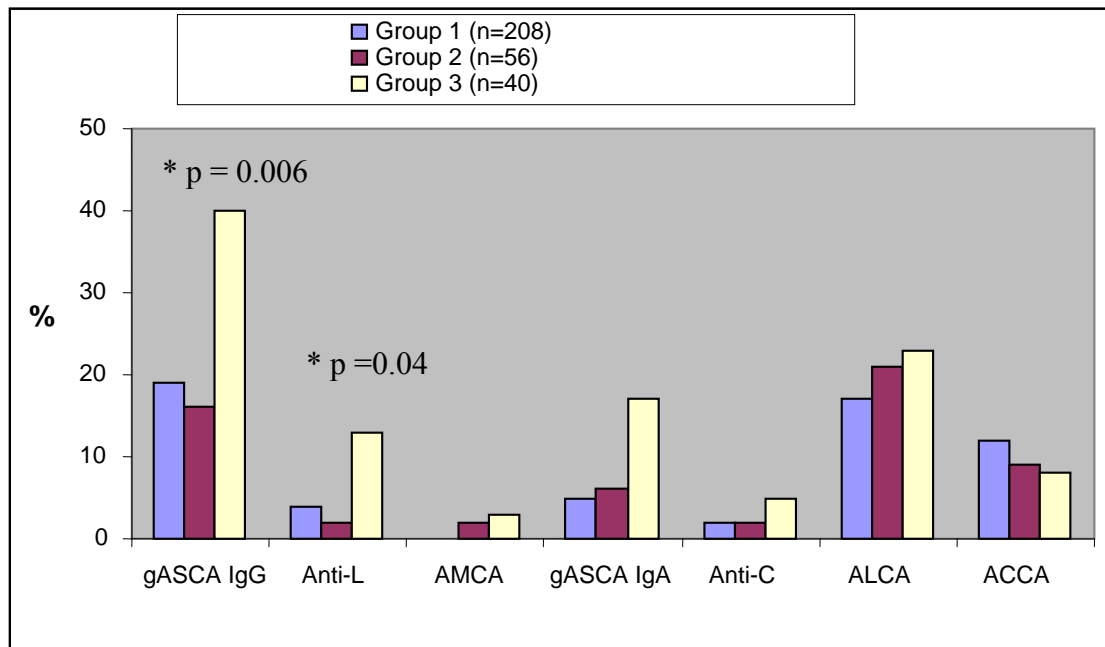
Table IV – 7: Association between Seropositivity and Post-IPAA Classification and Patient Cohort

Marker	Titer Cut-off Values	Categorization			p	All Patients n=304 (%)
		Group 1 n=208 (%)	Group 2 n=56 (%)	Group 3 n=40 (%)		
gASCA	<= 50 vs. >50	169/39 (18.8)	47/9 (16.1)	24/16 (40.0)	0.006* (0.0009)**	240/64 (21.1)
ASCA-A	<= 50 vs. >50	40/2 (4.8)	16/1 (5.9)	15/3 (16.7)	0.27 (0.33)	71/6 (7.8)
Anti - L	<= 60 vs. >60	199/9 (4.3)	55/1 (1.8)	35/5 (12.5)	0.04 (0.01)	289/15 (4.9)
AMCA	<= 100 vs. >100	207/1 (0.5)	55/1 (1.8)	29/1 (3.3)	0.40 (0.37)	301/3 (1)
Anti - C	<= 90 vs. >90	203/5 (2.4)	55/1 (1.8)	38/2 (5)	0.58 (0.11)	296/8 (2.6)
ALCA	<= 60 vs. >60	172/36 (17.3)	44/12 (21.4)	31/9 (22.5)	0.63 (0.96)	247/57 (18.8)
ACCA	<= 90 vs. >90	184/24 (11.5)	51/5 (8.9)	37/3 (7.5)	0.68 (0.26)	272/32 (10.5)

* - statistically significant using Chi-Square

** - p-value for regression model using continuous serum levels

Figure IV – 10: Prevalence of the Anti-Glycan Antibodies in Post-IPAA Groups



* - statistically significant using Chi-Square

A general linear model was used for serology as a continuous variable to allow for analysis of more than one independent variable (3 groups). Since the continuous serological data was not normally distributed, logarithmic transformation for the original data was performed, followed by use of a general linear model for the transformed data.

4.3.1. Multivariate analysis:

Multivariate analysis was performed for post-IPAA grouping of no/limited acute pouchitis vs. chronic/relapsing pouchitis vs. CD-like phenotype, for the following diagnostic factors: current smoking status, duration of pouchitis, gASCA positivity, and anti-L positivity. From these diagnostic factors, current smoking, and mean duration of years with the pelvic pouch were significant in the multivariate analysis ($p=0.0003$, and 0.02 , respectively), gASCA positivity was marginally significant ($p=0.07$), while anti-L positivity was not significant ($p=0.77$). The multivariate analysis was applied using a generalized logistic model. Each diagnostic factor found to be significant in the multivariate analysis was associated with the CD-like phenotype group, and hence in the direction expected.

4.4. GENETIC RESULTS

Twenty five SNPs associated with IBD, CD or UC were selected for analysis based on their potential role in immune and inflammatory pathways.^{226, 227, 232, 260, 271-273}

NOD2/CARD15 is a sensor for bacterial muramyl dipeptide that regulates antimicrobial immunity and inflammation via activation of kinases and transcription factors, including nuclear factor kappa B (NF- κ B). The NOD2/CARD15 protein is expressed in monocytes/macrophages, dendritic cells and intestinal epithelial cells.²⁷⁴ In this context, it is purported that an impaired innate immune response in CD might lead to an overly aggressive microbial-induced inflammatory response and expression of antibodies against self and nonself antigens.²²¹ Hence, it may be that the mechanism by which NOD2 mutations lead to intestinal inflammation is due to reduced ability to clear bacteria by innate immune mechanisms which leads to dysregulation of adaptive immune pathways²²⁷. Mechanisms by which NOD2 mutations lead to disease development remain controversial, but underscore the importance of immune response against the enteric microbiota.²⁷⁴

The IL23/IL12 pathway has an important area of study in immunology as it plays a critical role in determining differentiation of naïve T cells into effect Th1 cells (driven by IL12) or Th17 cells (driven by IL-23).²²⁷ IL-23 is produced by dendritic cells/macrophages and has been shown to be activated by bacterial ligands and expands IL-17 producing help T cells. Functional consequences of the IL23R gene variations have not yet been reported.²⁷⁴ In addition, it has been reported that specific bacterial components, such as peptidoglycan, can differentially induce antigen presenting cells to produce IL23 rather than IL12; as a result, this early regulatory mechanism may precipitate a distinct inflammatory response.²⁷⁵

The ATG16L1 gene is involved in autophagy which is a cellular pathway for degradation of long-lived proteins and cytoplasmic organelles. Autophagy is recognized as a key mechanism of innate immunity and recent studies have implicated autophagy in the processing of intracellular bacteria such as *Mycobacterium*.²⁷⁶ The ATG16L gene is expressed in intestinal epithelial cells,

lymphocytes and macrophages. The purported mechanism by which ATG16L influences CD susceptibility is by altered ability of autophagy to eliminate microbes in innate immune cells of the intestine.²⁷⁵ Recent studies have implicated autophagy in the processing of intracellular bacteria such as *Mycobacterium*.²⁷⁶

CD-associated loci, including the MST1 locus and variants in the IL-23 pathway, also contribute to UC susceptibility. Genes that are specifically associated with CD but not UC, including NOD2 as well as ATG16L1 and IRGM, affect intracellular handling of bacterial antigens, suggesting distinct pathogenic mechanisms relating to microbial processing. MST1 is known to suppress cell-mediated immunity by down-regulating IL-12 and has been associated with CD.²⁵⁹ MST1 encodes a protein that induces phagocytosis by resident peritoneal macrophages – variants associated with CD and UC.^{259, 273} IL-12B encodes a subunit shared by IL-12 and IL-23.²⁵⁹

Cytokines have been implicated in the pathogenesis of IBD and have an effector and regulatory role in the mucosal immune and inflammatory response in IBD. IL-1 is a major pro-inflammatory cytokine involved early in the inflammatory cascade. The IL-1RA is the natural inhibitor of these IL-1 agonists and acts by competitively binding to IL-1 receptors without eliciting signal transduction. These proteins are coded by genes on in the IL-1 gene cluster on chromosome 2. Increased production of IL-1 has been shown in the gut tissue of animal models. An imbalance in the IL-1RA/IL-1 ratio may contribute to the chronic inflammatory response in UC.¹³⁷

PTGER4, HERC2, CCNY and NKX2-3 further highlight the importance of the immune system in IBD pathogenesis. PTGER4 encodes prostaglandin receptor EP4, which has been strongly implicated in mouse model of IBD. PTGER4 gene expression is associated with polymorphisms mapping to large gene desert on chromosome 5p13, which has shown strong association with CD. HERC2 is involved in ubiquitination and has been found to be associated with CD. Ubiquitination, similar to phosphorylation, is a diverse cellular control mechanism.²⁷⁷

CCNY is involved in controlling cell cycling and is expressed in monocytes and dendritic cells. NKX2-3 is a poorly characterized transcription factor which is expressed in intestine, with variants associated with CD and UC.^{259, 273} NKX2-3-deficient mice develop gut-associated as well as splenic lymphoid tissue abnormalities with disordered segregation of T and B cells.²⁷⁸

Variants in the MHC in IBD have been studied in both genome-wide linkage scans as well as candidate gene studies. The MHC region, on chromosome 6, contains multiple immunoregulatory genes, including HLA and TNF- α gene.²⁷⁵ The HLA genes determine the specificity of the immune response, and they have been greatly studied functional candidate genes in IBD – a large body of evidence for an association between classical HLA loci and UC exists.²⁷⁹ Because of the complex pattern of linkage disequilibrium in HLA class II genes, it remains to be determined whether observed associations are due to variation in HLA class II genes themselves, at neighbouring loci, i.e. BTNL2, or both.²⁶⁰ IBD5 is attractive as a candidate region for IBD, as it contains the cytokine gene cluster.²²⁵

Pattern recognition receptors (PRRs), including TLRs, are critical components of the innate immune system as recognition of microbial products occurs by PRRs that are expressed by innate effector cells. Hence, microbial recognition results in efficient and fast immune response against invading microorganisms.²²⁶ The TLR4 +896A>G SNP (rs4986790) affects the leucine-rich repeat domain of TLR4 and is linked with hyporesponsiveness to LPS²⁸⁰ with increased susceptibility to severe bacterial infections and IBD²⁸¹ and may predispose to septic shock with Gram-negative microorganisms.²⁸² TLR9 is needed for the recognition of CpG motifs, short sequences of unmethylated DNA mainly present in bacterial DNA. CpG motifs have immunostimulatory activity by inducing dendritic cell maturation, B-cell proliferation and production of cytokines, including IL-12.²⁸³ The release of bacterial DNA from microflora might favour immune homeostasis, suggested by a study showing that TLR9 signaling mediated the resolution of intestinal inflammation in experimental colitis.²⁸⁴ CD14 is part of the

endotoxin/LPS receptor complex,²⁸⁵ and is important, along with TLR2 and TLR4, in the recognition of LPS.²⁸⁶

PTPN2 encodes a T cell protein tyrosine phosphatase (TCPTP) which is a key negative regulator of inflammation, such that dysregulation of TCPTP might lead to marked elevation of several pro-inflammatory cytokines such as TNF- α and IL12.²⁸⁷

ECM1 encodes a protein which is implicated in maintaining the barrier function of the gut wall. ECM1 variants most likely lead to altered intestinal permeability.²⁵⁹

Recently, loci on chromosomes 1p36 and 12q15 have been identified, where genes involved in inflammation and immunity include part of phospholipase A2, group IIE (PLA2G2E), interferon- γ (IFNG), IL26 and IL22. Significant association signals on chromosome 1p36 were located, including that of PLA2G2E. PLA2G2E is a member of the secretory phospholipase A2 family of proteins which release arachidonic acid from membrane phospholipids; ultimately, this leads to the production of proinflammatory lipid mediators, including prostaglandins and leukotrienes. {Silverberg, 2009 #364} PLA2G2E expression in lung and small intestine is induced with LPS stimulation, which suggests a role in bacterially associated inflammation. Chromosome 12q15 has shown a significant signal (rs1558744) in a region without established coding genes; however the IFNG, IL-26 and IL22 genes are located 44kb, 91kb and 137kb, respectively, telomeric to rs1558744. IFNG is critical in the immune response to pathogens, partially via regulation of macrophage function; it has been shown to regulate many levels of immune homeostasis, including T cell subsets, NK cells and NK T cells. {Silverberg, 2009 #364}

Of the 25 SNPs, 3 SNPs, rs2066845 (chr16, gene CARD15/NOD2), rs11209026 (chr1 IL23R) and rs2066844 (chr16 gene CARD15/NOD2), had low minor allele frequency (less than 5%) and were excluded from the association analysis. The remaining 22 SNPs were statistically analyzed using Pearson's chi squared, comparing genotypes based on three genetic models: the additive, dominant and recessive model (refer to Table IV – 8). No significant differences in genotype frequencies of the gene polymorphisms for IL23R, gene desert 1p36, ATG16L1,

MST1, Intergenic PTGER4, IBD5, IL12B, BTNL2, CCNY, NKX2-3, gene desert 12q14, HERC2, CARD15/NOD2, IL-1Ra, TLR4, and TLR9 were found between the three groups for both a three level and two level outcome (Group 1/Group 2 vs. Group 3 and Group 1 vs. Group 2/Group 3).

Table IV – 8: Summary of Chromosome, Gene/Region, SNP and Association to IBD Analyzed

Chromosome	Gene or Region	SNP	Association
1p31	IL23R	rs10889677	UC
1p36	Unknown	rs6426833	UC
1q21.2	ECM1 G290S	rs13294	UC
2q37.1	ATG16L1	rs2241880	CD
5p13	Intergenic (PTGER4)	rs9292777	CD
3p21.3	TLR9 (2848 G>A)	rs352140	Innate immunity
3p21	MST1	rs9858542	UC
3p21	MST1	rs3197999	CD
5q31	IBD5	rs2188962	CD
5q31	CD14 (-216C>T)	rs2569190	Pouchitis
5q33	IRGM	rs11747270	CD
5q33	IL12B	rs10045431	CD
6p21	BTNL2 to HLA-DQB1	rs2395185	UC
6p21.33	BTNL2	rs9268480	UC
9q32-q33	TLR4 (896 A>G)	rs4986790	
10p11.21	CCNY	rs3936503	UC
10q24	NKX2-3	rs10883365	UC
12q15	Unknown	rs1558744	UC
15q13.1	HERC2	rs916977	UC
16q12	CARD15(NOD2)	rs2076756	CD
18p11.21	PTPN2	rs2542151	CD
2q	IL-1RA(2018 T>C)	rs419598	Pouchitis

4.4.1. Three Level Outcome – Group 1 vs. Group 2 vs. Group 3

There was a marginally significant association with SNP rs2569190 (chromosome 5q31, gene CD14) between the three groups. The genotype frequency for this polymorphism is described in Table IV - 9. The carriership of allele CD14 -216A was more frequent in patients with chronic/relapsing pouchitis (81.5%) as compared to those with no/acute limited pouchitis (71%) and CD-like phenotype (67.5%) ($p=0.05$). The OR was 1.39 for Group 2 versus Group 1, and OR was 0.35 for Group 3 versus Group 1 ($p=0.05$).

Table IV - 9: Genotypes, P-values and Odds Ratio of the CD14 Gene Polymorphism in Subgroups of Patients

SNP (Gene)	Genotype (minor allele/major allele)	Total n=315 (%)	Group 1 n=216 (%)	Group 2 n=59 (%)	Group 3 n=40 (%)	p-value	OR
rs2569190 (CD14)	AA	74 (23.5)	52 (24.1)	18 (30.5)	4 (10)	0.05	1.39 ¹ 0.35 ²
	AG	154 (48.9)	101 (46.8)	30 (50.9)	23 (57.5)		
	GG	87 (27.6)	63 (29.1)	11 (18.6)	13 (32.5)		

* P-values and odds ratio were calculated with Pearson's chi-squared for SNP rs2569190 using a recessive model with OR¹ corresponding to Group 2 vs. Group 1 and OR² to Group 3 vs. Group 1 (unadjusted p-values).

Table IV – 10: Minor Allele Frequency of the CD14 Gene Polymorphism in Subgroups of Patients

SNP (Gene)	Chr	Minor Allele	Minor Allele Frequency			
			Total n=315	Group 1 n=216	Group 2 n=59	Group 3 n=40
Rs2569190 (CD14)	5q31	A	0.48	0.48	0.56	0.39

4.4.2. Two Level Outcome - Group 1/Group 2 vs. Group 3

An association analysis was applied to a two level outcome comparing patients in Group 1 and Group 2 to patients in Group 3 (Group 1/Group 2 vs. Group 3). A significant association between groups was found for two SNPs, rs13294 (chromosome 1q21, gene ECM1) and rs2569190 (chromosome 5q31, gene CD14) (p=0.04, OR=2.94; p=0.03, OR=3.07, respectively). Refer to Table IV – 11 and Table IV – 12 to see allele and genotype frequencies and associated p-values and ORs.

Table IV – 11: Genotypes, P-values and Odds Ratios of the ECM1 and CD14 Gene Polymorphisms in Subgroups of Patients

SNP (Gene)	Genotype (minor allele/major allele)	Total n=315 (%)	Group 1/ Group 2 n=275 (%)	Group 3 n=40 (%)	p-value	OR
rs13294 (ECM1)	AA	46 (14.6)	43 (15.6)	3 (7.5)	0.04	2.94
	AG	147 (46.7)	131 (47.6)	16 (40)		
	GG	122 (38.7)	101 (36.7)	21 (52.5)		
rs2569190 (CD14)	AA	74 (23.5)	70 (25.5)	4 (10)	0.03	3.07
	AG	154 (48.9)	131 (47.6)	23 (57.5)		
	GG	87 (27.6)	74 (26.9)	13 (32.5)		

* P-values and odds ratio were calculated using Pearson's chi-square (Group 1/Group2 vs. Group 3) using an additive model for SNP rs13294 and a recessive model for SNP rs2569190 (unadjusted p-values).

Table IV – 12: Minor Allele Frequency of the ECM1 and CD14 Gene Polymorphisms in Subgroups of Patients

SNP (Gene)	Chr	Minor Allele	Minor Allele Frequency		
			Total n=315	Group 1/Group 2 n=275	Group 3 n=40
rs13294 (ECM1)	1q21.2	A	0.38	0.40	0.28
rs2569190 (CD14)	5q31	A	0.48	0.49	0.39

4.4.3. Two Level Outcome – Group 1 vs. Group/Group 3

Lastly, association analysis was applied to a two level outcome, this time comparing patients in Group 1 versus those in Groups 2 and 3 (Group 1 vs. Group2/Group 3). The most significant association was obtained for SNP rs2542151 ($p=0.02$; $OR=1.88$) at the PTPN2 locus (protein tyrosine phosphatase, non-receptor type 2) on chromosome 18p11 (refer to Table IV – 13 and Table IV – 14).

Table IV - 13: Genotypes, P-value and Odds Ratio of the PTPN2 Gene Polymorphism in Subgroups of Patients

SNP (Gene)	Genotype (minor allele/major allele)	Total n=314 (%)	Group 1 n=215 (%)	Group 2/ Group 3 n=99 (%)	p-value	OR
rs2542151 (PTPN2)	CC	13 (4.1)	8 (3.7)	5 (5.1)	0.02	1.88
	CA	88 (28)	70 (32.6)	18 (18.2)		
	AA	213 (67.9)	137 (63.7)	76 (76.7)		

* P-values and odds ratio were calculated using Pearson's chi-squared (Group 1 vs. Group 2/Group 3) using a dominant model for SNP rs2542151 (unadjusted p-values).

Table IV – 14: Minor Allele Frequency of the PTPN2 Gene Polymorphism in Subgroups of Patients

SNP (Gene)	Chr	Minor Allele	Minor Allele Frequency		
			Total n=315	Group 1 n=216	Group 2/Group 3 n=99
rs2542151 (PTPN2)	18p11	C	0.18	0.20	0.14

In summary, this analysis was not adjusted by multiple comparisons. For SNP rs2569190, there is a signal for both a three level, and two level outcomes, which suggests that this SNP is worth further exploring. The weak signals of the association analysis may be due to a relatively small sample.

5.1. DISCUSSION - CLINICAL

Pouchitis is reported as the most frequent complication of surgery for UC patients.¹¹ Established risk factors for pouchitis include extra-intestinal manifestations (EIMs) of IBD, nonsmoking, and PSC.²⁸⁸ In the worst of cases, a patient will require pouch excision with creation of a permanent ileostomy.¹¹ All criteria, including symptoms, endoscopic findings, histology and/or radiology was used to assess the prevalence and severity of pouch inflammation.¹⁴⁰ Patients enrolled in this study had undergone colectomy between 1967 and 2007, and prior to final their inclusion, it was ensured that each patient had at least 1 year of exposure to the onset of pouchitis. Though this is a short exposure period, the fact that the mean number of years from IPAA to study start was 9.5 years, suggests that this long exposure time is adequate for optimal accuracy of our patient grouping. It is known that pouchitis may initially develop as late as 5 years after ileostomy closure; as a result, our post-op follow-up well surpasses this initial timeframe.²⁸⁹ We were able to contact only a fraction (30%) of patients who had restorative proctocolectomy despite multiple attempts. It would be impossible to compare those patients who had geographically relocated to patients involved in this study due to insufficient data. We attempted to enroll all patients with whom we established contact without selection bias. Historically, patients with IC are not ideal candidates for pouch surgery because of pouch failure, refractory pouchitis, and postoperative diagnoses of CD.¹⁴⁰ In retrospective surgical series, an incidence of pouch failure, ranging from 1.4% to 13%, has been described.^{290,}²⁹¹ In this study, seven patients had a pre-operative diagnosis of IC, of which 29% were classified in the CD-like phenotype group (Group 3) and 43% were classified in the chronic relapsing pouchitis group ($p=0.14$). Perhaps the numbers were too low to reach statistical significance.

The rate of chronic relapsing pouchitis was 18% in our IPAA patient cohort which falls below the range, 20-59%, of recent reported cases of pouchitis UC patients with IPAA.¹⁴² One explanation for this difference is that patients with only mild symptoms, or acute limited

pouchitis, were not considered apart of the “true pouchitis” group in this study; hence, a difference in definitions of what constitutes true pouchitis can result in discrepant results between studies. Also, patients with chronic problems post-IPAA, including many individuals who were refractory to medications, and who had clinical symptoms in line with our definition of CD of the pouch, were classified into the CD-like phenotype group. This classification is a more recent approach in deciphering between patients who have divergent phenotypes post-IPAA. This study illustrates that there are a wide variety of inflammatory and non-inflammatory complications following IPAA surgery for UC that must be considered in the differential diagnosis; in particular, it is a difficult process categorizing patients, post-IPAA, into any set number of groups due to the various phenotypes observed. The rate of CD-like phenotype (Group 3) of the pouch was 13% in this patient cohort. In previous studies, this group of patients may have been put into the pouchitis group due to broader definitions of pouchitis. Also, mean period of follow-up post-IPAA varies between series and based on previous studies with distinct definitions of pouchitis, the risk of pouchitis increases with duration of follow-up.¹³⁵ In our patient cohort, the mean number of years from IPAA to study initiation was 9.5 years for the patient cohort, with a significant difference between the CD-like group, 12 years, compared to the chronic relapsing pouchitis and no/acute limited pouchitis groups, 9 years ($p=0.01$). Clearly, the lack of a standard definition of pouchitis, explains the wide variation in the rate of pouchitis post-IPAA. For example, a study of 114 patients, from Seattle, Washington, reported that the incidence of pouchitis was 59%, and that the incidence increased with duration of follow-up.¹⁴² Furthermore, the incidence of pouchitis may seem uncharacteristically low in a patient population where patients are seen on a non-routine basis, i.e. only when showing symptoms of pouchitis. For example, Mount Sinai Hospital is a tertiary referral centre where patients from across Canada come for pelvic pouch surgery; subsequently, patients who encounter pelvic pouch problems may seek physician care near their residence which may also contribute to a lower reported incidence of pouchitis in this study compared to other similar studies.¹⁴⁰ On the other hand, MSH is a

tertiary referral center and patients who experience complications may return to visit their surgeon for follow-up more frequently for this reason.

A newer finding from our study is that the CD-like phenotype, a subset of patients who may have previously been classified as having pouchitis, have a significantly longer duration with their pelvic pouch than those individuals in Group 1 and Group 2 ($p=0.01$). One possible explanation is that these CD-like patients, who have experienced chronic inflammation over a longer period of time, may start to manifest more severe disease, such as fistulizing disease and pre-pouch ileal disease, which is a result of their genetic susceptibility. For example, of the forty five CD-like patients, twenty-five (64%) had a non-surgery related fistula and seven (16%) had small bowel disease which was significantly different from the other two groups ($p<0.0001$ and $p=0.0007$, respectively). Also, our study showed that a pre-colectomy diagnosis of perianal disease (refer to Table IV – 3 for definition) was significantly higher in those who developed a CD-like phenotype post-IPAA. This observation is in line with a study conducted by Richard *et al.* who found that 6.9% of their patients, who had a pelvic pouch produce with a pre-operative diagnosis of IBDU or UC, had pre-operative perianal disease and poor post-operative outcome. These authors used a similar definition of perianal disease.²⁶⁷ In order to determine the importance potential relevance of our results on perianal disease, follow-up chart review and clarification of definitions used to classify patients with pre-operative perianal disease in the IBD Database are necessary. My information on pre-operative perianal disease came from chart review and the IBD Database. The IBD Database requires that there be pathological confirmation for perianal complications in order for this information to be entered in the system.

Patient self-report of symptoms can be useful to determine patient's quality of life post-IPAA; however, symptoms do not necessarily indicate whether or not a patient has pouchitis on endoscopy, histology and/or radiology.¹⁴⁵ The chronic/relapsing pouchitis patients were the most likely to report the usual occurrence of urgency and cramping, 67.2%, compared to the CD-like and no/acute limited pouchitis patients, 47.1% and 37%, respectively ($p=0.001$). However, the

usual occurrence of fecal incontinence was associated with the CD-like group (41.2%) compared to the chronic/relapsing pouchitis (24.1%) and the no/acute limited pouchitis (15%) groups (P=0.0002). The CD-like patients and chronic/relapsing pouchitis patients reported similar frequency of fevers associated with pelvic pouch problems, 29% compared to 9% of no/acute limited pouchitis patients (p<0.0001). Overall, patient report of increased stool frequency seemed to be associated with diet and general daily life stresses, as well as pouch inflammation, where 92% of chronic/relapsing pouchitis, 71% of no/acute limited pouchitis and 68% of CD-like patients reported this symptom occurring since their surgery (p=0.003). Patients reported onset of pouch complications occurring a mean of 2.4 years post-IPAA, and there was a significantly shorter number of years reported by the chronic/relapsing pouchitis patients (1.8 years) compared to the CD-like (2.6 years) and the no/acute limited pouchitis group (2.9 years) (p=0.04).

In general, this clinical scenario of CD-like phenotype occurring post-IPAA underscores the imprecision when diagnosing and predicting the natural course of IBD that still exists today. The fact that the patients develop a CD-like phenotype or CD post-IPAA does not mean that patients were “misdiagnosed” with UC pre-operatively; our findings suggest that a patient’s natural course, and potentially true diagnosis of CD, was only revealed post-IPAA.²⁷⁴

5.1.1. Extra-intestinal Manifestations

There are many common extraintestinal manifestations (EIMS) in IBD which can be considered immune-mediated disease entities such as arthritis/ankylosing spondylitis, pyoderma gangrenosum, erythema nodosum, iritis, uveitis and PSC.²⁹² IBD patients have been shown to more commonly have AD than in non-IBD individuals, which suggests that IBD may share common etiologic factors with other immune-mediated diseases.^{131 145} Although the true interactions between immune-mediated diseases and pouch disorders are poorly understood, prior studies have demonstrated that patients who exhibit EIMS, such as PSC and arthralgias,^{135,293} and patients with positive autoantibodies, such as pANCA, have a high risk for pouchitis.¹³⁹ For

example, in a population-study with 3,876 UC and 4,192 CD patients, both groups of patients had a significantly greater chance of having arthritis, bronchitis, psoriasis, asthma and pericarditis than population controls.¹³¹ In our study, axial arthritis/ankylosing spondylitis was significantly more prevalent in CD-like patients (Group 3), 18%, compared to the no/acute limited pouchitis patients (Group 1), 5%, and chronic/relapsing pouchitis patients (Group 2), 5%, (p=0.004). Ankylosing spondylitis (AS) which is a more severe form of spinal arthritis, affects between 2-3% of IBD patients and is more common in CD patients than in UC patients. Our reported association between axial arthritis/AS and the CD-like patients is in line with similar data that suggests this specific association is characteristic of a CD phenotype than a UC phenotype. The effect of immune-mediated disorders on the disease course of pouch disorders is not clear but possible explanations include: shared etiological factors, disease condition may be causes or complications of the others; or association of these diseases that is coincidental and not related..^{134, 199} The present study is limited by the fact that we had only eight patients with PSC, so PSC prevalence in Group 2 (4.7%), Group 3 (4.7%) and Group 1 (1.7%) failed to reach statistical significant. HLA associations with PSC have also been described.²⁹⁴ Immune mechanisms for pouchitis and IBD have been extensively studied, and there is clearly overlap of tissue profiles of cytokines and other inflammatory mediators between pouchitis and UC.²⁹⁵⁻²⁹⁷ It would be of interest to compare post-operative outcome if preoperative EIMs were present. In our study, the presence of EIM at time of patient interview and chart review was recorded as present or absent; hence, it would be important to see if preoperative occurrence of EIMs in the joints is associated with postoperative outcome. Additional studies are needed to address whether potential associations between EIMs (PSC) and IBD relate to shared common etiologic factors, pathogenic pathways or are consequences of diseases – this remains a worthwhile avenue to explore to help shed light on IBD etiopathogenesis.¹³⁹

5.1.2. Post-operative Medications and Complications

The fact that pouchitis almost exclusively occurs in patients with underlying UC and that there exists similarities in terms of clinical presentation and immunological abnormalities between pouchitis and UC, suggests that a subset of pouchitis (e.g. antibiotic-refractory pouchitis) may represent the recurrence of a UC-like disease in the ileal pouch.^{113, 265} There are many lines of evidence to support this theory. For example, the presence of pouch stasis, exposure to fecal contents and an increased microbial load could cause inflammatory changes that lead to morphological alterations in the ileal pouch mucosa mimicking colon epithelia in UC.²⁹⁸ Similar to UC, pouchitis patients have altered mucin glycoproteins which may be more susceptible to enzymatic degradation by bacteria, making the mucous barrier less resistant.²⁹⁹ However, there are different groups of patients who are responsive to antibiotics and those that are not responsive.¹¹³ Antibiotic-responsive pouchitis is likely of bacterial etiology, while antibiotic-refractory pouchitis may be associated with immune-mediated chronic inflammation, similar to UC. Antibiotic-dependent individuals may represent the group of patients who are somewhere between a purely responsive state and that of a refractory one. This implies that disease phenotype resides along a spectrum and that other variables are at play in disease progression over time. For example, patients who started off antibiotic responsive initially post-IPAA became dependent or refractory over time, further emphasizing the fact that disease classification post-IPAA is in constant flux. In our study, the pouchitis patients had the highest frequency of antibiotic use, where 90% had been treated with ciprofloxacin and 97% with flagyl (metronidazole) compared to the CD-like patients (79%; 86%) and the no pouchitis patients (35%; 41%) ($p < 0.0001$). In addition, the pouchitis patients were more likely to take probiotics, 25%, compared to the CD-like (2.5%) and no pouchitis groups (5%) ($p < 0.0001$). A recent study showed that administration of oral probiotics in a highly concentrated mixture of bacterial strains was effective in the prevention of relapses in patients with chronic pouchitis, highlighting the etiologic role of alteration of gut flora in causing pouchitis. There was significantly higher

proportion of CD-like patients (47.5%) who were maintained on drug therapy supplementary to or instead of ciprofloxacin or flagyl compared to the other two groups ($P<0.0001$). Moreover, the idea of fecal stasis in the pelvic pouch is important to study since inflammation does not occur at end ileostomies in UC patients.¹¹⁶ The fact that 49% of patients have taken or continue to take Imodium to feel well, may indicate a contributing factor to bacterial stasis occurring in the pouch after IPAA ($P=0.70$). We found that 41.5% of CD-like patients were refractory to single or double antibiotic treatment compared to 17.5% of pouchitis patients and 0.5% of no pouchitis patients ($P<0.0001$). The use of CD-associated medications have proven to be beneficial in treating patients with a CD-like phenotype.²⁷⁴ Our study showed that the highest proportion of CD-like patients were treated with remicade, 45%, compared to patients in the pouchitis (8%) and no pouchitis (0.5) groups ($P<0.0001$). Nonetheless, the CD-like patients had the highest rate of pouch excision, 26%, compared to the pouchitis and no pouchitis patients, 6% and 1%, accordingly ($P<0.0001$).

5.1.3. Smoking Behaviour

As with UC, smoking tends to have a protective effect against development of chronic relapsing pouchitis.¹³⁵ There were a significantly larger number of patients who were current smokers and had a CD-like phenotype (24%) compared to the no/acute limited pouchitis (4%) and chronic relapsing pouchitis patients (6%) ($p<0.0001$). It is known that smoking is protective in UC but not CD, which is in line with our findings that smoking seemed to be protective in patients in Groups 1 and 2, which was comprised of individuals with a UC-like phenotype, compared to patients in Group 3, who had a CD-like phenotype. There was a lower proportion of patients with a CD-like phenotype who had never smoked (31%) compared to the patients in Group 1 (52%) and Group 2 (45%) ($p=0.04$). In general, compared to those who have never smoked, individuals who are former smokers have an increased risk of developing CD.⁷⁷ It is of interest that the CD-like patient group had a phenotype more frequently associated with CD, in

general. Many studies have demonstrated that smoking is associated with disease location such that a higher prevalence of ileal disease and a lower prevalence of colonic involvement in smokers.³⁰⁰⁻³⁰²

To date, the theory underlying the opposite effects of smoking observed in CD and UC remain obscure. Although there are numerous effects associated with smoking and nicotine, the fact that the pathogenesis of IBD remains only partially understood, discussion of potential mechanisms are speculative. Smoking, which has both specific and non-specific effects, has been shown to affect the immune system by impacting cellular and humoral immunity.³⁰³ Experiments on mouse colonic mucosa have shown that nicotine decreases the synthesis of proinflammatory molecules, i.e. IL-1 β and TNF- α , and decreases the production of mucosal eicosanoids.³⁰⁴ Further evidence for anti-inflammatory properties has come from studies on human mononuclear cells which have demonstrated that nicotine results in a decrease in proinflammatory cytokines, such as IL-8 and, partly by its action on nicotinic acetylcholine receptor 7a subunit.³⁰⁵ A study conducted on rats showed that chronic nicotine exposure impeded the antibody-forming cell response and impaired the antigen-mediated signaling in T-cells.³⁰⁶ Additional effects of smoking or nicotine include the reduction of smooth muscle tone (modulated by nitric oxide),²⁶⁷ decreased permeability,⁹⁸ increased lipid peroxidation and alteration in gut motility and microcirculation.³⁰⁷ In CD, the colonic mucosa is thicker, in contrast to UC where the colonic mucosal layer is thin or absent.³⁰⁸ It has been shown that nicotine increases mucin synthesis.³⁰⁹ Overall, IBD patients have a significant reduction in mucosal cytokine levels; specifically, UC patients have reduced levels of IL-1b and IL-8, whereas CD patients have reduced levels of IL-8.³¹⁰ The potential beneficial effects of nicotine in active UC may be associated with a reduction in IL-8 expression. An alternate possibility is that hypoperfusion of the rectum and of acutely damaged colonic tissue play a role in how nicotine affects UC patients.³¹¹ On the other hand, in CD, the total radical-trapping antioxidant potential is decreased,³¹² several plasma antioxidant potential is decreased and abnormalities are present in the microvasculature.³⁰⁷ Ischemia and

perpetuating ulceration and fibrosis may be caused by the increased carbon monoxide concentration from smoking, which amplifies the impairment in the vasodilation capacity of chronically inflamed microvessels.³¹¹ Smoking has been shown to cause an elevation in thrombotic potential associated with vascular damage. Moreover, a purported defect in bacterial clearance or a macrophage deficiency has been suggested to play an important role.³⁰³

5.1.4. DISCUSSION - SEROLOGICAL

Reported antibody prevalence has varied depending on studied cohort and methodology used. In our study, serum collection was on average 9.7 years after IPAA, with no significant difference between groups ($p=0.08$). Anti-glycan antibodies reflect both cellular and humoral immune responses, processes which are important in the development and expression of IBD. Subsequently, these biomarkers are important to study to determine potential clinical utility in differentiating between UC and CD and predicting disease course.¹⁸⁸ We found that the overall prevalence of the CD-associated gASCA was 21% in the total patient cohort, where 40% of CD-like patients were positive for gASCA compared to 16% of chronic relapsing pouchitis patients and 19% of no/acute limited pouchitis patients ($p=0.009$). ASCA recognize oligomannosidic epitopes of the yeast *S. cerevisiae* which can trigger a proliferative T-cell response to the yeast antigen mannan,⁴⁰ suggesting that ASCA expression represents loss of tolerance to yeast antigens. Units which may result in Chitobioside, laminaribioside and mannobioside are not auto-antigens; nonetheless, they are common structures in glycocalyx of pathogenic bacteria and yeast.¹⁸³ Chitobioside is a major component of the insect cuticle and the cell walls of infectious pathogens such as yeast and bacteria. Laminaribioside is the building block of laminarin, a polysaccharide of the β 1,3-glucan family which can be found in the cell walls of saprophytic and pathogen fungi and yeast, including *S. cerevisiae*. Mannobioside is a component of mannan from pathogenic fungi and yeast, such as *S. cerevisiae*. Chitin, mannan and β 1,3 glucans or fragments thereof, may bind to specific receptors on macrophages, neutrophils and NK cells, resulting in phagocytosis, stimulation of cell proliferation, and cytokine secretion. Overall, studies suggest that chitin and β 1,3 glucans have the potential to modulate the immune system, specifically the innate branch,¹⁸³ however, the resultant antibody production specifically against chitin and glucan and their association with CD suggests the intrinsic modulation of the adaptive immune system.³¹³

To date, elevated titer levels of ASCA IgG and/or IgA have been found in 50-80% of CD patients, but in <10% of UC patients and healthy controls.³¹⁴ Aisenberg *et al.* found that even though 15% of the 102 patients enrolled in their study were gASCA positive, no association between gASCA and pouchitis was reported. This lack of association confirms data from previous studies which discourages the hypothesis that pouchitis may signify occult CD, or that gASCA is simply a marker of ileal inflammation.³¹⁵ In accordance with these findings, our study did not find a difference between the proportion of patients positive for gASCA IgG in the chronic relapsing pouchitis (16%) and no/acute limited pouchitis (19%) groups; nonetheless, there was a significant difference in the prevalence of gASCA in the CD-like patients (40%) compared to the no/limited acute pouchitis and chronic relapsing pouchitis patients (p=0.006). Hence, the association between gASCA and the CD-like phenotype in our patient cohort would suggest that it is not pouchitis that signifies occult CD, but more so a subset of individuals who develop a CD-like phenotype specifically. Newer definitions and classifications of post-IPAA outcomes make it difficult to compare outcomes between studies; for example, previous series use a two grouping classification (pouchitis vs. no pouchitis) whereas this study takes into consideration a third distinct phenotype, that of the CD-like phenotype group.³⁸ In our study, there was a trend of a longer number of years from proctocolectomy to serum collection date in CD-like patients (mean 12 years) compared to the no pouchitis and pouchitis patients (mean 9 years) (p=0.07). There was a higher proportion of anti-L positivity in the CD-like patients (13%) compared to the other two groups (p=0.04). These markers have recently been associated with CD and support the association of a CD-like biomarker profile with our CD-like phenotype patients.¹⁸⁸

The prevalence of AMCA, ALCA, and ACCA was similar between the three groups (p=0.40, p=0.63, and p=0.68, respectively). In two European studies, ALCA, AMCA, and ACCA were shown to be associated with severe disease outcome in patients with CD, specifically; as a result, these markers were not shown to substantially improve differentiation

between CD and UC over the more commonly used gASCA.¹⁸⁸ Other studies have demonstrated that a variety of antibodies including ASCA, ACCA, AMCA, ALCA as well as anti-OmpC, Anti-I2, and anti-CBir1 are associated with more severe disease behaviour, including stricturing or penetrating disease, and with the need for abdominal surgery in both pediatric and adult populations.^{37, 38, 210} In keeping with existing data, recent data reported demonstrates that seropositivity to gASCA, ACCA, ALCA and AMCA as well as the two novel anti-glycans, anti-L and anti-C, independently indicates a more aggressive CD phenotype. In this study, the fact that these glycan antibodies were not significantly more prevalent in patients with CD-like phenotype compared to the other two groups suggests that the CD-like phenotype group is comprised of individuals who have some similarities as well as distinct differences in their serological profile compared to individuals with a diagnosis of CD. Perhaps these patients have a serological profile that is intermediate between those with UC and those with CD. Recent data is in accordance with the idea of an intermediate serological profile such that patients who had isolated inflammatory colonic CD had serological profiles that were intermediate between those with CD and UC.¹⁸⁸

Recently, the two novel anti-glycan antibodies anti-L and anti-C were shown to improve differentiation between CD and UC with a greater contribution from anti-L than anti-C. Specifically, these authors showed that 18.0% of CD patients were anti-L positive and 10.1% anti-C positive compared with 3.3% and 2.3% of UC patients respectively ($p < 0.0015$).¹⁸⁸ In our study, 12.5% of CD-like patients were anti-L positive and 5% were anti-C positive compared with 4.3% and 2.4% of patients in Group 1 and 1.8% and 1.8% of patients in Group 2, accordingly ($p = 0.04$ and $p = 0.58$, respectively). The modest association between anti-L seropositivity and CD-like patients compared to the UC-like patients is in line with data reported by Seow *et al.* who found that a significantly higher proportion of patients with isolated colonic inflammatory CD were seropositive for anti-L (18.0%) than those with UC (3.1%) ($p < 0.0001$).¹⁸⁸ In addition, Seow *et al.* have demonstrated that gASCA and anti-L have better discriminatory

ability between CD and UC than the other glycan markers.¹⁸⁸ These authors found that the most specific marker for CD was anti-C, with a specificity of 97.7% closely followed by anti-L with a specificity of 96.7%. In our study, only a small subset of patients had available information regarding gASCA IgA titer levels such that the difference between groups did not reach statistical significance (p=0.33). Recent data demonstrated that a significantly higher proportion of patients with isolated colonic inflammatory CD were seropositive for gASCA IgA than those with UC (20.5% vs. 6.2%, p=0.0023, respectively).¹⁸⁸ In general, if larger studies substantiate these serological findings, anti-L with ASCA may have a role in deciphering between patients post-operatively and may assist in directing more specific treatment in this IPAA patient cohort.¹⁸⁸ Nonetheless, recent data reinforces the low sensitivity of the newer glycan markers; consequently, larger scale prospective studies are needed to determine the predictive potential of these markers in IBD in general, and in IPAA patients, in particular.¹⁸⁸

In summary, serological markers should not replace thorough clinical evaluation, and should be used with caution when analyzing subgroups of IBD patients. These results suggest that patients who manifest a convincing CD phenotype after surgery may, in fact, have an intermediate phenotype somewhere between CD and UC. The differing serological profiles may indeed be indicative of a distinct subgroup of IBD patients, who reside somewhere along the spectrum of IBD, between UC and CD. Is this a new phenotype that results after surgery? Is this phenotype and serological profile similar to IBDU patients, underscoring the possibility that this third group is distinct from UC and CD altogether? Are other variables alone or together predisposing these patients to a poor post-operative outcome?

It is well documented that the combination of gASCA and pANCA is useful in differentiating UC from CD.^{37, 316, 317} Reese and colleagues performed a large meta-analysis demonstrating that a specificity of 92.8% and a sensitivity of 54.6% of ASCA and pANCA, respectively, in CD patients.³¹⁶ In a more recent study performed by our IBD group at MSH, both anti-L and anti-C when added to the combination of gASCA and pANCA were shown to

further improve differentiation between CD and UC with a greater contribution from anti-L than anti-C. The underlying implication is that if these markers are shown to be able to clearly differentiate between UC and CD as well as predict disease course, they may prove to be clinically useful and aid in pre-operative risk assessment.¹⁸⁸ Until this time, developing a better understanding, and conducting further studies to find supporting evidence for the use of the glycan antibodies in disease diagnosis and stratification is necessary. Testing the utility of these newer anti-glycan markers in this pelvic pouch cohort may shed light on significant serological differences between post-IPAA groups. The fact that patients manifest distinct phenotypes post-IPAA has raised many questions about pre-operative diagnosis and disease progression in general. Studying this anti-glycan panel in post-IPAA patients, with a pre-colectomy diagnosis of UC, may provide insight into the pathogenesis of pouchitis compared to CD of the pouch, specifically, and IBD in general. Furthermore, evaluating serological profile differences between groups may provide clues as to which patients are at risk for post-operative complications and pouch failure.

One of the purported benefits of serological markers is that they may determine which patients will benefit most from more aggressive therapy prior to complicated disease developing. The fact that existing studies are mostly cross-sectional in design, suggests an associative (diagnostic) rather than a predictive (prognostic) role. The fact that these antibodies have been shown to be present prior to disease onset implies that these biomarkers may reflect the immunopathogenesis of IBD.^{97, 318} As a result, these antibodies hold promise as prognostic tools, rather than simply being an epiphenomenon related to disease activity and duration.¹⁸⁸ Further studies on stability of these markers over time is warranted; to date, the stability of serum glycan antibody concentrations up to 13 weeks has been reported.¹⁸⁴ In order to determine the extent to which a patient's clinical phenotype alters their serological profile prospective, longitudinal studies, along with ongoing clinical assessment and serum measurement, are needed.¹⁸⁸

What questions remain from this study? Can serologic markers help guide clinical decision making? Are surgical or medical outcomes better or worse based on serologies? It is important to assess gASCA titer levels prior to colectomy and follow large groups of patients who undergo pelvic pouch surgery. This will be important to determine whether serologic tests change with surgical intervention and if serologic tests prior to proctocolectomy correlate with pouch-specific complications post-IPAA.¹⁴⁰ It is clear that prospective data is necessary to determine if these antibodies have a predictive role rather than just an associative role.³¹⁹

5.1.5. DISCUSSION - GENETICS

To date, there is strong evidence that enteric bacteria play a role in driving the inflammatory response in IBD and that genetic factors play a part in the pathogenesis as well the course and the extent of these disorders.^{79, 226} In order to identify UC and CD susceptibility loci in our patient cohort, 768 SNPs were systematically tested. From this SNP panel, 25 SNPs were screened to determine the frequency of these SNPs and investigate the relevance of these genes to disease susceptibility and severity. SNPs studied included those in innate immunity genes such as candidate genes of CD14, TLR4, TLR9, NOD2/CARD15 for their involvement in bacterial recognition and intracellular signaling pathways.²²⁶ It is well known that CD and UC have shared as well as disease-specific susceptibility loci – identifying these loci hold promise in elucidating the biologic relationship between the inflammatory bowel diseases.³¹⁹ To date, more than 30 CD susceptibility loci have been identified, yielding valuable insights into the pathogenesis of IBD.³¹⁹ These markers also represent important candidate susceptibility loci for UC.³¹⁹ In the clinical picture, systematic analysis of CD risk markers shows that many of them are in fact associated with UC including IL23R, IL12B, NKX2-3, CCNY and MST1 locus.²⁶⁰ Hence, an important issue regarding genetics and pathogenesis of IBD is how many of these susceptibility genes are shared by both CD and UC.⁴³ For example, UC has been shown to be more common in the relatives of CD patients, and vice versa, and approximately one third of extended pedigrees contain cases of both CD and UC.³²⁰

The study of polymorphisms in innate immunity genes may provide insight in a possible genetically determined susceptibility to chronic relapsing pouchitis and genetic differences that exist between pouchitis and CD-like phenotype individuals.²²⁶ Luminal bacteria are critical players in driving the inflammatory response in pouchitis, such that CD14 is an important candidate gene to explore.²²⁶ CD14 is part of the endotoxin/lipopolysaccharide (LPS) receptor complex and is important in the recognition of LPS, a membrane glycolipid on Gram-negative bacteria²²⁶ and in the detection of cell membrane components of Gram-positive mycobacteria and

viruses.²²⁶ CD14 exists in a soluble form in serum and in a membrane form on macrophages, monocytes and neutrophils.^{226, 321} The SNP rs2569190 is associated with enhanced transcriptional activity (located on chromosome 5q31 in the promoter region of the CD14 gene). Increased expression of CD14 in macrophages has been found in IBD,³²² and an association of CD14 - 260C>T gene polymorphism with IBD³²³ and atherosclerosis has been described.³²⁴ Analysis of the three groups of IPAA patients (i.e. no/acute limited pouchitis, chronic/relapsing pouchitis and CD-like phenotype) revealed a modest positive association with SNP rs2569190 when applied to a three level outcome ($p=0.05$). The risk genotype was more frequent in patients with chronic/relapsing pouchitis (81.4%) compared to those with no/acute limited pouchitis (70.9%) and those with CD-like phenotype (67.5%). There was an increased risk that patients in Group 2 carried the risk genotype compared to Group 1 (OR=1.39) and a decreased risk that patients in Group 3 carried the risk genotype compared to those in Group 1 (OR=0.35) ($p=0.05$). Given the fact that soluble CD14 has the ability to confer pathogen responsiveness to cells such as intestinal, epithelial and endothelial cells which do not express CD14 on their membranes, this suggests that genetically determined variation in CD14 serum levels may have functional consequences.²²⁶ The mechanism underlying increased risk of pouchitis may be due to a dysfunction in bacterial recognition or a lack of an adequate immune response to bacterial challenge. Soluble CD14 may facilitate reactivity to a broad array of bacterial components, and might confer epithelial cell responsiveness. For a two level outcome comparing Group 1/Group 2 versus Group 3, there was a significant association between SNP rs2569190 and post-IPAA outcome ($p=0.03$, OR=3.07). Patients in Groups 1 and 2 are comprised of individuals who are responsive, dependent or refractory to antibiotics or who have no need to take antibiotics. These patients have a phenotype that remains in line with UC. Response to antibiotics seems to reside along a spectrum from those who are responsive at one end to those who are non-responsive at the other end. Responsiveness to antibiotics may change over time due to various factors such as repeated antibiotic use, duration of years with the pelvic pouch (exposure to chronic

inflammation), other existing comorbidities all in conjunction with a predetermined genetic susceptibility. This implies that patients with a UC phenotype post-IPAA may share a similar genetic background which differs from those who develop a CD-like phenotype. The CD-like phenotype group consisted of patients who were more frequently refractory to antibiotic therapy (41.5%) and treated with remicade (45%) compared to the two other groups (<0.0001 ; <0.0001 , respectively). These findings suggest that the CD-like patients may have a divergent genetic susceptibility compared to the no/acute limited pouchitis and chronic/relapsing pouchitis patients. The modest evidence for the association detected may be due to linkage disequilibrium (LD) between this SNP and the main SNP responsible for the association signal, which may reside elsewhere along the CD14 gene.²⁶⁰ To date, there is no evidence of association between risk of various types of IBD and variants alleles of CD14.²³² Overall, it is likely that SNPs in different genes of the bacterial sensing network could act synergistically to enhance disease susceptibility in individuals.²³² The association of the CD14 gene polymorphism with a UC-like phenotype for both three level and two level outcomes (OR=1.39 and OR=3.07, respectively) is in line with studies that have found a positive association of this SNP in the promoter region of the CD14 gene and UC.^{232, 323} Overall, CD14 has been described as a risk allele in many studies on IBD such that it has received attention as a candidate IBD susceptibility allele.^{232, 325} To date, there is conflicting data on the association of the CD14 gene polymorphism and CD.^{326, 327}

The ECM1 SNP was chosen based on the study conducted by Fisher *et al.*²⁵⁹ There was a significant difference in genotype frequency of the gene polymorphism rs13294 between groups – Group 1/ Group 2 versus Group 3 ($p = 0.04$, OR=2.94). Again, these results imply that patients categorized as having a CD-like phenotype post-IPAA (Group 3) may have a dissimilar genetic background compared to the patients in Groups 1 and 2, who were classified based on a phenotype more line. ECM1 has been shown to be a plausible candidate gene for UC because it encodes extracellular matrix protein 1, a glycoprotein expressed in small and large intestine, and it interacts with the basement membrane and inhibits matrix metalloproteinase.²⁵⁹ ECM1

activates a key immune regulator, NF- κ B signaling. ECM1 expression is upregulated in colorectal cancer and metastases, implicating this gene in epithelial-stromal interaction.²⁵⁹ Altered intestinal permeability is the most probable mechanism at play.²²⁷ Hence, this new UC gene encodes a protein that is implicated in maintaining the barrier function of the intestinal wall which implies that an inherited defect in the intestinal wall may predispose to UC. Fine mapping is required to determine whether the causal variant maps within ECM1 or elsewhere in the haplotype block which spans 290 kb.²⁵⁹ Based on the association of rs13294 with Groups 1 and 2 (UC-like), this is in line with the recent UK-based study that demonstrated an association with ECM1 and UC susceptibility.²⁵⁹ The difference between Groups 1 and 2 versus Group 3 supports the hypothesis of a divergent genetic profile between patients who manifest a UC-like phenotype compared to those who manifest a CD-like phenotype.

The evidence for SNP rs2542151, which resides upstream of PTPN2 on chromosome 18p11, was of interest, as PTPN2 encodes a T cell protein tyrosine phosphatase (TCPTP) which is a key negative regulator of inflammation. The protein encoded by PTPN2 is a member of the protein tyrosine phosphatase (PTP) family. PTPs are signaling molecules which are known to regulate a variety of cellular processes such as oncogenic transformation, and cell growth differentiation. The rs2542151 SNP in PTPN2 has been shown to be associated with CD.²⁷³ Evidence from the knockout mouse suggests that dysregulated TCPTP leads to marked elevation of several pro-inflammatory cytokines including IFN- γ , TNF- α and IL12.²⁸⁷ Analysis of the two subgroups, Group 1 versus Group 2/Group 3 revealed an association of the rs2542151 SNP with Group 1 ($p=0.02$, OR=1.88).^{227, 273} Hence, SNP rs2542151 was not found to be associated with patients who demonstrated a CD-like phenotype. Fine mapping to identify causal variants at this locus and to determine their impact on TCPTP expression and function is required.²⁸⁷ The studied SNP is modestly associated on a genotypic level when considered individually, such that combining variants in genes affecting host-bacterial recognition may aid in more strongly confirming risk alleles.²³²

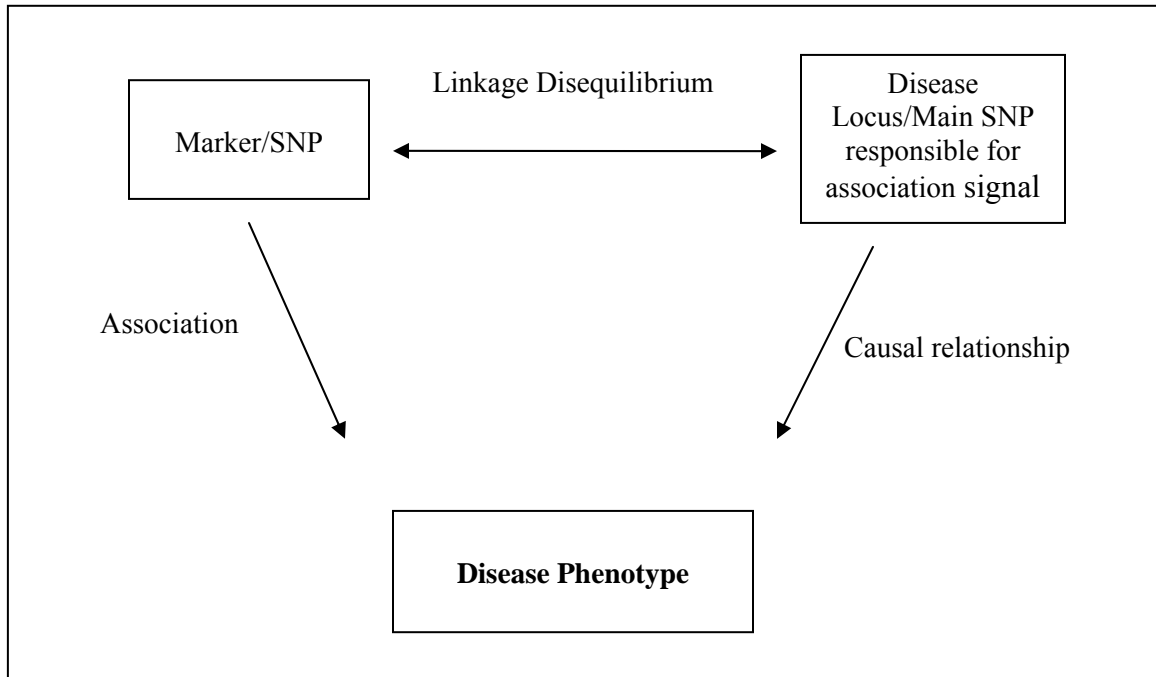
Analysis of the three groups of IPAA patients did not reveal a significant association for the other 19 SNPs and post-operative IPAA classification for both three level and two level outcomes. It cannot be excluded that the group of patients considered to have no/acute limited pouchitis may consist of patients who have not yet developed pouchitis due to a shorter period of time with the pouch or perhaps a lack of an environmental trigger that will turn on expression of the risk genes. In addition, the no/acute limited pouchitis group consisted of patients who may have had acute pouchitis – these patients may in fact be genetically susceptible to additional complications.

SNPs from different genes may in fact work synergistically and constitute a small to moderate relative risk of developing disease. Strong LD between variants can make it very difficult to identify the true causal variant (refer to Figure IV – 12). Our observations are based on a relatively small number of patients; nonetheless, this is one of the largest studies available on UC patients with IPAA.²²⁶ To what extent carriage of any of these variants can be functionally linked to susceptibility to poor post-operative outcome after IPAA in UC individuals in question can only be speculated.²⁶⁰ In order to determine if any of these SNPs are valuable predictive markers and to further understand the functional biological roles of the CD14, ECM1 and PTPN2, larger studies are required.²²⁶ Clearly, the causative variant(s) within these genes remain to be found.

The fact that UC and CD share common genetic susceptibility loci is seen from the fact that an intermediate phenotype – that of IBDU – comprises up to 10% of IBD cases. This underscores the findings of an increasing number of IBD susceptibility genes that are common to CD and UC and those that are distinct to each subgroup. Discovering gene profiles distinct to different phenotypes of IBD in subgroups of IBD patients (i.e. IPAA patients) requires further investigations of large samples. This study indicates that there are genetic differences between subgroups of UC patients with an IPAA after surgery. Further exploration of these signals may

shed light on pathways involved in disease progression in general, and may provide insight into factors associated with post-operative outcome.²⁸⁷

Figure IV – 12: Relationship between Genotype and Disease Phenotype



Certainly, the rapid progress in finding IBD susceptibility genes will assist in refining our ability to classify clinically important subgroups of patients based on immunodiagnostic testing.³²⁸ The multiplicity of genetic associations highlights that there are probably many pathways that may result in similar patterns of injury in the intestinal tract.²⁷⁴ Genetic studies on IBD share a commonality in that they generally indicate the important role of host-enteric microbiota interactions. Consequently, future characterization of patients will likely be based on both genetic polymorphisms in innate immune pathways and serological reactivity to the enteric microbiota. Currently, serologic markers have proven to be the most specific markers in predicting disease pattern, disease severity and response to therapeutic agents.²⁷⁴

5.2. LIMITATIONS OF THE STUDY

One limitation of this study is that not all patients received an endoscopy and biopsy to diagnose pouchitis, since in the late 1980s and 1990s, as well as now, gastroenterologists or surgeons frequently make this diagnosis of pouchitis based on clinical grounds.¹¹ Due to incomplete data collection, the use of more detailed diagnostic tools such as the Pouchitis Disease Activity Index (PDAI) was not used to help classify patients.¹¹⁴ Consequently, there are likely to be patients categorized and treated as having acute, or antibiotic responsive pouchitis, who may not have had endoscopic criteria for this diagnosis. Nonetheless, patients classified as having more significant pouchitis in frequency and severity had endoscopic and histological confirmation of this diagnosis in most cases.¹¹ A second limitation of the study is that it was performed retrospectively. Thirdly, one of the confounding variables which was difficult to control for was the migratory nature of disease categories. For example, a patient with IPS or a healthy pouch could later develop pouchitis or even CD-like phenotype of the pouch.¹⁴⁵ Hence, in order to minimize the effect of this confounding factor, prolonged and repeated follow-up is necessary. Furthermore, it is not known if STC with IPAA has favorable and harmful effects on disease course of AD or other EIMS; consequently, longitudinal studies before and after IPAA are warranted.²⁶⁵ As well, minor variation exists in reporting, likely due to the use of different populations, assays and cut-off values.

In epidemiology, questionnaire studies, results can be influenced by non-responder bias. The fact that participation in this study is elective, and response rate was 30%, there may have been a difference in the distribution of post-IPAA outcome if non-responders were added to the responders. MSH is a tertiary referral center such that people from all over the country come to Toronto to undergo pelvic pouch surgery. As a result, those who live in closer proximity to MSH may be more likely to see a gastroenterologist at MSH and those patients who experience complications may be more likely to go for follow-up appointments with their surgeon at MSH.

For this reason, if the non-responders were added to the post-IPAA grouping, there is a possibility that the distribution of post-IPAA outcome could change – subsequently, altering the associations found in this study. Even if it was known whether there were equal baseline characteristics among responders and non-responders, this would not preclude non-response bias. When interpreting results from population-based surveys, an important consideration is whether those who took part in the study differ in lifestyle than those who declined. As a result, such differences may cause an over- or under- estimation of the prevalence of disease/phenotype if the distribution of risk factors for those with the disease/phenotype who were surveyed differ from the risk factors for those in the rest of the target population. Nonetheless, it is important to note that the percentage of individuals with serious post-operative complications (i.e. chronic relapsing pouchitis) found in this study is modest and not inflated compared to similar literature.¹³ In order to ascertain if those who responded were in fact representative of the target population and if non-response bias exists, certain methods can be employed. To quantify differences in characteristics between responders and non-responders, a sample survey of non-responder characteristics or of their baseline characteristics can be compared to those of the responder's. This approach would ultimately depend on whether or not this information could be made available. An alternate approach is to compare individuals who responded and took part after first contact to those who decide to take part following subsequent contacts. The rationale is that this strategy may reflect a difference in characteristics between groups of people. Furthermore, improvement in response rates may be gained by ensuring that patients understand the trial procedures.³²⁹

Methods to improve the attribution of hidden SNPs are currently being pursued in order to improve power of association studies, as well as to reach SNPs that have not been genotyped. In addition, unlike genotypes which represent allelic information on both chromosomes, haplotypes represent information from one of the chromosomes only.³³⁰ Haplotypes improve the prediction of hidden SNPs and can enhance the ability to identify disease-related loci using LD.

This is because the number of haplotypes in any given region is smaller than the number of genotypes resulting in a larger sample size that is used to estimate any given haplotype.³³⁰

The important issue of disease progression of UC as well as CD over time is critical to address in studies relating genotype and phenotype; undoubtedly, disease behaviour and severity change over time and interviewing patients at different time points in their disease results in potentially critical differences in behaviour. Once again, the need for prospective studies is demonstrated.^{3, 19}

5.3. CONCLUSIONS

In summary, a positive test for gASCA, anti-L, axial arthritis/ankylosing spondylitis and smoking status were associated with a CD-like phenotype that can develop after IPAA for confirmed UC. These features are typically associated with CD – in our patient population, these same features were associated with the CD-like phenotype group with confirmed UC prior to colectomy. This raises a few key questions: Do pre-colectomy ASCA positive patients delineate a subset of UC patients at risk for developing CD-like complications post-IPAA? Does ASCA become positive after the development of a CD-like phenotype in the setting of IPAA for UC? Serologic testing has begun to unravel subgroups within IBD, but serologic dilemmas remain. For example, is ASCA positive UC a different disease? This study suggests post-operative ASCA titer levels are associated with different medical and surgical outcome in patients with a pre-operative diagnosis of UC. Understanding pouchitis etiology and pathogenesis is a requisite factor in developing appropriate diagnostic and classification tools which will enable improved strategies for prevention and treatment of pouchitis.¹⁰ Tracking patients postoperatively can assist in establishing whether serologic tests correlate with pouch-specific complications, and if chronic pouchitis patients will be determined as having CD.²

The recognition of genotypes/alleles linked to phenotypic characteristics of patients with IBD will assist in attempts to identify those at risk for specific clinical features and provide

information for additional research into mechanisms of the occurrence of such phenotypes. In order to aid the identification of important susceptibility genes in IBD, careful documentation of disease diagnosis and phenotypic manifestations is necessary. To date, prospective data to guide clinical practice simply does not exist.¹⁴⁰ Further evaluation of these biomarkers in pouchitis are needed to confirm these findings and will require larger populations, long-term prospective observation and studies that correlate polymorphisms with specific immunologic functions.¹¹ While there exist therapeutic challenges in IBD, including its chronicity, intermittence, and need for safety, a sound means of bypassing these obstacles calls for individualized approaches which integrate knowledge of genetic factors.⁷⁹ Further studies of gene function including genotype-phenotype relations, and gene-gene and gene-environment interactions need to be carried out on large patient cohorts in a prospective fashion. As the genetics and immunology of IBD are further solved, it is likely that CD and UC will be redefined, such that clinically important subgroups will be elucidated by immunogenetics. Newer definitions of these groups will likely provide useful information about natural history and response to medical and surgical therapy compared to the current gold standard which is based on clinical definitions established 80 years ago.¹⁴⁰ The translational potential of the data from this study demonstrates that a combination of clinical, serological and genetic markers hold promise in assisting diagnosis, pre-operative risk assessment, as well as post-operative risk assessment for IBD in general, and for UC patients who are deciding to undergo surgery, IPAA specifically. Based on the findings in the study, an individual who is considering an IPAA and is seropositive for anti-L and gASCA, has axial arthritis/ankylosing spondylitis, and is a current or ex-smoker may be an at-risk patient post-operatively. Future studies are needed to replicate these findings so that individual patients will soon be able to be assessed based on a wide range of clinical characteristics and biomarkers.

5.4. FUTURE DIRECTIONS

Further serological testing on UC patients with IPAA will be helpful in developing a better understanding of disease phenotype. Additional markers include pANCA, anti-I2, anti-CBir1, and anti-OmpC. These serum markers, in addition to ASCA, have been the most extensively studied biomarkers to date. Data on these markers has been shown to vary in independent studies; nonetheless, combinations of more than one of the five serological markers have been shown to have the most clinical value.³¹³

There still exist many unanswered questions regarding the translation of this scientific progress to clinical application, with a feeling of optimism among scientists and clinicians.⁸ The use of GWA studies and SNP screening to obtain genetic information on a cohort of patients in whom extensive phenotypic information is available, and following this cohort of patients prospectively represents an extremely promising mechanism of identifying important new connections between genotypes and disease course.⁷⁹ The use of GWA scanning represents an hypothesis-free survey of the human genome; as a result, with any type of screening approach, hits are identified purely on the basis of statistical support for association between markers in their vicinity and disease susceptibility.²⁸⁷ This approach has promise to elucidate biological processes previously unsuspected in the pathogenesis of the disease being studied. The use of expression studies also hold promise to help discover the link between genotypes, disease course and effects on disease expression. Cost-effective ways of comprehensively genotyping functional human polymorphisms and/or re-sequencing individual genomes is expected to be available as a diagnostic tool for clinicians in the near future.⁷⁹ The hope is that a diagnostic tool of this kind will be able to provide an abundance of crucial information. The capacity to predict disease course and severity is of enormous interest clinically, and requires reproducible, measurable endpoints of disease progression.⁷⁹ Ideally, the creation of novel IBD therapeutic algorithms involve integration of therapeutic interventions with information on individual genetic backgrounds.⁷⁹ Microarrays are anticipated to become a widely used tool for disease

classification, and could eventually be used as a routine diagnostic tool in “microarray readers” which would tailor treatments for individual patients.³³¹ The data from array analysis shows promise in providing information to identify the causes of disease, the mechanism of drug action and the discovery of gene products that are targets for therapy in various disorders.²⁴⁰ Microarray technology has the potential to assist in more accurate diagnosis and risk assessment of various diseases, as well as to enable more precise prognosis and new therapeutic interventions.²⁴⁰ Conclusively, the successful application of human genetics to clinical practice awaits improved understanding of genetic factors which contribute to disease pathogenesis, prognosis and disease course.⁷⁹ In the future, better knowledge of disease pathophysiology is expected to have an critical impact on clinical practice, and will lead to novel therapies, that are more effective and safe, compared to the current medical and surgical treatments available today.⁸

5.4.2. The Microbial Environment

As eluded to earlier, ileal inflammation does not develop post-IPAA until the fecal stream is re-established towards the pouch after closure of the protective ileostomy. This observation implicates the endogenous enteric flora as key players in onset of ileal inflammation. A recent study evaluating bacteria in the inflamed and non-inflamed ileal pouch mucosa and afferent limb found that there was a significantly lower microbial diversity in patients with ileal pouch inflammation compared to controls with non-inflamed pouches.²⁷⁴ In addition, the authors established that the diversity of the microbiota of the afferent limb did not significantly differ from that of the pouch mucosa.²⁷⁴ What was of interest was that the clone libraries revealed that the non-inflamed status was associated with higher diversity of bacterial species which included members of the normal, anaerobic enteric microbiota.¹¹⁷ Accordingly, one of the critical factors involved in the development of ileal inflammation in IPAA, and IBD, in general, is identification of species present on the epithelium directly prior to development of inflammation. Microbial analysis is an important area to study. Key questions that are important to address in future studies include: 1. Are the species and numbers of microorganisms present in healthy and

inflamed pouches similar or different? 2. What microbial populations are present in the various subtypes of ileitis of the pouch above? 3. Are microbial populations in the pouch and in the afferent limb similar or different in the presence or absence of afferent limb inflammation? 4. Are microbial populations in UC and FAP similar or different?

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7.1. Letter of Participation for Patients in MSH Surgical Database

	<p>The Dr. Zane Cohen Digestive Diseases Clinical Research Centre THE JOSEPH AND WOLF LEBOVIC CENTRE, MOUNT SINAI HOSPITAL 60 MURRAY STREET, L3-016, PO BOX 23 TORONTO, ONTARIO, CANADA M5T 3L9 PHONE: 416-586-4800 X8399 FAX: 416-586-5932</p>
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PATIENT INFORMATION AND CONSENT FORM v.8
PRINCIPAL INVESTIGATOR: Dr. Mark Silverberg
THE IDENTIFICATION OF GENES FOR INFLAMMATORY BOWEL DISEASE

Introduction

You are being asked to take part in a research study. Before agreeing to participate in this study, it is important that you read and understand the following explanation of the proposed study procedures. The following information describes the purpose, procedures, benefits, discomforts, risks and precautions associated with this study. It also describes your right to refuse to participate or withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is known as the informed consent process. Please ask the study doctor or study staff to explain any words you don't understand before signing this consent form. Make sure all your questions have been answered to your satisfaction before signing this document.

Inflammatory Bowel Disease (IBD) is the term used to describe two similar, but distinct gastrointestinal tract diseases - Crohn's Disease and Ulcerative Colitis. IBD affects people of all ages and the symptoms include abdominal pain, bleeding, fatigue and diarrhea. The cause of IBD is not known and likely involves many different factors. Recent scientific studies indicate that genetic factors are important in the development of IBD.

DNA (deoxyribonucleic acid) is organized into genes which contain the instructions required for all processes in the body, including appearance, growth and development. Humans have approximately 30 000 genes which are arranged on separate chromosomes and genes contain the codes used by the body to generate proteins. Serum is the yellow-coloured liquid component of blood in which blood cells are suspended. Serum contains proteins that may act as messengers and help run the processes our body needs to function. When there is a problem with the genetic code or the subsequent steps involved in making the proteins, the proteins can be defective, or made in either excessive or insufficient quantities than what the body needs. This may cause the body to malfunction and can lead to disease. Analyzing serum can be helpful in identifying "serological markers" that help detect specific diseases as well as the stage or severity of disease. Serological markers have been useful in diagnosing and guiding treatment for diseases such as rheumatoid arthritis and some types of cancer and have recently been studied in the context of IBD but their roles are still being investigated.

RNA (ribonucleic acid) is the "message" created from our genes or our DNA. RNA serves as a template in the steps involved in the creation of proteins from genes and is a reflection of gene expression (i.e. genes are turned "on" or "off"). Differences in gene expression may be tissue-specific e.g. some genes may be expressed in the blood but may

have a completely different gene-expression profile in a specific tissue e.g. the colon. Thus to study which genes are turned on or off in the blood and in other tissue samples may help us understand why genes cause disease.

A portion of this research is done locally, at Mount Sinai Hospital, a portion of this research is being conducted with 5 other North American centres (the NIDDK IBD Genetics Consortium) and a portion is conducted with other investigators internationally to advance the understanding of the genetic causes of Inflammatory Bowel Disease and with the hope of identifying the specific genes associated with IBD.

These studies will investigate: 1. Identifying gene-containing areas which are involved in IBD 2. Identifying the specific biomarkers (e.g. genes, gene expression patterns, serum markers, etc.) which are directly involved in IBD. 3. Understand the role these genes play in causing IBD allowing for the possibility to discover new diagnostic tests and better, safer therapies.

Details of the Study

What is involved in this study?

People who are willing to participate in this study will be asked to provide a small blood sample, and possibly, a tissue sample (e.g. endoscopic biopsy or tissue from a surgical procedure) for collection of DNA, RNA and serum for testing; a variety of clinical and demographic information will be collected either by phone or in person. In order to have accurate and complete information about the extent of IBD to help in our genetic study, access to medical information is also required. We may ask you for permission to contact your family members (affected or unaffected parents, siblings, children) to participate as determining the inheritance patterns of IBD is difficult without their information. Family members would be asked to also provide a blood sample as well as clinical and demographic information. If family members are affected with IBD, access to medical documentation of IBD would also be required. With your permission, your sample may be sent to and stored within a central repository in the United States to permit the collaborating centres to pool their samples for a more powerful genetic analysis. Analysis of samples may be conducted at centres outside of Mount Sinai Hospital. In this event, all samples will be kept completely anonymous and your identity will not be linked to your sample.

Who is eligible to participate in the study?

Any individuals who have been diagnosed with IBD (either Crohn's Disease or Ulcerative Colitis) are eligible to participate. Individuals without IBD may also be asked to participate to act as controls. These individuals may be healthy or may have other gastrointestinal illnesses.

Risks

Risks associated with drawing blood from your arm include pain, bruising, lightheadedness and, on rare occasion, infection. Precautions will be taken to avoid these difficulties.

Benefits

There will be no direct benefit to you as a result of the genetic research performed with the material obtained from your blood sample. A possible indirect benefit is that your participation may help to find the causes of IBD, or may help in developing early diagnosis methods or new treatments.

Withdrawal of consent

You may withdraw your consent and discontinue your participation in the genetic research described above at any time without affecting your medical care. In such case, you should notify the study doctor and let them know that you want to withdraw your consent and discontinue your participation in the research.

Destruction of samples

The study doctor will keep records linking your identity with your blood sample for an indefinite period. Your blood sample and material obtained from your blood sample will be destroyed if you choose to withdraw from the study. Otherwise, your sample will be kept indefinitely.

Confidentiality

Information obtained from performing research with material obtained from your blood will be filed and kept confidential. Your blood sample and material derived from your blood sample will be given a code, and it will not be possible for anyone other than the principal investigator and research coordinator to link your name or any other information identifying you with the sample you provide.

Compensation:

If you become ill or are physically injured as a result of participation in this study, medical treatment will be provided. In no way does signing this consent form waive your legal rights nor does it relieve the investigators or involved institutions from their legal and professional responsibilities.

Consent

In taking part in this study, I agree and understand that:

- I freely and voluntarily give my permission to participate in this Inflammatory Bowel Disease Genetic research study
 - I will be asked to provide information regarding the history of my disease. This information will be kept strictly confidential and will be used only for the purposes of the study
 - I will provide a blood sample (approximately 3-7 tubes – no more than 3-4 tablespoons) from a vein in my arm for the purposes of genetic and serological testing
- *Note:** I am aware that blood taking may result in temporary bruising, local discomfort, pain or rarely may result in a fainting episode.
- **Note:** I may also be asked to provide additional samples in the future, if necessary.
- The blood (or tissue) sample will be used to extract serum, RNA and genetic material (DNA) to look at markers or genes involved in IBD
 - It will not be possible to link my information resulting from the analysis of my samples with my identity for any purposes other than this research project
 - I can withdraw from the research study at any time. This decision will in no way affect my medical care now or in the future. If I choose to withdraw my samples will be destroyed.
 - Any member of my family can withdraw from the research study at any time. This decision will in no way affect the medical care received by any members of my family now or in the future and their samples will be destroyed.
 - Analysis of my samples may be conducted at centres outside of Mount Sinai Hospital. In this event, all samples will be kept completely anonymous and my identity will not be linked to my sample.
 - With your permission, your anonymized sample will be sent to the Rutgers University Cell and DNA repository, overseen by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in the National Institutes of Health (NIH) in the United States. The repository will allow investigators to share DNA samples to study the genetics of IBD and eventually, other biomedical research problems. Anonymized samples in the repository will not be under control of the investigators at Mount Sinai Hospital and the NIDDK will have control over the disposition of repository samples. This repository will also retain immortalized cell lines allowing for an indefinite supply of your DNA to be available.
 - The analysis of my samples may contribute to the future identification of new medications, diagnostic tools or other events that may have commercial value. My participation in this study does not entitle me to any share in such economic benefits, if any.

If you, or your family, have any questions regarding this study, please contact:

Joanne Stempak

Dr. Mark Silverberg

Research Coordinator
416-586-4800 x 8399 (jstempak@mtsinai.on.ca)

or

Principal Investigator
416-586-4800 x 8236

If you have any questions about your rights as a research subject, please call Dr. R. Heslegrave, Chair of the Mount Sinai Hospital Research Ethics Board at (416) 586-4875. This person is not involved with the research project in any way and calling him will not affect your participation in the study.

I hereby consent to participate. I have read, understood and agree with all of the points mentioned above.

Participant Signature

Participant Name (Print)

Date

Person Obtaining Consent – Signature

Person Obtaining Consent - Name (Print)

Date

Study Information (for study staff):

Family ID:

Member Id:

Sample #:

Proband: Yes No

Relationship to proband: _____

7.2. Questionnaire



Dr. Zane Cohen Digestive Diseases Clinical Research Centre

Family ID: _____ Member ID: _____ Sample ID: _____
 [Old IBD # _____ Member ID: _____ js # _____]
 Old Genetics _____ ACD/Yellow Lavendar Red PaxGene
 Hospital ID/Record #: _____ Individual case

Family

NIH Consortium ID

#: _____
 Alternate ID# _____ NIDDK

ID#: _____

Last Name: _____ First Name: _____
 Middle Name: _____ Maiden Name: _____
 Gender: M F Date of Birth: _____

Street Address: _____
 City/Town: _____ Province: _____ Postal
 Code: _____
 Phone (Home): _____
 (Business): _____
 Email address: _____

What is your main diagnosis?

IBD CD UC IC FAP Healthy Control * Unaffected N/A
 *If Spouse, duration of cohabitation (years) _____

Family History of IBD: Yes No # of unaffected siblings: _____
Other IBD Family Members: # of unaffected children: _____
 IBD CD UC Relation: _____
 IBD CD UC Relation: _____
 IBD CD UC Relation: _____

Family History of FAP: Yes No # of unaffected siblings: _____
Other FAP Family Members: # of unaffected children: _____
 Relation: _____
 Relation: _____
 Relation: _____

Centre: MSH TGH TWH SHSC WCH SMH HSC N/A

Multiple Birth: Are you a twin/triplet?

No Twin Identical Twin Unidentical Twin Triplet Other

Name of physician who diagnosed your IBD/FAP:

Original Physician: _____ **Phone:** _____
Address: _____

Name of physician currently treating your IBD/FAP:

Current Physician: _____ Phone: _____

Current Address: _____

Marital Status: What is your current marital status?

Single Married Widow(er) Separated Divorced

Education: What is the highest level of education you have received?

No formal 1° 2° Post-2° Bachelor Master PhD Other
Unknown

Employment Status: What is your current employment status?

Employed Unemployed Disability Insurance Retired Unknown

Occupation: What are your primary and current occupations?

Primary _____ Current: _____

Income (\$): What is your personal annual income?

<\$20K \$20-50K \$50-100K >\$100K Unknown

Psychological Stress: Near time of symptom onset, were you affected by any of the following psychological stresses, such as:

Death Family Stress Divorce/Separation Financial Stress Other

Physical Stress: Near time of symptom onset, were you affected by any of the following physical stresses, such as:

Motor vehicle accident Surgery Other injury Major infection

Oral Contraceptive: Have you taken oral contraceptives?

Unknown Never

Current Previous Start Date: _____ End Date: _____

of Pregnancies: _____ #of Live Birth: _____

Menopause: Are you currently experiencing menopause?

Yes No Unknown

Are you currently taking (or have you previously taken) hormone replacements?

Current Hormone Replace: No Estrogen Alone Estrogen + Progesterone

Previous Hormone Replace: No Estrogen Alone Estrogen + Progesterone

Ethnicity:

	Caucasian	Black	Asian	Hispanic	Jewish Ashkenazi	Jewish Sephardic	N.A.'n Indian	Other	BIRTH COUNTRY/City
--	-----------	-------	-------	----------	---------------------	---------------------	------------------	-------	-----------------------

<i>Subject</i>									
Mother									
Father									
Maternal G.Mother									
Maternal G. Father									
Paternal G. Mother									
Paternal G. Father									

Other Diagnosis:

No CAD DVT Lymphoma Psoriasis Stroke Other

Diagnosis Date (mm/dd/yy): _____ **Age of Diagnosis:** _____

Date of Onset (mm/dd/yy): _____ **Age of Onset:** _____

Diagnosis documented by:

	NO	YES
Clinical		
Pathology		
Radiology		
Endoscopy		

	NO	YES
CRC		
CRC Family Hx.		

Details (e.g. Other Family Hx.):

Site: Do you know the location of your disease?

Oral Esophagus Stomach Duodenum Jejunum
 Ileum
 Terminal Ileum Cecum Ascending Colon Transverse Colon
 Descending Colon Sigmoid Colon Rectum Perianal

Pouch
Updated: _____

Site Date

Behaviour: Fibrostenotic
Updated: _____

Fistulizing Inflammatory **Beh.Date**

On(mm/dd/yy): _____ **Size of Resection:** _____

Indication:

Obstruction Fistula/Abscess **Dysplasia/Cancer(UC)** Chronic continuous dis.(UC) Acute fulminant dis. (UC)

2. Procedure: _____ **Dr./Hospital:** _____

On(mm/dd/yy): _____ **Size of Resection:** _____

Indication:

Obstruction Fistula/Abscess **Dysplasia/Cancer(UC)** Chronic continuous dis.(UC) Acute fulminant dis. (UC)

3. Procedure: _____ **Dr./Hospital:** _____

On(mm/dd/yy): _____ **Size of Resection:** _____

Indication:

Obstruction Fistula/Abscess **Dysplasia/Cancer(UC)** Chronic continuous dis.(UC) Acute fulminant dis.(UC)

4. Procedure: _____ **Dr./Hospital:** _____

On(mm/dd/yy): _____ **Size of Resection:** _____

Indication:

Obstruction Fistula/Abscess **Dysplasia/Cancer(UC)** Chronic continuous dis.(UC) Acute fulminant dis.(UC)

5. Procedure: _____ **Dr./Hospital:** _____

On(mm/dd/yy): _____ **Size of Resection:** _____

Indication:

Obstruction Fistula/Abscess **Dysplasia/Cancer(UC)** Chronic continuous dis.(UC) Acute fulminant dis.(UC)

Dx Active Score: _____ **Date:** _____ **Index:** CDAI HBI PDAI UCAI

Steroid Side Effects: Yes No If Yes, please specify: _____
Hemoglobin : _____
Albumin : _____

Perianal Disease:

Fistula-in-ano: Superficial	Fistula-in-ano: Complex	Rectovaginal Fistula
Fissure	Hemorrhoids/Skin Tags	Abscess (no fistula
observed)		
Other	Unknown	

Disease Activity of the Rectum:

Quiescent/None	Mild	Moderate
Severe	Unknown	Not appropriate
Not applicable	Missing	

Medical Treatment for IBD/FAP:

Medication	Av.Daily Dose	Start Date	End Date	<i>Indication</i>	Response	Reason Discont'd.
6-MP						
<i>Azathioprine</i> (Imuran)						
<i>Asacol</i>						
<i>Solucortef</i>						
<i>Oral 5-ASA</i>						
<i>5-ASA Enemas</i>						
<i>Pentasa</i>						
Salofalk						
<i>Sulfasalazine</i> <i>(Salazopyrine)</i>						
<i>Ciprofloxacin</i>						
<i>Gentamicin</i>						
<i>Neomycin</i>						
<i>Vancomycin</i>						

<i>Metronidazole</i> (Flagyl)						
Infliximab (Remicade)						
Medication	Av. Daily Dose	Start Date	End Date	Indication	Response	Reason Discont'd.
Budesonide (Entocort)						
Steroid Enemas						
Methotrexate						
Prednisone						
Ancef						
Other (1) Specify:						
Other (2) Specify:						
Other (3) Specify:						
Other (4) Specify:						
Other (5) Specify:						

Pouch Section

Date of Colectomy: _____

Number of Surgeries after Colectomy: _____

Type of Surgeries:

Colectomy Date: _____

Pouch Construction Date: _____

Ileostomy Closure Date: _____

Symptoms 6 months after IPAA Surgery:

Symptom	Date of onset (month/year)	Date of resolution (month/year)	Number of episodes from onset to resolution	Duration of episode (days)
Faecal urgency/ Abdominal Cramping				
Loss of control of Bowel Mov'ts				
Fever				
Blood in Bowel Mov't/Rectal Bleeding				
Other Specify:				

Do you have trouble feeling that you have emptied your pouch? Yes No

Baseline Stool Frequency:

Daytime stool frequency (number/24h): _____

Nocturnal stool frequency (number/24h): _____

Incontinence/leakage (number/24h): _____

Change in Stool Frequency (6-9 months postoperatively):

Daytime stool frequency (number/24h): _____

Nocturnal stool frequency (number/24h): _____

Incontinence/leakage (number/24h): _____

Diagnosis of Pouchitis:

Date of Pouchitis onset after IPAA: _____

Received an endoscopy at the time of diagnosis: Yes No

Medical Treatment for Pouchitis:

Medication	Av.Daily Dose	Start Date	End Date	<i>Indication</i>	Response	Reason Discont'd.
6-MP						
<i>Azathioprine</i> (Imuran)						
<i>Asacol</i>						
<i>Pentasa</i>						
Salofalk						
<i>Sulfasalazine</i> <i>(Salazopyrine)</i>						
<i>Ciprofloxacin</i>						
<i>Metronidazole</i> (Flagyl)						
Infliximab (Remicade)						
Prednisone						
Budesonide (Entocort)						
Methotrexate						
Probiotics						
Imodium/ Lomotil (Loperamide)						
Other (1) Specify:						

Other (2) Specify:						

Other Treatment(s) (Naturopathic, etc...):

Duration of pouchitis episodes: _____

Frequency of episodes of pouchitis/yr:

- | | |
|---------------|----------------|
| 1 episode/yr | 4 episodes/yr |
| 2 episodes/yr | 5 episodes/yr |
| 3 episodes/yr | >6 episodes/yr |

Revision of Pouch: _____

Time Since Stoma Closed: _____

Type of Pouch:

J S W

Any Findings at Sx:

- | | | |
|-----------------------------|------------------------------------|-------------------|
| Long Outlet | Evidence of Pelvic/Perianal Sepsis | Ileal Resection |
| R/V Fistula | Pouchitis | Jejunal Resection |
| Other Fistula | Small Bowel Disease (excl. Pouch) | IAA Stricture |
| Other Please Specify: _____ | | |

Construction of IAA:

IAA	Type:	Handown	Linear Stapler	N/A
		Unknown	Missing	

Length of rectal cuff: _____

Length of Mucosa left: _____

Intraoperative Difficulties:

- | | | | |
|-----------------------------|-------------------|-----------------|-------|
| Anal Anastomosis Related | Rectum Related | Length Related | Pouch |
| Rectal Mucosa Related | Adhension Related | Stapler Related | |
| Other Please Specify: _____ | | | |

Dysplasia

Colon

Rectum

Anus

Findings of Rectal Mucosa:

Degree of

Inflammation: _____

Presence of Cancer:

Yes

No

Diagnosis documented by:

	NO	YES
Clinical		
Pathology		
Radiology		
Endoscopy		

Endoscopy Inflammation:

Oedema

Granularity

Friability

Loss of vascular pattern

Mucoid exudate

Ulceration

Other Please Specify: _____

Acute Histological

Inflammation:

Polymorphic nuclear leucocyte infiltration:

Mild

Moderate + crypt abscess

Severe + crypt abscess

Ulceration per low-power field (mean)

<25%

25-50%

>50%

Use of Meds:

Occasional Meds

Const. Meds

Antibiotic Treatment:

Antibiotic-responsive pouchitis

Antibiotic-dependent pouchitis

Antibiotic-refractory pouchitis

Categorization of Pouchitis:

Acute

Relapsing (intermittent)

Chronic (continuous)

Complication and Treatment Data For Pouch Procedure:

Date of Related Surgery (MM-DD-YYYY)

See Table 1 and 2 for Treatment and Complications Codes respectively

Complication 1 *Enter code from table	Date of Complication (MM-DD-YYYY)	#	Treatment	Date of Treatment (MM-DD-YYYY)	Success (1) Yes (2) No (3)Unk
		1			
		2			
		3			
		4			
		5			

Complication 2	Date of Complication (MM-DD-YYYY)	#	Treatment	Date of Treatment (MM-DD-YYYY)	Success (1) Yes (2) No (3)Unk
		1			
		2			
		3			
		4			
		5			

Complication 3	Date of Complication (MM-DD-YYYY)	#	Treatment	Date of Treatment (MM-DD-YYYY)	Success (1) Yes (2) No (3)Unk
		1			
		2			
		3			
		4			
		5			

Complication 4	Date of Complication (MM-DD-YYYY)	#	Treatment	Date of Treatment (MM-DD-YYYY)	Success (1) Yes (2) No (3)Unk
		1			
		2			
		3			
		4			
		5			

Pelvic Pouch Complications:

Table 1:

compspec	comptreattype
None	0
Leak from I.A.A.: with Abscess	1
Leak from I.A.A.: no Abscess	2
Leak from I.A.A.: cutaneous fistula	3
Leak from I.A.A.: vaginal fistula	4
Leak from I.A.A.: radiological only	5
Leak from Pouch	6
Fistula – pouch cutaneous	7
Fistula – entero-pouch	8
Fistula – enterocutaneous (not ileo)	9
Fistula - Other	10
Intra-Abdominal Sepsis	11
S.B. obstruction before closure	12
S.B. obstruction after closure	13
Leak from ileostomy closure site	14
Wound infection with P.P. procedure	15
Wound infection with ileo closure	16
Stoma complications	17
High ouptput/dehydration	18
Hemorrhoid related	19
Perianal disease after closure	20
Suspected Leak	21
Stricture of I.A.A. before closure	22
Stricture of I.A.A. after closure	23
Mucocele of rectal cuff	24
Poor functional result	25
Bladder dysfunction	26
Pouch Related Bleeding	27
Pouchitis before closure	28
Pouchitis after closure	29
Perianal disease pre-closure	30
Fever of unknown origin	31
Death	32
Rectovaginal Fistula	33
S.B. perforation	34
Sexual dysfunction	35
Late Pouch/IAA fistula	36

Table 2:

compspec	comptreattype
I-A Bleeding	56
Other	666
Unknown	888
Missing	999