Perianal Crohn's Disease in Canterbury, New Zealand

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2. M Lion, R B Gearry, A Day, T Eglinton. The cost of paediatric and perianal Crohn's disease in Canterbury, New Zealand. NZMJ accepted 2nd August 2011.

3. Eglinton T, Reilly M, Chang C, Barclay M, Frizelle F, Gearry R. Ileal Disease is Associated with Surgery for Perianal Disease in a Population-Based Crohn's Disease Cohort. British Journal of Surgery 2010; 97: 1103-1109.

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3. T. W. Eglinton, A. G. Balasingam, L. Dixon, R. B. Gearry, F. A. Frizelle. Magnetic resonance imaging (MRI) in perianal Crohn's disease (CD): does it change intra-operative decision making? Tripartite Colorectal Meeting, Cairns, July 2011.

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List of Abbreviations

AGA	American Gastroenterological Association
ASCA	anti-Saccharomyces cerevisiae antibody
ATG16L1	autophagy related 16-like one
CARD 8	caspase recruitment domain family, member 8
CD	Crohn's disease
CDAI	Crohn's disease activity index
CDHB	Canterbury District Health Board
CI	confidence interval
CT	computed tomography
DRG	Diagnosis related group
EAUSS	endo-anal ultrasound scan
EUA	examination under anaesthetic
GP	general practitioner
GWA	genome-wide association
HUHC	high user health card
HWE	Hardy-Weinberg equilibrium
IBD5	inflammatory bowel disease 5
IBD3	inflammatory bowel disease 3
IL2_21	interleukin 2-to-21
IL23R	interleukin 23 receptor
IRF1	interferon regulatory factor 1 gene (*IRF1*),
IRGM	immunity-related guanosine triphosphatase protein type M
OCTN1	carnitine/organic cation transporter 1
OCTN2	carnitine/organic cation transporter 2
MAF	Minor allele frequency
MRI	magnetic resonance imaging
NADPH	nicotinamide adenine dinucleotide hydroxylase
NALP3	NACHT, LRR and PYD domains-containing protein 3
NCF4	nuclear cytosolic factor 4
NOD2/CARD15	nucleotide-binding oligomerization domain 2/capsase recruitment domain-containing protein 15
OR	odds ratio

PCR	polymerise chain reaction
PDAI	perianal disease activity index
PHARMAC	Pharmaceutical Management Agency in New Zealand
PHO	primary health organisation
PSI	perianal surgical intervention
SNP	single nucleotide polymorphism
TNFα	tumour necrosis factor-α

Chapter 1

Perianal Crohn's Disease Overview.

1.1 Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease characterised by transmural segmental inflammation that can occur in any portion of the gastrointestinal tract from the oral cavity to the anus. It can be complicated by the development of fibrotic strictures, perforation, abscess formation, and fistulisation [1]. The aetiology and pathogenesis remain undetermined although on-going research suggests a multi-factorial aetiology combining genetic and environmental factors [2]. The fact that the incidence of CD is increasing worldwide [3] and that the peak age of onset is in the productive years of life between ages 15 and 35 [4] mean it is an increasingly significant health problem in terms of both morbidity and cost.

Crohn's disease has long been recognised as a protean disorder that incorporates a range of disease phenotypes. A percentage of patients with CD will develop a variety of perianal complications during the course of the disease which range from mild problems requiring minimal intervention, such as anal skin tags, to severe fistulising disease resulting in proctectomy. In addition to the morbidity associated with the perianal lesions themselves, research suggests that patients with perianal CD suffer from more severe intestinal disease [5, 6] requiring more intensive medical and surgical management. Previously it was considered that perianal fistulas were closely related to intestinal fistulas and were simply a manifestation of the same pathologic process occurring higher in the gastrointestinal tract. However, recent research suggests penetrating perianal disease often occurs independently of penetrating intestinal disease [7-9] raising the possibility of separate underlying aetiological and pathogenic factors for these disease phenotypes.

While much progress has been made in our understanding of inflammatory bowel disease, perianal CD has attracted less attention and important gaps in our knowledge exist. The present discussion reviews the current literature pertaining to perianal CD incidence, classification, diagnosis, management and cost. In doing so, a

number of research questions are highlighted which are subsequently addressed in the five separate but related studies presented in this thesis.

1.2 Classification and Incidence

Penner and Crohn first described the presence of a perianal fistula in a patient with CD in 1938 [10]. This was six years after the original description of regional ileitis [11] and a decade after Gabriel's finding of perianal granulomas in the absence of tuberculosis [12]. Since then, perianal CD has become an increasingly recognised entity with the documented perianal manifestations including; skin tags, haemorrhoids, fissures, ulcers, perianal abscesses, fistulas, strictures and cancer.

1.2.1 Classification

1.2.1.1 Anal Skin Tags

Skin tags are common but often asymptomatic. While they may be associated with fissures or fistulas, they can occur independently, possibly as a result of lymphoedema from lymphatic obstruction. They have been reported to become larger and firmer during an active CD flare.

1.2.1.2 Haemorrhoids

Haemorrhoids are a common problem in the general population but appear to cause significant symptoms infrequently in CD patients. In a series of 50,000 patients with haemorrhoids, 20 were noted to have CD (0.04%) [13].

1.2.1.3 Anal Fissures and Ulcers

Anal fissures in CD tend to be broad based and deep with undermining of the edges. There may be associated skin tags and a bluish tinge to the surrounding skin. Idiopathic anal fissures, not associated with CD, occur in the posterior midline, are painful and are associated with high resting anal canal pressure. In contrast, fissures in CD have classically been described as painless although reported series suggest pain

is a feature in a significant proportion of patients [14, 15]. CD fissures are more often multiple and lateral than idiopathic fissures but around 40% still occur in the posterior midline [14]. Early series of perianal CD patients also suggested CD fissures differed from their idiopathic counterparts in that they were not associated with high resting sphincter pressures [16]. However, more recent evidence suggests CD patients have high resting anal canal pressures suggesting compromised anal circulation may also play a role in the pathogenesis of CD fissures [17]. Fissures appear to be a relatively common lesion in perianal CD occurring in 21-59% of patients in various series [18-20]. Deep cavitating ulcers are less common than fissures and are usually associated with rectal inflammation. They may erode into the sphincters and progress to anorectal strictures or complex fistulas.

1.2.1.4 Perianal Abscess

Perianal abscesses are common and usually associated with a fistula-in-ano. Up to 62% of perianal CD patients will develop an abscess at some stage [19]. Any of the potential anorectal spaces may become infected with an abscess, however, ischiorectal and transphincteric fistulas are particularly prone to abscess development meaning the ischiorectal space is a common site with around 40% of abscesses occurring in this location [21]. After surgical drainage, recurrence is common and is likely related to the location of the associated fistula.

1.2.1.5 Fistula-in-ano

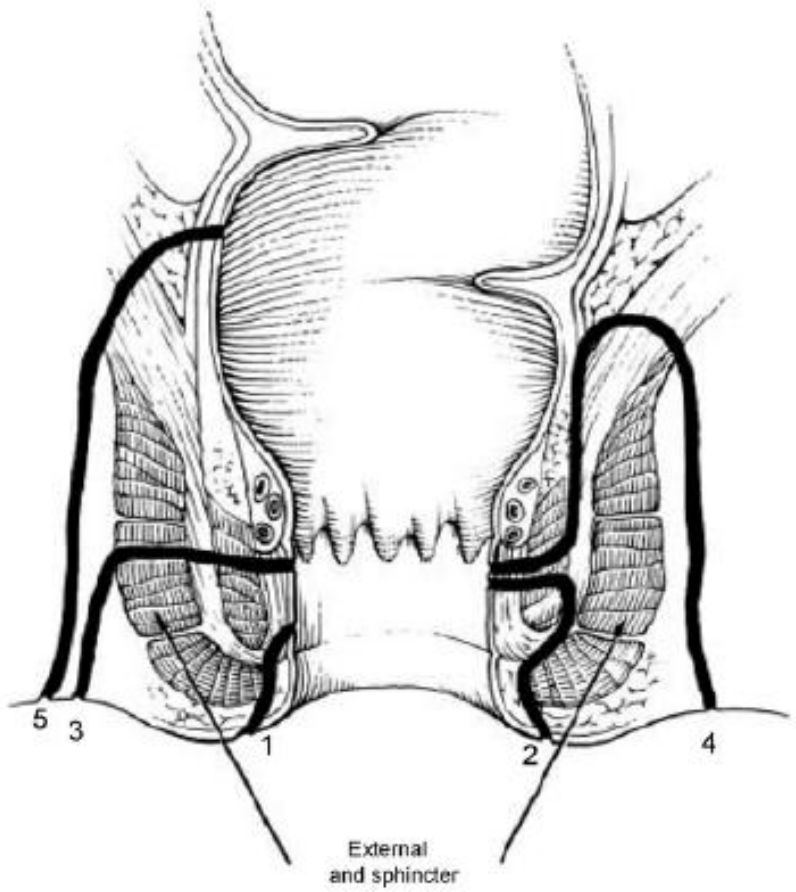


Figure 1.1. Park's classification of cryptoglandular fistula-in-ano. (1. Superficial; 2. Intersphincteric; 3. Transphincteric; 4. Suprasphincteric; 5. Extrasphincteric; Reproduced from reference [22])

Crohn's perianal fistulas are common lesions and a significant source of morbidity. They are often described using Parks' classification (Figure 1.1) developed for fistulas of cryptoglandular origin [23]. The aetiology of fistulas in CD is unclear. They may arise as a result of infected anal glands similar to that suggested in the cryptoglandular theory. Alternatively, or in addition, anorectal inflammation with cavitating ulcers may result in direct extension into the perianal spaces and subsequent abscess then fistula formation. Perianal CD fistulas can be classified as either simple or complex based on their anatomic and associated features [24]. Simple fistulas are low (superficial, low intersphincteric or low transphincteric) without associated abscess or stricturing. Complex fistulas, on the other hand, are high (high

intersphincteric, transphincteric, extrasphincteric or suprasphincteric) and may be associated with multiple external openings, an abscess, rectovaginal fistula or stricture. This classification has significant clinical relevance as simple fistulas are more straightforward to treat and have higher rates of healing [25].

Rectovaginal fistulas have been reported in up to 23% of women with perianal CD [26]. As with other fistula-in-ano, they can be described using Park's classification based on the relationship of the fistula tract to the sphincters; intersphincteric, transphincteric, suprasphincteric and extrasphincteric. In the majority (85%), the opening into the rectum or anal canal is anterior [27]. The aetiology is likely from anorectal inflammation and ulceration penetrating into the vagina although infected anal glands may contribute in some cases.

1.2.1.6 Anorectal strictures

These often occur in the low rectum and may be the result of chronic ulceration, perianal sepsis or severe, active luminal inflammatory disease. Strictures appear less common than other perianal manifestations but represent a severe form of the disease; 43% in one series requiring proctectomy [28].

1.2.1.7 Cancer

Both adenocarcinoma and squamous cell carcinoma (SCC) of the anus occur with perianal CD. Patients with longstanding CD colitis are recognised to be at increased risk for colorectal adenocarcinoma similar to that seen in ulcerative colitis (UC) [29]. However this risk appears to predominantly involve colonic rather than rectal cancer [30] when all CD patients are considered. Smaller studies looking at perianal CD patients specifically suggest the incidence of adenocarcinoma is increased and likely related to duration of disease and activity [31].

Anal SCC also appears to occur with greater frequency in perianal CD than the general population. Slater et al found the relative incidence of anal cancer as a proportion of all colorectal cancer was 14% in CD patients compared with 1.4% in patients without CD [32]. Cancer risk is likely to be increased as a result of chronic,

long standing perineal inflammation and risk appears to be related to the duration of disease. Delay in diagnosis may also occur as cancers have been demonstrated in long standing fistulas and other perianal lesions.

1.2.1.8 Classification Systems for Perianal CD

Due to the variety of perianal CD lesions discussed above, systems have been developed in an attempt to accurately classify the anatomic, pathologic and clinical features of the disease. Hughes produced the Cardiff classification in 1978 which is an anatomic and pathologic classification system which grades the major manifestations of perianal CD (ulceration, fistula or stricture) on a scale from 0 to 2 (table 1.1) [33].

Table 1.1. The Cardiff Classification of Crohn's Disease (reproduced from reference [34])

Cardiff classification		
U Ulceration	F Fistula or abscess	S Stricture
0 Not present	0 Not present	0 Not present
1 Superficial fissures	1 Low or superficial	1 Reversible stricture
(a) Posterior and/or anterior	(a) Perianal	(a) Anal canal—spasm
(b) Lateral	(b) Anovulval/anoscrotal	(b) Low rectum—membranous
(c) With gross skin tags	(c) Intersphincteric	(c) Spasm with severe pain (no sepsis)
	(d) Anovaginal	
2 Cavitating ulcers	2 High	2 Irreversible stricture
(a) Anal canal	(a) Blind supralevator	(a) Anal stenosis
(b) Lower rectum	(b) High direct (anorectal)	(b) Extrarectal stricture
(c) With extension to perineal skin (aggressive ulceration)	(c) High complex	
	(d) Rectovaginal	
	(e) Ileoperineal	
Subsidiary classification		
A Associated anal conditions	P Proximal intestinal disease	D Disease activity (in anal lesions)
0 None	0 No proximal disease	1 Active
1 Haemorrhoids	1 Contiguous rectal disease	2 Inactive
2 Malignancy	2 Colon (rectum spared)	3 Inconclusive
3 Other specify	3 Small intestine	
	4 Investigation incomplete	

This classification has never been prospectively validated using clinically relevant endpoints and has not been widely adapted as, while it is accurate and comprehensive, it lacks clinical utility [24]. Buchmann and Alexander-Williams also applied a classification of perianal CD using three categories; skin lesions, anal canal lesions and fistulas. While this system is also useful for describing the anatomical features of the disease it does not involve any measure of severity and effect on the overall wellbeing of the patient, factors which are necessary to aid in clinical decision making and assessing treatment efficacy for research purposes. The perianal disease activity index (PDAI), developed by Irvine [35], is a scoring system similar to the

Crohn's disease activity index (CDAI) used in intestinal CD, designed to allow assessment of these more global measures (table 1.2).

Table 1.2 The Perianal Disease Activity Index. (Reproduced from reference [35])

Perianal disease activity
Discharge
0 No discharge
1 Minimal mucous discharge
2 Moderate mucous or purulent discharge
3 Substantial discharge
4 Gross fecal soiling
Pain/restriction of activities
0 No activity restriction
1 Mild discomfort, no restriction
2 Mod. discomfort, some limitation activities
3 Marked discomfort, marked limitation
4 Severe pain, severe limitation
Restriction of sexual activity
0 No restriction sexual activity
1 Slight restriction sexual activity
2 Mod. limitation sexual activity
3 Marked limitation sexual activity
4 Unable to engage in sexual activity
Type of perianal disease
0 No perianal disease/skin tags
1 Anal fissure or mucosal tear
2 <3 Perianal fistulae
3 ≥3 Perianal fistulae
4 Anal sphincter ulceration or fistulae with significant undermining of skin
Degree of induration
0 No induration
1 Minimal induration
2 Moderate induration
3 Substantial induration
4 Gross fluctuance/abscess
Total score

While this does provide some indication of response to treatment, its utility in the clinical setting is limited. The drawbacks of these proposed systems led the American Gastroenterological Association (AGA) to recommend an empiric classification in their recent Technical Review on Perianal Crohn's Disease [24].

Table 1.3 Definition of the various types of perianal lesions in CD. Reproduced from reference [36].

Type of lesion	Description
Skin tag	Two types: 1. Large, edematous, hard, cyanotic skin tags. Typically arising from a healed anal fissure or ulcer. Excision contraindicated due to problems with wound healing. 2. "Elephant ear" tags that are flat and broad or narrow, soft painless skin tags. May cause perianal hygiene problems and can be safely excised.
Hemorrhoids	Prolapsing internal hemorrhoids. Uncommon in Crohn's disease. Often present as large external skin tags.
Fissure	Anal fissures are broad based and deep with undermining of the edges. There may be associated large skin tags and a cyanotic hue to the surrounding skin. They tend to be multiple and may be placed either eccentrically around the anal canal or in the midline in contrast to idiopathic fissure-in-ano, which tend to lie in the midline. Typically painless (pain should raise suspicion for perianal abscess or acute/chronic conventional anal fissure). Conventional anal fissures occasionally are treated by conventional fissure treatment including lateral sphincterotomy.
Anal ulcer	Anal ulcers are usually associated with rectal inflammation and may lead to destruction of the anorectum, anorectal strictures, complex anorectal fistulas, and perianal abscess.
Low fistula	Superficial, low intersphincteric, or low trans-sphincteric fistulas. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum.
High fistula	High intersphincteric, high transsphincteric, suprasphincteric, extrasphincteric fistulas. Arise from penetrating ulceration of the anal canal or rectum.
Rectovaginal fistula	Superficial, intersphincteric, transsphincteric, suprasphincteric, extrasphincteric fistulas. Arise from penetrating ulceration of the anal canal or rectum into the vagina.
Perianal abscess	Potential anorectal spaces may become infected with an abscess, including perianal, ishiorectal, deep postnatal, intersphincteric, and supralevator.
Anorectal stricture	May be short annular diaphragm-like strictures <2 cm in length or longer tubular strictures arising from rectal inflammation. May arise from either the anal glands (cryptogenic) or from penetrating ulceration of the anal canal or rectum.
Cancer	Squamous cell carcinoma, basal cell carcinoma, or adenocarcinoma arising from malignant degeneration of nonhealing perianal fistulas or sinus tracts.

This paper recommends clinical examination to document the presence of any of the perianal lesions discussed above. Fistulas are then classified either as simple or complex based on the aforementioned criteria. This simple and clinically relevant approach was adopted for use in this thesis. The definitions used for the various perianal lesions are summarised in table 1.3.

1.2.1.9 Classification systems for all CD.

Perianal disease is one of a wide variety of clinical presentations that are manifest under the banner of CD. The heterogeneous nature of the clinical manifestations of CD mandated the development of classification systems using recognizable clinical and epidemiological features to facilitate both clinical decision making and research. Both the anatomic location of the disease and the disease behaviour were shown to have implications for response to medical therapy, requirement for surgery and association with certain serological and, more recently, genetic markers. This led to the development of the Vienna Classification in1998 following the World Congress of Gastroenterology in Austria. This classification, shown in table 1.4, grouped patients based on the categories of disease location, behaviour and age.

Table 1.4 The Vienna Classification of CD (adapted from reference [37])

Age at Diagnosis	A1	<40 years
	A2	≥40 years
Location	L1	Terminal ileum
	L2	Colon
	L3	Ileocolon
	L4	Upper gastrointestinal
Behaviour	B1	Inflammatory
	B2	Stricturing
	B3	Penetrating

The classification system was useful in predicting aspects of disease presentation and natural history. In addition, using the Vienna Classification, associations in disease location and behaviour with anti-Saccharomyces cerevisiae antibody (ASCA) [38, 39] and the presence of the nucleotide-binding oligomerization domain 2/capsase recruitment domain-containing protein 15 (*NOD2/CARD15*) gene mutations [39-41] were demonstrated. However, significantly, the Vienna classification did not separate luminal and perianal fistulising disease into different categories. Subsequent evidence suggested that while perianal fistulas and luminal fistulas are related, they tend to have distinct clinical associations and often occur completely independently of each other [7]. This evidence was incorporated into the subsequent Montreal modification of the Vienna classification, in which, instead of perianal fistulas being included under the B3 category, a separate perianal modifier was added that can coexist with any disease behaviour. The Montreal Classification, shown in table 1.5, also incorporated a separate sub-category for those diagnosed aged 16 and under and the upper GI disease sub-category was changed to allow existence alongside or independently of other intestinal disease. The Montreal system has been subsequently validated [42] and phenotypic classification using this system allows more direct and meaningful comparison amongst cohorts.

Table 1.5 The Montreal Classification of CD (adapted from reference [42])

Age at Diagnosis	A1	Less than 17 years
	A2	Between 17 and 40 years
	A3	Above 40 years
Location	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper disease
Behaviour	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
		p perianal disease modifier*

* p is added to B1-B3 when perianal disease is present

1.2.2 Incidence

The variety of perianal lesions that can complicate CD combined with the different classification systems has led to a wide range in the reported frequency of

perianal involvement in CD which varies from 10-80% [43]. In addition to differences in the definition of perianal disease and the criteria for inclusion in different series reporting on incidence, the wide range is also a result of whether the samples originate from referral centres or population based cohorts. Given that perianal CD is associated with a more complicated disease course, single centre, tertiary hospital based studies can be expected to produce a different prevalence to that seen in the CD population as a whole. Table 1.6 summarises the studies published to date which document the prevalence of perianal CD. In general the lowest prevalence is reported in population based cohorts including only fistulas and the highest prevalence in referral centre cohorts that include all perianal lesions including less significant lesions such as skin tags, haemorrhoids and fissures.

The duration of follow up will also impact on the reported frequency of perianal involvement as the temporal relationship of the perianal to the intestinal manifestations is variable. Previous research suggests that in up to one third of patients the perianal disease precedes the intestinal CD [18, 44] and of those that develop perianal CD, 75% do so within 10 years of the diagnosis of intestinal disease [44]. In less than 5% of patients perianal disease is the sole manifestation of CD. Hence, the longer the duration of follow up, the higher one would expect the cumulative incidence of perianal CD to be.

Table 1.6 Studies reporting the frequency of perianal involvement in CD

Study	Year	n	PD	%PD	Definition of PD
Population studies					
Hellers [45]	1980	826	224	23	Perianal fistula
Lapidus [46]	1998	507		37	Perianal and rectal fistulas and fissures. Note: colonic CD only
Schwartz [47]	2002	165		26	Perianal fistula (after 20 yrs)
Lapidus [48]	2006	1293		13.7	Fistula
Tang [7]	2006	1595	201	21.7	Fistula
Referral center studies					
Fielding [49]	1972	153		80.4	Abscess/sinus/fistula, tags, fissures
Lockhart Mummery [50]	1975	400		61	Ulcers, skin tags, skin changes, abscesses, fistulas
Rankin [51]	1979	569	205	36	Abscess, fistula, fissure
Buchmann [52]	1980	153	109	71	Fistulas, fissures, skin tags
Williams [44]	1981	1098		22	Anal fissures, fistulas and abscesses
Keighley [53]	1986	202	110	54	Abscess, fistula, tags, fissures, stricture, haemorrhoids, ulcer
Halme [54]	1995	112	35	31	Fistula
Platell [55]	1996	306	129	42.4	Fissure, abscess,fistula, skin tags, ulcers
Michelassi [56]	2000	717	224	31	Abscess, fistula, stenosis, incontinence
Veloso [57]	2001	450	91	20	Anal/rectovaginal fistulas or abscesses
Cosnes [58]	2002	2065	540	26	Fistula, abscess, large ulceration
Leong [59]	2004	80		36	Perianal fistulae, fissures or abscesses
Beaugerie [6]	2006	1123	282	25.1	N/G

The wide range in incidence demonstrated in this review illustrates the need for greater consistency in reporting perianal CD in future research. Employing clear definitions such as that provided here from the AGA allows for more meaningful comparison between series when assessing aetiological factors, natural history and response to treatment. To date there is no data from a population based cohort classifying symptomatic perianal lesions. In chapter 2 the results of a study describing

the rate and classification of symptomatic perianal CD in a population based cohort are presented.

1.3 Aetiologic factors in perianal CD

1.3.1 The aetiology of CD

The aetiology and pathogenesis of CD remain incompletely understood. Current evidence, however, overwhelmingly favours a dysregulated immune response to resident intestinal bacteria in genetically susceptible hosts [60, 61]. Familial aggregation suggests that CD is heritable [62]. A significantly higher rate of disease in monozygotic versus dizygotic twins [63], together with genome-wide association (GWA) studies, has confirmed a definitive genetic component in the pathogenesis of this disorder and this is discussed in detail in section 1.3.4 below. In addition to genetic risk factors, a number of lifestyle factors have been implicated in CD aetiology. Smoking has been shown to double the risk of CD [63] and also to aggravate the disease course. While the data is inconsistent, being breast fed likely has a protective role [64] and the duration of breast feeding may also be significant [65]. In addition, stringent sanitation and hygiene may have a deleterious effect[66]. Luminal antigens, in particular associated with luminal flora, are recognised as critical in CD pathogenesis, however, recent studies implicate a disordered host immune response, rather than microbial composition *per se*, as the critical factor in disease development [67, 68]. Defects in the innate and/or adaptive immune response ultimately result in uncontrolled expression of pre-inflammatory cytokines, chemokines and adhesion molecules, resulting in amplification and perpetuation of the cycle of inflammation [69]. This in turn produces the clinical manifestations of CD including full thickness inflammation of the intestinal wall with penetrating and stricturing complications. In around one quarter of patients, these clinical manifestations include perianal disease. Why only a subset of these patients develop perianal manifestations is not clear. Previously perianal fistulas were thought to be the result of a similar process to luminal fistulas, however, recent evidence suggests that fistulas in the perianal region in fact occur independently of luminal penetrating complications [7]. As previously discussed, this led to the Montreal modification of the Vienna Classification of CD and raises the possibility that penetrating perianal

disease represents a distinct disease phenotype. A review of the aetiological factors in perianal CD patients indeed reveals emerging associations with phenotypic, patient and genetic influences.

1.3.2 Phenotypic associations

The phenotypic categories from the aforementioned Montreal Classification (Age at diagnosis, disease location and disease behaviour) have all been investigated for associations with perianal CD. Of these, disease location appears the most consistent predictor of the risk of perianal complications, with the risk increasing the more distal the intestinal disease. In an early study, Rankin *et al* investigated the relationship of perianal disease to intestinal disease location in 569 CD patients from tertiary referral centres. Of these patients, 205 had perianal complications defined as anal fistula, fissure or abscess. This paper demonstrated perianal complications occurred more frequently in patients with colitis ($p=0.0027$) and ileocolitis ($p=0.0005$) than those with only small bowel disease [51]. These findings have been replicated in several other referral centre based cohorts that have also demonstrated rectal involvement shows the strongest association [44, 49, 50, 53, 55, 57]. The evidence from population based cohorts is less consistent. A study from Manitoba suggested patients with Crohn's colitis were three times more likely to develop perianal fistulae compared with those with ileitis [7]. Schwartz et al reported data from Minnesota and showed that ileocolitis was associated with an increased risk of all fistulising disease (intestinal and perianal) when compared with ileitis only [47]. In contrast, a study from Stockholm of 1389 patients demonstrated no relationship of perianal disease to intestinal disease location [48]. Explanations for this lack of association demonstrated in the Swedish study have included relatively limited follow up and low rates of colonoscopy, thereby potentially under diagnosing colonic disease.

Studies pertaining to the association of disease behaviour and perianal disease have also yielded varying results. Smith *et al* [8] found that perianal disease was not associated with penetrating intestinal disease and similarly Veloso *et al* [57] demonstrated that the presence of perineal fistulas was independent of penetrating intestinal disease. In contrast, Tang *et al* found there was a strong association between intestinal and perineal fistulas (OR 5.02, 95% CI 3.4-7.42, $P<0.0001$), but this was

much stronger for colonic disease location compared with ileal. These authors also noted that the two fistula types often occurred exclusively [7]. Sachar *et al* pooled data from six cohorts analysing 5491 CD patients and found an association between colonic luminal fistulas and perianal disease, but not ileal fistulas [9]. This evidence led the Montreal Working Group to conclude that perianal and enteric fistulas represent two different but associated phenotypes [70].

A number of studies suggest that young age at diagnosis is associated with perianal disease. Halme et al reported a series of 112 patients and found the average age of those with perianal disease was 27.7 years compared 37.4 years in those without ($p<0.01$) [54]. Roberts *et al* described a cohort of CD patients over 50 years of age and concluded they were less likely to present with perianal complications [71] and Cosnes *et al* found fistulising complications, including perianal disease, were more common in those younger than 40 years [58]. This data from referral centre based cohorts is also supported by population based studies. Lapidus *et al* found increased incidence of perianal fistulas with decreased age of diagnosis in an update of the Stockholm county cohort [48]. Tang *et al* demonstrated that all fistulising disease was most common in the 20-29 age range and least likely in those diagnosed after the age of 49 [7]. However, the relationship of age to perianal disease is not consistent across studies as both Platell *et al* [55] and Hellers *et al* [45] found no difference in age in patients with or without perianal disease. In addition, many studies do not control for duration of disease and the effect of young age at diagnosis may simply be a result of longer disease duration allowing perianal complications to develop. The impact of disease duration on needs to be considered in studies assessing association of age at diagnosis and perianal CD.

1.3.3 Other patient related factors

Several other patient related factors have been investigated for associations with perianal CD. There are conflicting reports on whether gender predicts the occurrence of perianal disease. Sangwan *et al* and Hellers *et al* both reported that significantly more males than females in their studies had perianal CD [18, 45]. On the contrary, Bell [25] noted a slight female predominance for perianal fistulae and Fry [72] reported more than twice as many females as males had perianal CD.

In a disease such as CD, thought to have a significant genetic aetiological component, ethnicity may play a role in determining the phenotypic manifestations and studies have shown that the risk of perianal disease varies in different ethnic groups. In particular non-Caucasian race has been associated with perianal disease in at least three studies [7, 58, 73]. In addition it has been demonstrated that Sephardic Jews have a higher rate of perianal disease than Ashkenazi Jews [74].

The documented rising incidence of CD over the last 50 years also suggests a strong environmental influence on the aetiology. A number of environmental factors have been repeatedly associated with CD including tobacco smoking [75-77], diarrhoeal illness in childhood [78] and oral contraceptive pill use [79]. Other less consistent associations include previous tonsillectomy [80, 81], and antibiotic use [82, 83]. Breast feeding has inconsistently been shown to have a protective effect against developing CD [64, 84].

The relationship between tobacco smoking and CD has been extensively investigated in epidemiologic studies and smoking is an accepted environmental risk factor for CD [75-77]. In addition, previous studies demonstrate the association of smoking with intestinal penetrating disease [85] and smoking accelerates the rate of progression to complicated disease [86]. There is, however, a paucity of data on the impact of smoking specifically on the development of perianal disease. The Manitoba population based cohort was investigated for the relationship between smoking and fistulising disease. For smoking history, there was no significant difference when comparing all fistulising with non-fistulising disease and comparing perianal versus intestinal fistulising disease [7]. Unfortunately, the perianal disease and non-perianal disease groups were not compared for smoking history in this study. If both smoking and perianal disease are associated with a more complicated disease course then the relationship between these factors deserves further consideration. In addition none of the other possible aforementioned environmental risk factors have been investigated separately for perianal disease.

1.3.4 Genetic Factors

1.3.4.1 Evidence for heritability of CD

The preceding discussion highlighted the fact that CD incidence has increased markedly in the last 50 years suggesting strong environmental influences. However, a significant amount of epidemiological evidence exists that demonstrates heritability is also a major aetiological factor, reinforcing the current theory that CD results from a dysregulated immune response to some environmental factor(s) in genetically susceptible hosts.

CD demonstrates a familial pattern of disease that was first recognised by Crohn *et al* in 1932 [11]. Subsequent research has documented the relative risk to the sibling of an affected individual is in the range of 15-35 for CD compared with only 6-10 in UC [87-91]. In fact, family history is the strongest risk factor for developing IBD [92] and the evidence for a genetic basis is further strengthened by twin studies which demonstrate higher disease concordance in monozygotic twins compared with dizygotic twins. Once again, this effect is more pronounced in CD than UC [93-96].

1.3.4.2 Techniques of gene identification

The two methods employed are candidate gene testing and genome wide scanning, both by linkage and association. The two techniques are complimentary and used together, with genome wide scanning identifying possible locations for subsequent candidate gene testing. Genome wide linkage utilises 'sibling pair' families which are genotyped using polymorphic DNA microsatellites located at intervals throughout the genome. Linkage analysis then measures excess sharing of the same allele between affected siblings suggestive of a correlation between inheritance of the disease and inheritance of that particular allele. Subsequently the search can be focused more specifically on that area of the genome [97]. More recent research has utilised single nucleotide polymorphisms (SNPs) as opposed to microsatellite markers in GWA studies. Single nucleotide polymorphisms are defined as single base pair variations where the least frequent allele has a population frequency of 1% or more. Single nucleotide polymorphisms may represent the genetic

defect themselves or act as a marker for the actual functional defect [97]. Candidate gene analysis, comparing allele frequencies between affected patients and controls, can then be performed; either using the results of GWA studies, or based on the known function of a gene.

1.3.4.3. Susceptibility Genes in CD

The first susceptibility gene identified for CD was *NOD2* on chromosome 16q12 [98, 99] and its association has been widely replicated in European cohorts, but not in Asian and African populations [97, 100]. A recent meta-analysis demonstrated the relative risk for developing CD in homozygotes for the mutant allele was 17.1 [101]. In addition, genotype-phenotype association studies have demonstrated that *NOD2* is associated with earlier age of onset [102-104] and ileal disease location [40, 102, 105, 106]. The mechanism by which *NOD2* mutations lead to CD is unknown. The gene encodes an intracellular receptor expressed in monocytes that is involved in response to bacterial muramyldipeptide. This may result in reduced ability to clear bacteria leading to dysregulation of adaptive immune pathways.

Since the discovery of *NOD2*, the progression of genetic technology has led to a rapid expansion in the identification of susceptibility genes in IBD. A recent meta-analysis of six genome-wide association studies defined 71 distinct susceptibility loci for CD [107]. This study analysed data from 15,694 cases and 14,026 controls and the genetic associations demonstrated are summarised in tables A1 and A2 provided in appendix 1. A complete discussion of all these genes is beyond the scope of this review, however, from the above discussion of *NOD2* it is clear that different susceptibility genes also confer risk for different phenotypes in CD, raising the question as to whether separate genetic susceptibility exists for perianal CD and this will be the focus of the subsequent discussion.

1.3.4.4 Genetic associations with perianal CD

There is still relatively sparse literature on genetic associations in perianal CD. To date, three genetic loci, *IBD5*, *IRGM* and *TNFα*, have been documented to be associated with perianal CD, albeit with varying strength and consistency.

1.3.4.4.1 IBD5 and perianal CD

The *IBD5* susceptibility locus on chromosome 5q31 is associated predominantly with CD but also UC. The association of this locus with CD itself has been widely replicated [108-111] and it was first associated with perianal CD by Armuzzi *et al* in 2003 [110]. These authors used three SNPs to create an H2 risk haplotype to span the *IBD5* gene. Perianal CD patients in the cohort had a relative risk of 3 (95% CI 1.8-5.0) for H2 homozygosity compared with healthy controls and a relative risk of 2 (95% CI 1.1-3.7) compared with non-perianal CD patients. In addition the H2 haplotype was also associated with ileal disease location [110]. The SNPs in this haplotype were selected as positional rather than functional variants and to date the exact causal variant in this region remains unclear.

Peltekova *et al* [112] identified potential functional variants with SNPs within the carnitine/organic cation transporter genes *OCTN1* and *OCTN2*. Several theories of how these variants could contribute to the pathogenesis of CD have been proposed. *OCTN* variants may produce reduced carnitine transport and thereby reduced oxygen burst mediated pathogen killing resulting in altered handling of intestinal bacteria. In addition, impaired fatty acid oxidation which occurs in the setting of impaired carnitine transport, has been shown to lead to colitis in animal models [113]. Vermeire *et al* investigated recognised SNPs in the *OCTN1* and *OCTN2* genes and while they found no association overall with CD in the Flemish population, they did demonstrate that the *OCTN2* variant was associated with perianal CD in a dose dependent manner. The same univariate analysis also demonstrated a trend to association between perianal CD and *OCTN1* [114]. These findings were essentially replicated by Palmieri *et al* who demonstrated that the *OCTN1-2* TC risk haplotype frequency was 55.7% in CD patients with perianal fistulas compared with 40.7% in CD patients without perianal fistulas (p=0.007) [115]. In addition this relationship was maintained in a logistic regression analysis including clinical factors.

Despite these positive associations, two further studies failed to show a significant effect of the *OCTN* variants on perianal CD. Newman *et al* found the *OCTN1-2* TC risk haplotype was associated with CD overall but not perianal CD

[116]. Most recently, Karban *et al*, found no association with perianal CD in any of the *OCTN1*, *OCTN2* or *OCTN1-2* TC risk haplotypes [74]. Hence, the *OCTN1* and *2* polymorphisms are not consistently associated with perianal CD and further research has also suggested they are not independent of the background risk haplotype for CD itself [117]. There are a number of other potential causal variants in the IBD5 region and the uncertainty surrounding the *OCTN* polymorphisms has focused attention on genes such as the cytokine cluster, the interferon regulatory factor 1 gene (*IRF1*), the *PDZ* and *LIM* domain and others [97].

The most recent study of the *IBD5* region and perianal disease used another positional variant *IGR2063b_1* and confirmed a higher risk of perianal disease in homozygotes for this SNP [117]. Explanations for this variation in results amongst studies include the inclusion and classification of perianal and intestinal CD, the ethnic makeup of the cohorts and also statistical under powering. Certainly, four of the six studies addressing the subject suggest an association with perianal CD and the IBD5 region, however further study is required both to confirm this association and the causal variant producing it.

1.3.4.4.2 IRGM and perianal CD

The immunity-related guanosine triphosphatase protein type M (*IRGM*) gene was associated with CD susceptibility in a large GWA study [118]. The fact this is an important autophagy gene also provides a plausible biological link to CD pathogenesis. The *IRGM* protein is involved in the elimination of intracellular bacteria by generating large autolysosomal organelles after binding to GTP [119]. Commensal bacteria likely contribute to the pathogenesis of CD and ineffective autophagy allowing persistence of intracellular bacteria may provide an opportunity for antigenic stimulation of the adaptive immune/inflammatory pathways. In addition to the discovery of *IRGM*, several other autophagy genes have been associated with CD in GWA scans including the autophagy related 16-like 1 (*ATG16L1*) and nuclear cytosolic factor 4 (*NCF4*) genes [120].

A single study of 823 CD patients did demonstrate the *IRGM* rs4958847 polymorphism was associated with perianal CD (OR 1.61) [121]. Significantly this

SNP was also associated with intestinal fistulising behaviour and after logistic regression analysis, combining clinical factors including age at diagnosis, disease location and smoking status, both associations persisted. The potential pathogenic mechanism and association with both perianal and fistulising CD make this an attractive target as a true susceptibility locus. However, sub phenotypic analysis of the same cohort used in the aforementioned GWA study failed to show an association with perianal disease and in addition, sequencing of the gene by Parkes *et al* did not show any causal amino acid changes [118]. Further studies in well characterised cohorts are required to clarify the relationship of perianal CD and the *IRGM* susceptibility locus. In addition, no studies have considered the other autophagy genes *ATG16L1* and *NCF4* and their association with perianal disease.

1.3.4.4.3 TNFα and perianal CD

The proinflammatory cytokine TNFα has an established role in CD pathogenesis and as a result the *TNFα* gene, located on chromosome 6 in the *IBD3* susceptibility locus, has been the subject of considerable interest. TNFα has a pivotal role in the inflammatory response and also apoptosis. TNFα levels have been shown to be elevated in the stool of IBD patients compared with healthy controls and anti-TNFα monoclonal antibody therapies have demonstrated considerable efficacy in CD treatment [122]. Several SNPs in the promoter region of the *TNFα* gene have been identified at positions 308, 857, 863 and 1031 [97] and downstream functional effects have been documented including increased TNFα production in carriers of the -308A polymorphism [123-125] and the -857 C→T SNP [126].

Despite the biological plausibility of a role for the *TNFα* gene in the pathogenesis of CD, results of association studies of the frequency of these SNPs in CD have been varied. A recent meta-analysis of 31 case control studies found an association only in the -1031 TC+CC genotype (OR 1.32; 95% CI 1.03-1.70) in all patients. While *TNFα* SNPs have not uniformly contributed to overall susceptibility to CD, individual studies have demonstrated association with different CD phenotypes [127-130]. However, the same meta-analysis stratified patients using the Vienna classification and did not find any association between these SNPs and CD location or behaviour. Fistulising disease was included in the analysis but perianal fistulas were

not considered separately. Only one study has separately considered the relationship of perianal CD to *TNFα*. Kim *et al* investigated genotypes in 63 patients with CD and published the results in the Korean Journal of Gastroenterology in Korean with an English abstract. They found the frequency of -238A allele of *TNFα* was significantly higher in CD patients with a perianal lesion than those without. Hence while *TNFα* is an attractive candidate as a susceptibility locus, the results for CD overall are variable and only one small study has directly considered the perianal disease group.

This discussion of aetiology demonstrates that while major advances have been made in the understanding of the environmental and genetic risk factors for CD overall, further research is necessary to clarify the relationship of perianal disease to intestinal CD and determine whether different risk factors exist for patients with this important subgroup of disease. Better defining the group at risk for perianal disease and therefore a more severe CD course will allow the appropriate use of investigations and management which also continue to evolve and are the subject of the remainder of this review.

1.4 Assessment of Perianal CD

CD patients with perianal symptoms require clinical examination to document the presence of the perianal lesions discussed in section 1.2. In addition, the assessment of perianal disease must be accompanied by an assessment of proximal luminal disease as this may influence decisions regarding management of the perianal disease. Treatment of the perianal CD depends on the nature of the lesion and ranges from conservative through medical and surgical intervention. Simple clinical examination is often inadequate and not well tolerated secondary to pain or the pathology present. Hence, further imaging and or examination under anaesthetic (EUA) is often necessary to allow accurate diagnosis and formulation of an appropriate management plan.

Further assessment with EUA or imaging is useful to;

- Document the presence of undrained sepsis
- Accurately define fistula anatomy in particular the relationship to the anal sphincter complex

- Identify any secondary tracts arising from fistulas
- Assess sphincter integrity in patients with previous perianal surgery
- Monitor response to medical and surgical treatments

At EUA the surgeon can perform a full examination including visual inspection and proctoscopy. Fistula anatomy is defined using probes and instillation of hydrogen peroxide to aid in identification of any internal fistula opening(s). Drainage of sepsis and other surgical management including fistulotomy or seton placement can be performed at the time. However, EUA is not 100% accurate and missed sepsis or fistula extensions can lead to recurrence and further tissue damage. In addition, both incorrect identification of the relationship of the fistula to the anal sphincters leading to inappropriate fistulectomy and injudicious probing can lead to iatrogenic injury and inadequate fistula treatment. Therefore, preoperative imaging is often employed and the most frequently used modalities are magnetic resonance imaging (MRI) and endo-anal ultrasound (EAUSS). Computed tomography (CT) occasionally has a role and historically fistulography was used, but has limited application in the modern era.

1.4.1 MRI in Perianal CD

For the reasons outlined above, MRI is now frequently used in perianal CD, particularly in complex perianal sepsis, to define fistula anatomy and to monitor response to treatment. Imaging techniques and experience vary amongst institutions. Both T2 and T1-weighted images are obtained with and without fat suppression. Active fistulas appear hyperintense on T2-weighted images (figure 1.2).

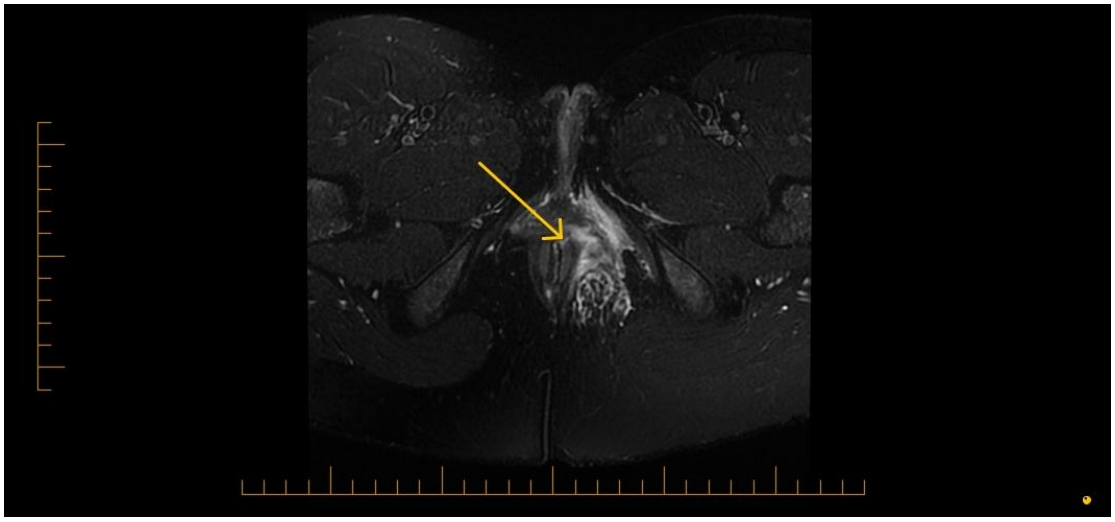


Figure 1.2 MRI of perianal CD. (T2 weighted magnetic resonance image documenting anal fistula; arrow)

These images can be obtained using either an endoanal or a surface external coil. The endoanal coil provides better spatial resolution, but over a more limited viewing field and is also subject to motion artefact. The surface coil does not require anal insertion (which can be problematic with severe perianal CD) and provides better definition of fistula extension over a wider field of view in the perianal region [131].

The reported accuracy of MRI in this setting varies with the technique used and the institutional experience. In 1992, Lunnis *et al* assessed perianal fistulas of cryptoglandular origin with preoperative MRI and found agreement with independently documented operative findings in 14 of 16 patients [132]. However CD fistulas tend to be more complex than those of cryptoglandular origin and in a similar study which included both cryptoglandular and CD patients, the only discordance between MRI and EUA was found in those patients with CD [133]. Reduced accuracy in CD was further supported by an early report of MRI exclusively in perianal CD demonstrating only 50% accuracy when compared with EUA [134]. In this study Orsoni *et al* used a body coil as opposed to pelvic phased array which may have affected the accuracy. In addition, studies assessing the accuracy of MRI against EUA are problematic as it is recognised that EUA itself is operator dependent and an imperfect gold standard that may have low sensitivity for undrained sepsis and fistula extensions. To address this problem Schwartz *et al* used a consensus gold standard for comparison of the accuracy of MRI, EUA and EAUSS. Thirty-four patients, all with

CD, underwent all three modalities and discordance was resolved by review of the findings of all the modalities together to reach the consensus gold standard. Using this methodology, the accuracies of MRI, EAUSS and EUA were 87%, 91% and 91% respectively. In addition any two of the modalities taken together resulted in an accuracy of 100% [135]. While this study did, to some extent, strengthen the reference used for comparison amongst these techniques, no clinical follow up was included. Given the complexity of perianal CD fistulas, it may only become clear which modality was correct when the outcome of treatment is known hence, the results of this type of study can be further strengthened by including the clinical outcome in the determination of the gold standard. This very approach was taken in a study from St Mark's Hospital in the United Kingdom of 104 patients. After mean clinical follow up of 23 months, an outcome based gold standard was derived to compare clinical examination (not under anaesthetic) with MRI and EAUSS. This study found a significant linear trend in the proportion of fistula tracks correctly identified by clinical examination (61%), EAUSS (81%) and MRI (90%) [136]. While this study suggests that MRI is accurate in defining the anatomy of fistulas, only nine of the 104 patients had CD. Hence, to date the literature contains variable results regarding the accuracy of MRI in perianal CD.

Given the cost and restricted availability of MRI in some areas, it is important to determine not only its accuracy but also whether adding MRI to the diagnostic algorithm in CD actually produces clinical management changes. This question has been addressed by studies that initially blind the surgeon performing EUA to the MRI result and after intraoperative revelation of the result, operative management changes resulting from the MRI are documented. The first of these studies involved only cryptoglandular fistulas in 34 patients. It demonstrated a relatively high discordance of 50% between MRI and EUA and concluded that MRI caused significant change to surgical management in 10% of patients [137]. With the increased complexity of CD perianal fistulas it could be expected that MRI may have a more significant impact on management in CD patients. Using similar methodology, Beets-Tan *et al* investigated 56 patients of which 15 had CD and found that MRI produced important information that lead to additional surgical management in 21% overall. Of note, in the 15 CD patients, 40% had surgical management altered [138].

In addition to its role in initial management of perianal CD, MRI is also increasingly used to monitor response to therapy. As discussed in the following section, modern biologic treatments have demonstrated considerable efficacy in healing CD fistulas. However, serial MRI of these fistulas has demonstrated persistent tracts despite apparent clinical healing [139, 140] suggesting a role for assessing response to treatment and potentially guiding decision making regarding continuing or changing biologic agents.

1.4.2 EAUSS in Perianal CD

EAUSS is performed using a specialised probe placed in the anal canal. The standard endoanal ultrasound probe obtains axial 360˚ images and allows accurate definition of the sphincter components, fistulas and abscesses. Modern probes contain a mobile crystal which can obtain multiple axial images to produce three-dimensional reconstructions giving enhanced views of the anal canal anatomy. Instillation of hydrogen peroxide into fistula tracts may also enhance visualisation [141, 142]. The proposed advantages of EAUSS over MRI are that it is simple and quick to perform and relatively inexpensive. In addition, it can be used in the operating theatre to guide surgery. However, the technique is highly operator dependent and the pathology may limit its use, particularly in the case of painful perianal lesions or strictures preventing probe insertion. As documented in the comparative studies discussed above [135, 136], the accuracy of EAUSS ranges from 81-91% and appears similar to that of MRI in assessing and classifying CD fistulas.

As with MRI, EAUSS may have a role in monitoring response to therapy. Ardizonne *et al* reported its use in 30 patients with perianal CD after treatment with infliximab. Of the 15 fistulas that ceased to drain, only 5 disappeared on EAUSS and closure on EAUSS was associated with a lower relapse rate [143]. Similarly, Schwartz *et al* followed 21 patients after infliximab treatment with serial EAUSS. Seven of 11 patients with no persistent fistula were taken off infliximab and no recurrences occurred in this group (the other four patients without evidence of persistent fistula were left on infliximab for luminal disease) [144].

1.4.3 Other Imaging Modalities

While computed tomography is often used to assess luminal and intra-abdominal complications of CD, it has little application in assessment of perianal CD. Detailed anatomic definition of the relationship of a fistula to the pelvic floor structures is not possible to the same extent as in EAUSS and MRI, as the CT attenuation of fistulas and the pelvic floor tissues is very similar. For this reason sparse literature exists on its accuracy and the only comparative study of CT and EAUSS in perianal CD demonstrated sensitivities of 24% and 82% respectively [145]. In the future CT fistulography using multi-detector imaging and contrast may offer improved accuracy but will still carry the disadvantage of radiation exposure to patients [131].

Fistulography was used historically to assess perianal fistulas however it had several disadvantages including poor contrast penetration into secondary fistula extensions and poor patient tolerability. The main downside compared with the more modern techniques of EUASS and MRI was the inability to visualise the sphincter mechanism and pelvic floor musculature and therefore accurately classify the fistula relationship to these.

1.4.4 Summary: Assessment of Perianal CD

Magnetic resonance imaging and EAUSS have emerged as the imaging techniques of choice in perianal CD and this is reflected in the most recent guidelines published by the AGA. These recommend either EAUSS or MRI in all perianal CD patients where surgery is the initial treatment strategy or in the presence of pain, fluctuation or stricture [24]. However, as the preceding discussion demonstrates, questions regarding the accuracy and utility of these modalities remain. While MRI and EAUSS appear reasonably accurate, much of the literature to date applies to cryptoglandular fistulas. The sparse literature available pertaining to more complex CD fistulas suggests the concordance between EUA and imaging reduces. Chapter 4 addresses this question presenting the results of a clinical study comparing the accuracy of MRI to EUA and its effect on operative management, including an assessment of outcomes.

1.5 Management of Perianal CD

The current management of perianal CD involves combined medical and surgical approaches and relies on close liaison between gastroenterologists and surgeons. Generally, surgical conservatism is advocated as there is a high rate of disease recurrence and poor wound healing. Inappropriate surgery can lead to sphincter damage and incontinence and it has previously been stated that "incontinence in CD is usually related to aggressive surgery, not progressive disease" [146]. If medical and local surgical approaches fail, treatment will culminate in proctectomy in a proportion of patients. Specific management depends on the nature of the perianal lesion producing symptoms.

1.5.1 Management of perianal abscess and fistula-in-ano

In the setting of perianal sepsis, the first priority is adequate drainage of abscesses including insertion of loose seton ligatures if fistulas are identified. With sepsis adequately drained, attention is turned to fistula healing and both medical and surgical approaches are available. The approach depends largely on the whether the fistula is classified as simple or complex and whether rectal disease activity is present. Surgical intervention in the presence of severe proctitis is associated with lower success rates. Simple and complex fistulas require different surgical approaches as discussed below and often, medical treatment is required in the first instance.

1.5.1.1 Medical Management of fistula-in-ano

1.5.1.1.1 Antibiotics

Antibiotics, most often metronidazole and/or ciprofloxacin, are widely used to treat perianal CD sepsis. This is despite a relative paucity of randomised evidence. A review of the literature published in 2003 documented several case series which demonstrated reduced pain and tenderness and up to half of patients achieving complete healing however relapse rates were high with dose reduction or cessation of therapy[36]. Subsequent to this review, a randomised, double blind, placebo

controlled, pilot study was performed. In 25 patients, this demonstrated clinical remission in 30%, 0% and 12.5% with ciprofloxacin, metronidazole and placebo respectively [147]. Despite the lack of evidence, antibiotics remain a common treatment due to perceived relative safety and lack of other therapeutic alternatives.

1.5.1.1.2 Immunomodulators

There is also a lack of randomised evidence pertaining to the use of azathioprine and 6-Mercaptopurine in perianal CD with fistula closure as the primary end point. A post-hoc meta-analysis of 5 studies considering fistula closure did demonstrate a 54% rate of healing of fistulas in the treatment group compared with 21% with placebo [148]. A single study also considered the combination of antibiotics and azathioprine and found improved response when both treatments were used together [149].

1.5.1.1.3 Anti-TNFα antibody therapy

The introduction of the anti-TNFα antibody treatments infliximab and more recently adalimumab have provided another option to attempt healing of perianal CD fistulas and these agents are now central to the treatment algorithm. The efficacy of infliximab was established in two randomised, placebo controlled trials. The first, ACCENT I, demonstrated a 68% clinical response rate (defined as a 50% reduction in the number of draining fistulas) and complete fistula healing in 55% [150]. The second study, ACCENT II, considered maintenance therapy and found that in the 69% of patients that responded at week 14, 39% maintained the response at 54 weeks following 8 weekly dosing of infliximab [151].

While infliximab does appear to have medium term efficacy in healing perianal CD fistulas, questions remain as to the necessary duration of therapy, the role of combination therapy both with other medical agents and surgery and whether any patient factors will predict response. These are all important issues given the expense and potential short and long term toxicity of these agents including infusion reactions, formation of human antichimeric antibodies, infectious complications and malignancy [152, 153]. Evidence accumulated since the ACCENT trials suggests a high relapse rate after cessation of therapy [154]. Combined medical therapy was investigated by a

post-hoc assessment of the ACCENT II data that did not demonstrate any benefit in the one-third of patients who were administered both infliximab and immunomodulators [155]. Combining surgery with infliximab infusion has the potential advantage of ensuring all sepsis is identified and facilitating on going drainage with seton placement and several small series report success with this approach [156-159]. However, data from the ACCENT II study did not show any evidence of increased perianal abscess formation in the infliximab treated group [160] and EUA prior to infliximab therapy is not considered mandatory.

A number of factors have been investigated in an attempt to predict those patients who will respond to infliximab, but the only association has been the presence of proctitis predicting a poor response [139]. In those that do not respond to infliximab, the alternative anti-TNFα antibody, adalimumab, has still been shown to have some efficacy albeit reduced compared with first line response rates to infliximab [161].

1.5.1.1.4 Other medical options

A number of other medical therapies have been trialled in small series including tacrolimus [162-165] and cyclosporine [166-172] which have shown some success but there use is restricted to patients who have failed other modalities. In addition there are recent experimental reports of the local injection of infliximab into fistula tracts [173, 174]. Several case reports found that hyperbaric oxygen therapy was associated with fistula healing [175-178] suggesting further investigation into this modality is warranted, however, the limited availability of hyperbaric chambers and duration of therapy are likely to limit its widespread use.

1.5.1.2 Surgical management of fistula in ano

Successful surgical management requires adequate drainage of all abscesses, accurate classification of the fistula as simple or complex and an assessment of luminal disease, especially in the rectum. This assessment dictates the specific operation performed.

1.5.1.2.1 Fistulotomy

For low fistulas involving minimal sphincter muscle, laying open of the fistula is an option and the reported healing rates range from 8% to 100% with majority of studies demonstrating healing rates of 80-100% [36]. However, there is also a broad range of reported recurrence (6-60%) and incontinence (0-50%) [36]. Healing rates are lower in the presence of macroscopic rectosigmoid inflammation hence fistulotomy should be avoided in this situation.

1.5.1.2.2 Seton ligature

If fistulotomy is not appropriate, due to rectal inflammation or the presence of a complex fistula, then a non-cutting seton ligature is the treatment of choice. Cutting setons, which effectively cause a slow fistulotomy and are associated with non-healing and incontinence, are not widely advocated in complex CD fistulas. A non-cutting seton can be left in situ long term to prevent abscess formation, whilst proctitis is controlled medically, then fistula healing can be attempted with medical treatment or a definitive surgical procedure.

1.5.1.2.3 Rectal advancement flaps

An endorectal advancement flap involves raising a flap of rectal wall containing the internal opening of the fistula, excising the mucosal opening, then suturing the healthy mucosa over the fistula tract in the muscle. This should only be attempted in the absence of rectal inflammation otherwise, failure rates are high. Even in the absence of rectal inflammation success rates are only around 50% in CD fistulas, compared with 70-80% in the setting of cryptoglalndular fistulas [179, 180].

1.5.1.2.4 Anal fistula plugs and fibrin glue

Recent developments in the treatment of cryptoglandular fistulas have included the insertion of either fibrin glue or collagen plugs into the fistula tract. These techniques have been extended to CD fistulas and have the advantage of avoiding sphincter damage. An initial study of CD patients treated with collagen fistula plugs

demonstrated 80% healing at 10 months follow up [181]. However, more recent small studies with both collagen fistula plugs and fibrin glue have shown healing rates in CD patients of only 26.6% [182] and 38% [183] respectively. Further randomised studies with larger numbers of CD patients would be necessary before advocating these treatments.

1.5.1.2.5 Faecal diversion and proctectomy

The severity of perianal sepsis and the aforementioned difficulties in treatment has seen proctectomy rates of 12-20% overall [34]. The presence of rectal involvement with CD is associated with even higher rates of proctectomy [45]. In severe perianal disease, faecal diversion with temporary ileostomy is occasionally employed. While several studies confirm it is rare for the ileostomy to be reversed [184, 185], it may allow the perianal disease to be brought under sufficient control to facilitate proctectomy with improved perineal wound healing. Poor perineal wound healing is a major complication of proctectomy in this setting with over one third of patients unhealed at 6 months. Yamamoto *et a*l documented that 23% of patients had perineal wound sinuses after proctocolectomy and attributed this lower rate to the use of defunctioning prior to proctectomy [186]. Other strategies to avoid perineal wound problems include the use of gracilis transposition flaps [187] or performing a low Hartmann's procedure as opposed to proctectomy [188].

1.5.2 Surgery for other perianal CD conditions

As documented in section 1.2, skin tags occur frequently in perianal CD and excision is best avoided due to poor healing. In contrast to skin tags, symptomatic haemorrhoids are rare in CD but when they do occur, operative management should also be avoided on the basis of high rates of wound complications and anal stenosis, which can potentially lead to proctectomy [36].

Typical CD anal fissures are painless and not necessarily associated with increased anal sphincter tone. Surgical intervention is generally not advised as spontaneous healing occurs in greater than 80% of patients [52] and the risk of incontinence with sphincterotomy needs to be carefully considered in patients with a

chronic diarrhoeal illness. However, in selected patients with appearances more typical of an idiopathic fissure in ano, that is; in the posterior midline, painful and associated with anal spasm, lateral sphincterotomy can be performed. Glycerol trinitrate or diltiazem ointments have efficacy in idiopathic fissures as does injection of botulinum toxin into the anal sphincter. Little data exists on the efficacy of these conservative measures in CD although they are simple measures that can be used prior to sphincterotomy. Symptomatic anal strictures require dilation which often needs to be repeated and some patients will go on to require proctectomy.

1.5.3 Summary of management approach for perianal CD

The array of treatment options available and the variability of presentation of perianal CD mean that treatment is tailored to the individual patient with close liaison between surgeon and gastroenterologist. The key factors in management are drainage of sepsis, accurate classification of fistulas and assessment of luminal, especially rectal, disease activity. Figure 1.3 summarises how this information is integrated into the contemporary treatment algorithm for Crohn's perianal fistulas.

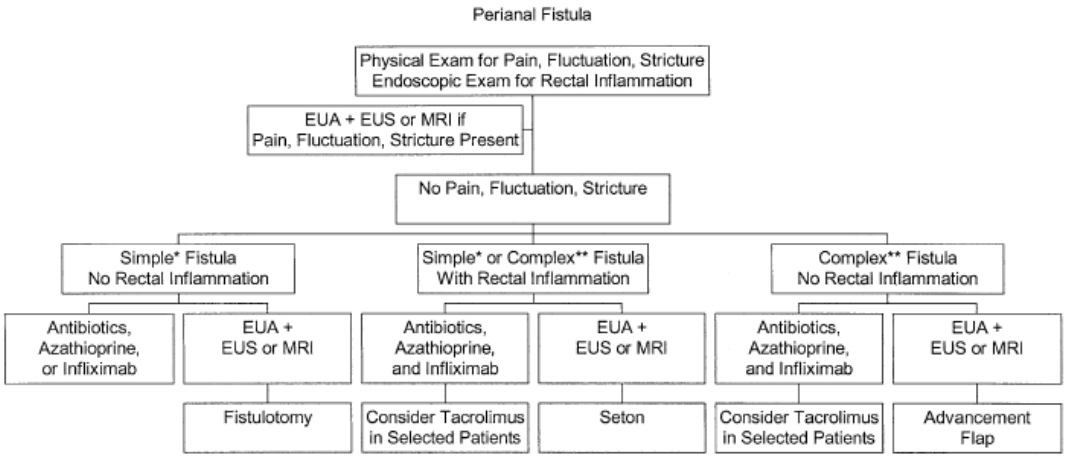


Figure 1.3 Treatment algorithm for perianal CD fistulas (reproduced from reference [36])

1.5.4 Frequency and risk factors for perianal surgical intervention in CD

The foregoing discussion and resulting treatment algorithm demonstrate that surgery is an integral part of treatment and therefore likely a frequent occurrence in perianal CD patients. However, there is surprisingly little data documenting the overall frequency of perianal surgical intervention (PSI), or the patient characteristics that predict the requirement for this. The few studies that have documented rates of PSI display a wide variation from 4% to 42% [18, 55, 56]. As with estimates of the incidence of perianal CD overall, these studies may not represent the true rate of PSI in the CD population as the subjects were drawn from referral centre cohorts where severe disease is likely to be over represented. A better understanding of the true rate of PSI, the operations performed and the patient factors associated with PSI is necessary to aid in refinement of the treatment algorithm, particularly in the era of biological therapy. In chapter 5 the results of a study investigating the rates of PSI and factors associated with PSI from the Canterbury IBD Project are presented.

1.6 The cost of perianal Crohn's disease

In section 1.1 it was emphasised that the incidence of CD is increasing worldwide[3] and the peak age of onset is between 15 and 35 years [4]. The foregoing discussion of management demonstrates that while advances in treatment have been made, particularly with the introduction of modern biological medications, CD remains incurable. A proportion of patients will endure recurrent, prolonged periods of illness requiring extensive medical and surgical interventions during what would otherwise be a highly productive time of life. Hence CD presents an increasingly significant health problem not only in terms of morbidity, but also cost to the individual patient and society.

The introduction of these modern biological treatments has been associated with significant expense and has created a need for government agencies to consider the economic impact of therapeutic alternatives. To achieve this, the cost of CD and its societal burden requires further study. Previous studies have indicated that the majority of the total cost associated with this disease relates to "extensive interventions required by a small proportion of severely affected individuals" [189].

The fact that perianal CD is a marker of a more severe disease course, and that perianal disease patients develop local perianal complications requiring frequent intervention means this group is more likely to use significant health resources and incur the largest costs as a result of the disease. A number of international studies have considered the cost of inflammatory bowel disease overall [190-199], however, to date, there has been no published research documenting the average patient cost of IBD in New Zealand or Australia and no previous work specifically investigating the perianal CD group. Chapter 6 presents the results of a study performed to assess the cost of perianal CD from the societal point of view to provide baseline information on which to guide future decisions regarding CD therapy.

Chapter 2

The Spectrum of Perianal CD

Abstract

Background

Perianal Crohn's disease represents a phenotype distinct from luminal Crohn's disease (CD) and may follow a different course. To date, the only detailed classifications of perianal CD arise from referral centre cohorts that do not reflect the spectrum of disease in the population as a whole. This study aimed to document the frequency, classification and time course of symptomatic perianal CD in a population based cohort.

Methods

All patients with inflammatory bowel disease (IBD) in Canterbury, New Zealand, were eligible for recruitment over a three year period. The clinical records of all CD patients were reviewed and all symptomatic perianal CD was classified according to the American Gastroenterological Society position statement.

Results

An estimated 91% of IBD patients in the region were recruited. 715 patients had CD of which 190 (26.6%) had symptomatic perianal CD. The median age of patients with perianal CD was 37 years (range 4-82 years) and 58.4 % were female. Median follow up was 9 years (range 2 months to 45 years) from CD diagnosis. Onset of perianal CD ranged from 18 years pre-CD diagnosis, to 33 post-CD diagnosis. Fistulas were the most common lesion (50% of patients), followed by perianal abscesses (42.1%), fissures (32.6%), skin tags (11.1%), strictures (7.4%) and haemorrhoids (1.6%). The cumulative probability at 20 years of any perianal CD was 42.7% and of a perianal fistula 28.3%.

Conclusions

This study provides the first detailed description of perianal CD in a population based cohort and confirms that the onset of perianal symptoms relative to CD diagnosis is highly variable.

2.1 Introduction

It is commonly accepted that perianal lesions complicate a significant proportion of CD cases and that these patients represent an important subgroup in CD, as they tend to have a more severe course. However, the discussion in section 1.2 highlighted the fact that the reported proportion of CD patients affected by perianal complications varies widely. This appears to be the result of discrepancies in the classification of perianal lesions included in the studies and also whether the studies drew subjects from tertiary referral centre practices or the population as a whole. Studies which include only anal fistulas and abscesses, not unexpectedly, report a lower rate of perianal involvement than those which include other perianal lesions in addition, such as skin tags, fissures and haemorrhoids. Likewise, population based studies also produce lower estimates of the rate of perianal involvement as the more severe CD cases and, therefore, those with a higher rate of perianal involvement are over-represented in referral centre cohorts. Evidence continues to mount that perianal disease may represent a distinct CD phenotype and research into clinical and genetic risk factors for perianal disease is on-going. To allow meaningful comparison of these factors, both within and amongst cohorts, accurate classification and standardised definitions of perianal lesions are required. In 2005 the Canterbury Inflammatory Bowel Disease Project completed data acquisition on IBD patients in the Canterbury region as part of a PhD Thesis [200]. This resulted in the finding of an extremely high incidence of CD within the Canterbury region by global standards [201]. The existence of this well characterised population based cohort provided the opportunity to study the perianal CD subgroup in detail.

2.2 Aims

The aims of this study were to;

- determine the rate of symptomatic perianal CD lesions in a population based cohort
- classify these lesions according to the American Gastroenterological Association Guidelines

- determine the temporal relationship of the onset of perianal disease to CD diagnosis

2.3 Methods

This study was performed as part of the on-going Canterbury Inflammatory Bowel Disease Project, a large population based study aimed at describing the epidemiology of IBD in the Canterbury region and to study the clinical, environmental and genetic risk factors for IBD. The methods used in data collection in this project have been described in detail previously [200, 201] and are summarised here.

The Canterbury region is located on the east coast of the South Island of New Zealand and had a population of 464,700 in 2005 when recruitment for the study was completed [202]. Ethnic diversity in Canterbury is limited, with approximately 90% of the population being Caucasian (descended predominantly from migrants from the United Kingdom and Ireland) and the remainder consisting of Maori (7.2%), and other (mainly Asian) ethnicities. Young people less than 20 years of age make up 20.3% of the population while 13.8% are aged over 65 years [202].

Patients were initially recruited to the project as part of a PhD Thesis (RBG) and a multifaceted approach to recruitment was used. Over 3 years (2003–2005) surgeons and gastroenterologists referred all inflammatory bowel disease patients from public and private specialist clinics. In addition, patients self-referred for inclusion following advertising in public media, general practices, specialist clinics and with the Crohn's and colitis support group. Validation of the completeness of recruitment using representative general practices indicated 91 per cent of patients with IBD in the Canterbury region were included in the study. IBD cases were confirmed using standard diagnostic criteria [203]. Demographic information and clinical data, together with IBD phenotype and IBD-related surgical procedures, were extracted retrospectively from patient medical records. This information was then entered into a custom-built Microsoft Access (Microsoft, Redmond, Washington, USA) database. In 2009/2010 TE reviewed the CD patient cohort to classify perianal cases. All patients with symptomatic perianal lesions noted in the medical record at

any time point relative to the diagnosis of IBD were included. This was achieved by reviewing the hospital medical records and where necessary those of General Practitioners and Private Specialist Gastroenterologists and Surgeons of those patients identified to have perianal disease. The perianal lesions were classified according to the AGA guidelines as described in section 1.2.1.8. Kaplan–Meier curves were used to demonstrate the proportion of patients developing perianal CD over time. Statistical analysis was performed using SPSS® version 19 (SPSS, Chicago, Illinois, USA). Ethical approval was obtained from the regional ethics committee and all patients entering the study provided written informed consent.

2.4 Results

A total of 1421 patients were recruited into the Canterbury IBD Project of which, 715 had CD. The median age was 40 years (range 4 to 93 years) and 422 (59.0 per cent) were female. The median follow up from CD diagnosis was 6.3 years (range 2 months to 65 years). One hundred and ninety patients (26.6 per cent) had symptomatic perianal lesions. The median age of the patients with perianal involvement was 37 years (range 4 to 82 years) and 111 (58.4 per cent) were female. Median follow up from CD diagnosis was 9 years (range 2 months to 45 years).

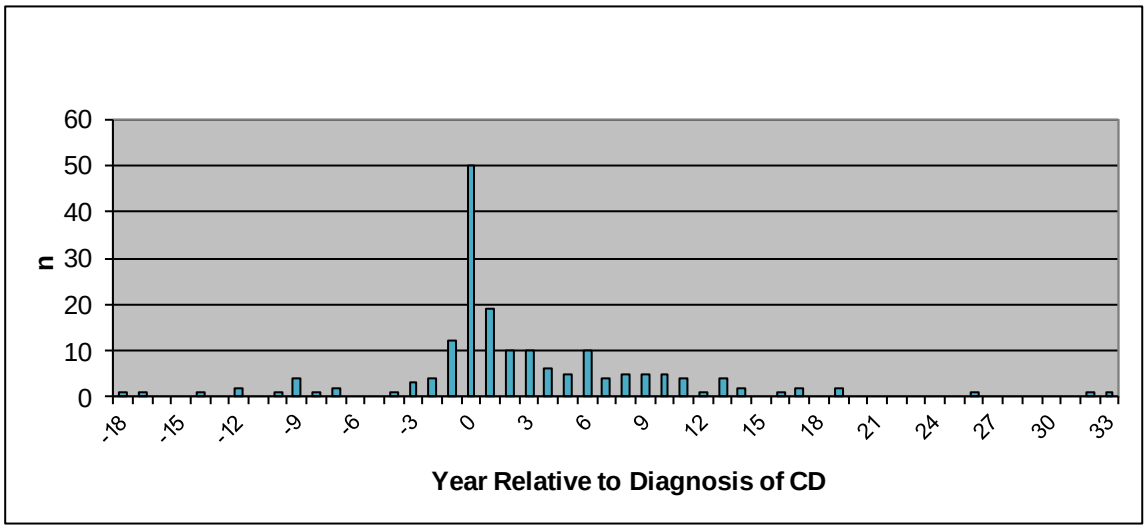


Figure 2.1 Temporal Relationship of perianal involvement to diagnosis of CD

One hundred and eighty six patients had complete data on the date of first presentation with perianal disease. Figure 2.1 summarises the temporal relationship of the presentation of perianal lesions to the diagnosis of CD. There was a broad range of onset of perianal symptoms from 18 years prior to diagnosis to 33 years after diagnosis. In 32 patients (17.2 per cent of the perianal CD group), the perianal lesion preceded the diagnosis of CD by more than six months. In 50 patients (26.9 per cent), perianal disease presented from six months prior to six months after the diagnosis of CD, while perianal disease was first observed more than six months after CD diagnosis in the remaining 104 (55.9 per cent) patients. Using Kaplan-Meier methods, the cumulative probabilities of developing any perianal CD were 0.295 and 0.427 at 10 and 20 years post diagnosis respectively (Figure 2.2). When considering only perianal fistulas, the cumulative probabilities were 0.169 and 0.283 at 10 and 20 years post diagnosis respectively (Figure 2.3).

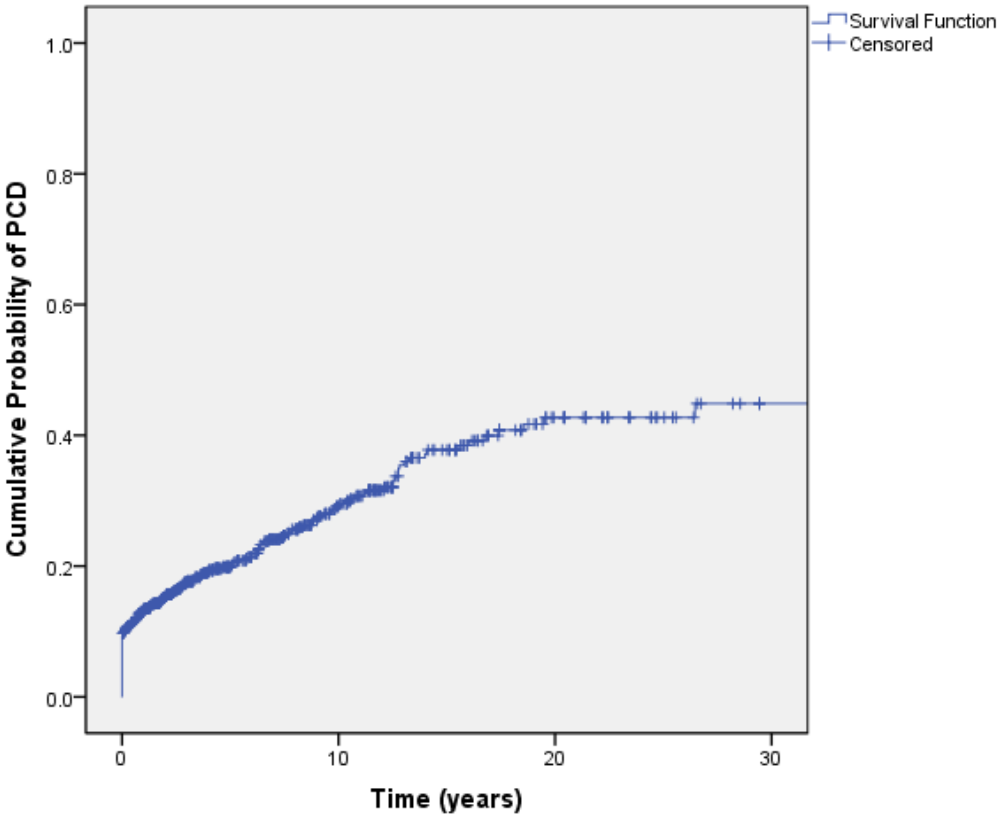


Figure 2.2 Kaplan-Meier curve showing the proportion of patients developing perianal Crohn's disease (PCD).

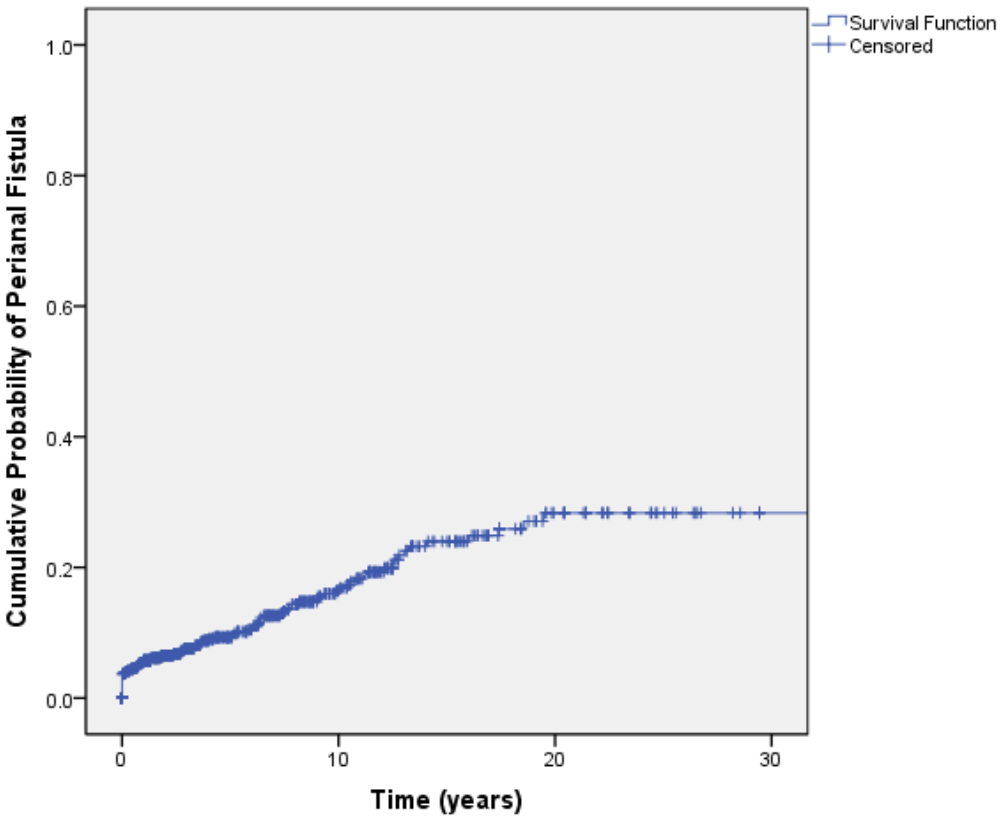


Figure 2.3 Kaplan-Meier curve showing the proportion of patients developing perianal fistulas.

The frequencies of different perianal lesions classified according to the AGA guidelines are given in table 2.1. Perianal fistulas represented the most common lesion identified (50% of perianal CD patients) and more than half of these fistulas were classified as complex. Perianal abscesses were also common. Fissures, Skin tags, stenosis and haemorrhoids made up the remainder of the lesions in decreasing order of frequency. The perianal lesion included an abscess and/or fistula in 131 patients or 18.3 per cent of the total CD sample.

Table 2.1 Classification of Symptomatic Perianal CD lesions in the Canterbury IBD Cohort

	Number	% of Perianal CD Patients (n=190)	% of Total CD Patients (n=715)
Abscess	**80**	**42.1**	**11.2**
Fistula (total)	**95**	**50.0**	**13.3**
Simple	22	11.6	
Complex	57	30.0	
Rectovaginal	15	7.9	
Not Classifiable	1	0.5	
Fissure/Ulcer	**62**	**32.6**	**8.7**
Stenosis	**14**	**7.4**	**2.0**
Skin Tags	**21**	**11.1**	**2.9**
Haemorrhoids	**3**	**1.6**	**0.4**

2.5 Discussion

The reported frequency of perianal CD in this cohort lies within the range of previously reported studies of 10-80 per cent (see table 1.6). The wide range of reported frequency is due to variable definitions of perianal disease, the population from which the samples have been taken and the duration of follow up. No population based study has previously reported and classified all symptomatic perianal lesions, although three studies from population based cohorts report on frequency of perianal fistulas in the range 13.7-26 per cent [7, 47, 48]. Schwartz *et al* found a cumulative incidence of perianal fistula of 26% at 20 years in 169 patients in Olmstead County [47], similar to the cumulative rate documented in this study. Similarly, Tang et al reported 21.7% of patients had perineal fistulas at median follow up of 24 years [7]. The Stockholm county reported perianal fistulas in 13.7% however this figure is difficult to compare as median follow up time is not provided [48].

This is the first report from a population based cohort of CD patients to classify the spectrum of symptomatic perianal CD lesions. The most frequently cited data

documenting the spectrum of perianal lesions comes from a referral centre cohort published by Keighley and Allan [53]. This series reported on 202 CD patients presenting to a CD follow up clinic over 1 year who were examined to determine the presence of perianal lesions. One hundred and ten of the patients had evidence of perianal CD and spectrum of lesions is shown in table 2.2 .

Table 2.2 Types of perianal lesions in 202 consecutive patients in a Crohn's disease follow-up clinic (reproduced from reference [53])

Type of lesion	**Number of patients (%)**
Skin tag	75 (37)
Fissure	38 (19)
Low fistula	40 (20)
High fistula	12 (6)
Rectovaginal fistula	6 (3)
Perianal abscess	32 (16)
Ischiorectal abscess	8 (4)
Intersphincteric abscess	7 (3)
Supralevator abscess	6 (3)
Anorectal stricture	19 (9)
Haemorrhoids	15 (7)
Anal ulcer	12 (12)
Total patients with perianal lesions	**110 (54)**

Keighley and Allan demonstrate a slightly higher rate of significant perianal lesions including abscesses and fistulas than that demonstrated in the present study, as would be expected from the preceding discussion of the nature of referral centre cohorts. That the difference is not greater between the two studies may be explained by the longer duration of follow up in the present study (median 9 years) compared with Keighley and Allan's data, which considered only patients presenting to a follow up clinic over the course of one year. The present study also demonstrates that there is a wide variation in the timing of perianal disease presentation relative to the diagnosis of; the longer follow up in this study thus provides a more representative estimation of the burden of perianal CD over time in these patients.

The timing of perianal symptoms relative to the diagnosis of CD has been explored in other cohorts with comparable results to those presented here. Williams *et al* reviewed 1098 patients with CD presenting to the Lahey Clinic in Massachusetts over a 10 year period. In the majority (64%) of the 242 patients with perianal CD, the perianal manifestations developed after intestinal disease over a wide range of time from 2 weeks to over 10 years. Hellers investigated perianal fistulae in a population based cohort from Stockholm and found that in 20% the fistula preceded diagnosis by more than six months, in 38% perianal fistulae occurred between six months prior and three months after the diagnosis and in the remaining 40% occurred more than three months after diagnosis. The present study similarly demonstrated that a significant proportion of patients develop perianal symptoms prior to intestinal disease with 17% presenting more than six months prior to a CD diagnosis and 32.2% prior to or at the time of diagnosis of CD. In addition, the present study, with longer follow up, documents an even more dramatic range of first perianal presentation from 18 years prior to 32 years after the diagnosis of CD.

This study assumed that all perianal lesions that occurred in patients with an eventual diagnosis of CD were related to CD. Many of the lesions associated with perianal CD also occur commonly in the general population in the absence of CD. Idiopathic anal fissure and cryptoglandular fistula-in-ano are two examples of such conditions. Retrospectively differentiating these lesions was not possible; hence some patients with perianal lesions not necessarily related to CD may have been included. This dilemma is common to any retrospective study of CD and indeed remains an issue in prospective studies and clinical practice itself. In practice any CD patients with perianal lesions, whether truly CD related or idiopathic, require special consideration. Different management approaches are required in these patients; hence the inclusion of all lesions is justified.

The retrospective identification and classification of perianal CD lesions employed in this study may be inaccurate in some cases. Patients who moved into, or out of, the district may have had perianal lesions not included in this follow up. Classification was performed using public and private hospital records including inpatient and, outpatient notes, operation and radiology reports. Where classification

remained inconclusive despite these sources, treating gastroenterologists and surgeons were contacted for clarification. Using this methodology, only one of 95 fistulas could not be classified according to the AGA guidelines. In addition, the stringent capture-recapture methodology used in assembling this cohort of patients resulted in inclusion of an estimated 91% of IBD cases within the region. This amounts to true population based data and, to the author's knowledge, the classification provided is the only detailed classification of perianal CD to come from a population based cohort to date.

2.6 Conclusion

The reported rate and definition of perianal CD has varied widely in studies to date. Using stringent methodology to obtain population based data; this study estimated the rate of perianal fistulae at 16.9 per cent and all perianal lesions at 29.5 per cent at 10 years post diagnosis. While the onset of perianal symptoms in CD was clustered around the time of diagnosis, the temporal relationship between perianal symptoms and CD diagnosis was highly variable. This purely descriptive study was undertaken to accurately document the true rate of perianal CD and highlight the importance of a standardised definition of perianal CD, recognition of the relevance of the population under investigation and the importance of adequate duration of follow up.

Chapter 3

Risk Factors for Perianal CD

Abstract

Background

Perianal Crohn's disease (CD) affects around one quarter of CD patients and represents a distinct disease phenotype. This study investigated a large population-based cohort of inflammatory bowel disease (IBD) patients to identify clinical and genetic risk factors for perianal CD.

Method

Data was collected in the Canterbury IBD database, estimated to include 91% of all patients with IBD in Canterbury, New Zealand. Genotyping was performed for selected loci previously demonstrated to be associated with CD. Patients with perianal disease were then compared to both CD patients without perianal disease and healthy controls to assess the presence of potential phenotypic, environmental and genetic risk factors.

Results

Of the 715 CD patients in the database, 190 (26.5%) had perianal disease. 507 patients with genotype data available were analysed. Perianal disease was associated with younger age at diagnosis ($p<0.0001$), complicated intestinal disease ($p<0.0001$) and ileal disease location ($p=0.002$). There was no association with gender, ethnicity, smoking or breast feeding. Genotype analysis revealed an association with the Neutrophil Cytosolic Factor 4 (*NCF4*) gene compared with both non-perianal CD patients (OR 1.47; 95% CI 1.08-1.99) and healthy controls (OR 1.47; 95%CI 1.10-1.95). There was no association identified with other genes, including *IBD5* (OR 0.91; 95% CI 0.69-1.20), *TNFα* (OR 1.04; 95%CI 0.56-1.85) and *IRGM* (OR 1.21; 95% CI 0.80-1.82).

Conclusion

This study suggests that younger age at diagnosis, complicated disease behaviour, and ileal disease location are risk factors for perianal CD. In addition, this paper represents the first report of an association of the NCF4 gene with perianal disease.

3.1 Introduction

Chapter two demonstrated that perianal manifestations occur in around one quarter of CD patients and that these manifestations occur at a variable time relative to the diagnosis of CD itself. However, why only a subset of CD patients develops perianal manifestations and at time points so distant from the onset of luminal disease activity, is unclear. Perianal CD bears pathologic resemblance to luminal CD with common lesions such as stricturing and fistulas. However, it is evident from the discussion in chapter one, that perianal and luminal fistulas occur independently, suggesting that perianal CD represents a distinct disease phenotype [7, 9]. While the aetiology of CD itself is incompletely understood, it is clear that both genetic and environmental influences contribute [60-62]. Analysis of these factors reveals a number of emerging associations in patient characteristics, environmental influences and genotype as documented in chapter one. If perianal CD does in fact represent a separate disease phenotype then it may be expected that patients with perianal CD would have different characteristics to those CD patients without perianal lesions. Characterising the genetic, clinical and environmental risk factors for perianal CD not only has the potential to contribute to unravelling the pathogenesis of CD but also has substantial clinical relevance. Perianal CD is a marker of luminal disease severity and these patients require multiple surgical interventions and intensive medial therapy for both the perianal and luminal manifestations of the disease. Therefore, identifying those at risk of perianal disease has both prognostic and therapeutic implications.

3.2 Aims

The aim of this study is to identify environmental, clinical and genetic risk factors for perianal CD.

3.3 Methods

This study was performed as part of the on-going Canterbury Inflammatory Bowel Disease Project. The study population and methods for recruitment for this project were described in section 2.3. Following recruitment, demographic information and clinical data were extracted retrospectively from medical records.

The diagnosis of IBD was confirmed according to recognised and accepted criteria [203]. The IBD phenotype was classified according to the Montreal classification (see table 1.5) and symptomatic perianal lesions occurring at any time were recorded.

All patients recruited into the project underwent the Canterbury IBD Questionnaire; a self-administered survey developed to determine the presence, absence and timing of exposure to a number of environmental factors. Participants were encouraged to corroborate answers with family members and Plunket Books to minimise recall bias. The survey used categorical yes, no or unsure answers to minimise information bias. Responses to this survey were used for data pertaining to smoking and breast feeding.

Control subjects were identified at random from the Canterbury electoral roll and details of their recruitment and characteristics have been published elsewhere [65]. None of the controls had IBD.

DNA was collected from peripheral blood of study participants using phenol chloroform extraction and stored at -20˚C in Tris-EDTA buffer until analysis. Genotyping for the *NOD2* single nucleotide polymorphisms (SNPs), rs2066842 (802C>T, P268S), rs2066844 (2104C>T, R702W), rs2066845 (2722G>C, G908R) rs2066847 (3020insC, 1007fs) was conducted using a previously described multiplex allele-specific polymerase chain reaction (PCR) assay [204] Briefly, PCR primers specific for the wildtype and variant allele of each SNP were divided across two PCRs per sample. An additional pair of primers, specific for a 569bp region of the beta-2-microglobulin (β2M) gene, was included in each reaction as an independent control for DNA amplification. PCR products were resolved using 3% agarose electrophoresis. Study participants were genotyped for all other risk loci using pre-designed TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). TaqMan® assays were performed in 384-well plates following the recommendations of the manufacturer and run on a Roche LightCycler 480 machine (Roche Diagnostics Corporation, Indianapolis, IN, USA). The accuracy of the *NOD2* multiplex allele-specific PCR and each TaqMan® assay was confirmed by repeat analysis of 10% of samples. Concordance between original and repeat genotype calls was 100%.

All collected data was entered into a custom-built Microsoft Access (Microsoft, Redmond, Washington, USA) database. Statistical analysis was performed using the R language for statistical computing Version 2.10.1 [205]. For the purposes of this study, only those with complete genetic data were included. Patients were grouped into those with perianal disease and those without. The presence of demographic, clinical and phenotypic risk factors was compared using X^2 tests without continuity correction for count data and t-tests (unequal variance) for continuous variables. All results were considered significant at $p<0.05$, however p values less than 0.1 are shown in the tables. The Bonferroni correction for multiple testing of 13 SNPs (10 genes) adjusts the significance level to 0.0038 for these results, however this is a conservative correction which does not account for lack of independence between the SNPs tested. Genotypes were compared between both the CD cohort and healthy controls and within the CD cohort between the groups with and without perianal CD. Each genetic marker was tested for Hardy-Weinberg equilibrium (HWE) in both the patient and control group before inclusion in the analysis (P value ≥ 0.05). The significance of the difference in allele frequencies was compared using the X^2 test. Genetic association was tested under both allelic and genotypic models. The genotypic model presented is the log additive model on the number of minor alleles, tested by using the Likelihood ratio test against the null model. Multiple logistic regression was then used to investigate the effect of *NCF4* on perianal disease within the CD cohort. Covariates fitted were gender, age over 40 years old, age over 30 years old at diagnosis, family history of IBD, breast feeding history, smoking, disease behaviour and location along with *NCF4* genotype fitted as the number of T alleles (the log additive genetic model).

3.4 Results

Of the 715 CD patients in the study, 507 patients had had blood samples available for DNA extraction and were included in this analysis. The mean age was 45 years (standard deviation 17.9 years) and 320 (62.9 per cent) were female. The mean follow up from CD diagnosis was 9 years (standard deviation 9.1 years). One hundred and thirty seven patients (26.9 per cent) had symptomatic perianal lesions. The mean age of the patients with perianal involvement was 41 years (standard

deviation 16.8 years) and 83 (60.6 per cent) were female. Mean follow up from CD diagnosis was 10.9 years (standard deviation 9 years).

Clinical, phenotypic and environmental risk factors for the genotyped CD patients with and without perianal disease are compared in table 3.1. The mean age at diagnosis was 8.1 years younger for those with perianal CD than those without ($p<0.0001$) and disease duration was also significantly longer in the perianal disease group. Of those with perianal disease, 55.5 per cent had complicated intestinal disease compared with 38.6 per cent in the group without perianal disease ($p<0.0001$). The percentage of patients in the perianal disease group with ileal involvement was higher (69.3 per cent) compared with the no perianal disease group (53.8 per cent, $p=0.002$). The patients in the perianal disease group had higher rates of surgery and immunomodulator use. There was no association found between perianal disease and smoking status or breastfeeding history.

The genotypes of CD cases for the genes that were selected for testing for association with perianal disease are shown in table 3.2 which also demonstrates the data for the same genes in the healthy controls. Significant deviations in HWE were observed for the SNPs *IRGM* rs13361189 and *NOD2* rs2066847 in controls, but not for other SNPs in these genes, hence rs13361189 and rs2066847 were excluded from the analysis. In addition, significant deviations in HWE were observed for the SNPs *IL2_21* rs6822844, *NOD2* rs2066844, *NOD2* rs2066845, and *IL23R* rs11209026, in cases but not controls. Such deviations are common in cases when the variant has a significant effect on disease risk, hence these SNPs were included [206].

Minor allele frequencies (MAF) in the CD group were compared with those in healthy controls. CD patients had significantly different MAF to healthy controls at the following loci; CARD 8, *IL2_21*, *IRGM*, *ATG16L1*, *NOD2* (rs2066844), *IL23R* and *IBD5* (rs2631367), with *IL2_21* and *ATG16L1* exceeding the Bonferroni significance level (Table 2). Restricting analysis to patients with perianal disease found the MAF of *IL2_21, IRGM, NCF4, ATG16L1 and NOD2* (including all three SNPs analysed) differed significantly between patients and healthy controls (Table 3.3).

MAF were then compared within the Crohn's disease cohort for the perianal disease and no perianal disease groups. The only significant difference in MAF was a higher frequency of the minor allele of the nuclear cytosolic factor 4 (*NCF4*) gene (table 3.4), albeit not at the Bonferroni level.

The clinical and demographic factors: sex, age, smoking, diagnosis age, any family history, breastfed, complicated disease and any ileal involvement were then included in the additive genotypic model to produce the adjusted P values (tables 3.3 and 3.4) showing that they did not modify the association of *NCF4* with perianal disease above the 0.05 significance level.

Power calculations demonstrated that the study was adequately powered to compare the associations of *CARD8, ATG16L1, NOD2, IL21, NCF4* and *IBD5* between the perianal disease group and both the no perianal disease group and healthy controls as these genes had sufficiently high MAF. The sample was large enough to detect MAF as low as 18 per cent against a MAF of 30 per cent in the perianal disease group with 80% power. However for the genes with lower MAF, that is *IL23R, NALP3, TNFα* and *IRGM,* there was insufficient power to conclude that the null results supports no association.

Table 3.1 Clinical characteristics of CD patients with and without perianal disease

	No Perianal Disease (*n*=370)	**Perianal (*n*=137)**	**CD (*n*=507)**	OR/diff (CI)	P
Female (%)	63.7%	60.6%	62.9%	0.9 (0.6,1.3)	NS
Age in 2008 (yr) (SD)	46.5 ± 18.1	40.8 ± 16.8	45 ± 17.9	-5.6 (-9,-2.3)	0.001
Age at diagnosis (yr) (SD)	38.1 ± 18.1	30 ± 15.7	35.9 ± 17.9	-8.2 (-11.4,-4.9)	$<10^{-5}$
Duration (yr) (SD)	8.4 ± 9.1	10.9 ± 9	9 ± 9.1	2.5 (0.7,4.3)	0.006
Familial IBD	33.0%	37.1%	34.1%	1.2 (0.8,1.8)	NS
Location					
Not Ileal (L2)	46.2%	30.7%	41.9%	1.9 (1.3,2.9)	0.002
Ileal (L1+L3)	53.8%	69.3%	58.1%		
Behaviour					
Inflammatory (B1)	61.4%	44.5%	56.8%	2 (1.3,2.9)	$<10^{-3}$
Complicated (B2+B3)	38.6%	55.5%	43.2%		
Any EIM	17.6%	24.1%	19.3%	1.5 (0.9,2.4)	NS
Surgery (resection)	28.6%	51.8%	34.9%	2.7 (1.8,4)	$<10^{-5}$
Immunomodulators	50.0%	72.3%	56.0%	2.6 (1.7,4)	$<10^{-5}$
Smoker	54.1%	48.5%	52.6%	0.8 (0.5,1.2)	NS
Breast fed	67.0%	66.9%	60.5%	1 (0.7,1.4)	NS

P values with Odds Ratios (OR) or differences (diff) with 95% Confidence Intervals (CI) are from perianal disease versus no perianal diseases using t or chi-square tests for continuous and count data respectively.

EIM=extra-intestinal manifestations; NS=non-significant (p>0.05); SD=Standard Deviation

Table 3.2 Allelic and genotypic associations between Crohn's disease and controls.

Gene	Locus	alleles	MAF		HWE		Allelic			Additive		
			Controls	CD	Controls	CD	OR	CI	P	OR	CI	P
CARD8	rs2043211	A/T	33.7	29.5	0.84	0.75	0.82	(0.68-0.99)	0.044	0.82	(0.68-0.99)	0.044
NALP3	rs35829419	C/A	4.0	3.8	0.19	0.52	0.97	(0.61-1.51)	0.878	0.97	(0.62-1.50)	0.880
IL2_21	rs6822844	T/G	17.4	34.0	0.77	$<10^{-4}$	9.17	(7.50-11.26)	$<10^{-15}$	12.23	(9.22-16.56)	$<10^{-15}$
IRGM	rs4958847	G/A	9.7	12.7	0.79	0.31	1.35	(1.01-1.81)	0.039	1.37	(1.02-1.85)	0.035
NCF4	rs4821544	C/T	27.5	29.7	0.16	0.10	1.11	(0.92-1.35)	0.280	1.11	(0.92-1.35)	0.281
ATG16L1	rs2241880	T/C	49.7	41.9	0.39	1.00	0.71	(0.60-0.85)	$<10^{-4}$	0.71	(0.59-0.84)	$<10^{-4}$
NOD2	rs2066842	C/T	25.1	28.7	1.00	0.26	1.2	(0.97-1.50)	0.094	1.21	(0.97-1.52)	0.088
NOD2	rs2066844	C/T	4.6	7.3	0.33	0.002	1.61	(1.11-2.35)	0.012	1.54	(1.08-2.21)	0.017
NOD2	rs2066845	G/C	1.5	2.6	0.12	$<10^{-6}$	1.72	(0.92-3.27)	0.083	1.70	(0.93-3.21)	0.088
IL23R	rs11209026	G/A	6.2	3.7	1.00	$<10^{-6}$	0.58	(0.38-0.87)	0.009	0.57	(0.37-0.87)	0.008
IBD5	rs1050152	T/C	43.4	44.9	0.15	0.09	1.07	(0.89-1.3)	0.484	1.06	(0.89-1.3)	0.499
IBD5	rs2631367	C/G	47.3	47.6	0.24	0.01	1.23	(1.02-1.5)	0.033	1.21	(1.01-1.4)	0.041
TNFα	rs361525	A/G	7	6.2	0.32	0.70	0.88	(0.61-1.2)	0.465	0.88	(0.62-1.2)	0.469

MAF: Minor Allele Frequency. HWE: P value from Hardy Weinberg Equilibrium test.

OR: odds ratio. CI: 95% Confidence Interval for OR. P: P value

Table 3.3 Allelic and genotypic associations between perianal disease and controls.

Gene	Locus	alleles	MAF		HWE		Allelic			Additive		
			Controls	PA	Controls	PA	OR	(CI)	P	OR	(CI)	P
CARD8	rs2043211	A/T	33.7	29.3	0.84	0.83	0.82	(0.61-1.10)	0.180	0.82	(0.60-1.09)	0.175
NALP3	rs35829419	C/A	4.0	4.1	0.19	0.01	1.05	(0.50-2.00)	0.920	1.03	(0.51-1.94)	0.922
IL2_21	rs6822844	T/G	17.4	34.2	0.77	0.12	9.09	(6.77-12.28)	$<10^{-15}$	10.03	(6.92-15.11)	$<10^{-15}$
IRGM	rs4958847	G/A	9.7	14.3	0.79	1.00	1.56	(1.02-2.32)	0.033	1.61	(1.04-2.46)	0.032
NCF4	rs4821544	C/T	27.5	35.8	0.16	0.85	1.47	(1.10-1.95)	0.009	1.50	(1.11-2.01)	0.008
ATG16L1	rs2241880	T/C	49.7	40.5	0.39	0.72	0.67	(0.51-0.88)	0.004	0.66	(0.50-0.87)	0.004
NOD2	rs2066842	C/T	25.1	32.4	1.00	1.00	1.44	(1.05-1.95)	0.021	1.43	(1.05-1.95)	0.023
NOD2	rs2066844	C/T	4.6	8.8	0.33	0.01	1.99	(1.17-3.28)	0.008	1.84	(1.12-2.97)	0.018
NOD2	rs2066845	G/C	1.5	3.4	0.12	$<10^{-5}$	2.28	(0.95-5.10)	0.045	2.16	(0.93-4.75)	0.071
IL23R	rs11209026	G/A	6.2	3.4	1.00	$<10^{-5}$	0.55	(0.25-1.06)	0.083	0.54	(0.25-1.04)	0.066
IBD5	rs1050152	T/C	43.4	43.1	0.15	0.21	0.99	(0.75-1.30)	0.93	0.988	(0.75-1.29)	0.93
IBD5	rs2631367	C/G	47.3	48.5	0.24	0.84	1.05	(0.78-1.42)	0.75	1.048	(0.78-1.41)	0.76
TNFα	rs361525	A/G	7	6.4	0.32	0.40	0.91	(0.50-1.56)	0.73	0.909	(0.51-1.53)	0.73

MAF: Minor Allele Frequency. HWE: P value from Hardy Weinberg Equilibrium test. OR: odds ratio. CI: 95% Confidence Interval for OR. P: P value

Table 3.4 Allelic and genotypic associations for perianal disease within Crohn's disease

Gene	Locus	alleles	MAF		Allelic			Additive			
			no PA	PA	OR	(CI)	P	OR	(CI)	P	P adjusted
CARD8	rs2043211	A/T	29.5	29.3	0.99	(0.73-1.35)	0.95	0.99	(0.73-1.34)	0.95	0.84
NALP3	rs35829419	C/A	3.7	4.1	1.11	(0.52-2.22)	0.79	1.10	(0.52-2.18)	0.79	0.48
IL2_21	rs6822844	T/G	34	34.2	1.01	(0.75-1.36)	0.94	1.01	(0.73-1.40)	0.94	0.50
IRGM	rs4958847	G/A	12.1	14.3	1.21	(0.80-1.82)	0.36	1.23	(0.80-1.86)	0.35	0.35
NCF4	rs4821544	C/T	27.4	35.8	1.47	(1.08-1.99)	0.012	1.43	(1.07-1.92)	0.02	0.03
ATG16L1	rs2241880	T/C	42.4	40.5	0.92	(0.69-1.23)	0.58	0.92	(0.69-1.23)	0.58	0.42
NOD2	rs2066842	C/T	27.3	32.4	1.28	(0.94-1.74)	0.12	1.30	(0.94-1.78)	0.11	0.59
NOD2	rs2066844	C/T	6.7	8.8	1.35	(0.79-2.25)	0.27	1.29	(0.78-2.06)	0.31	0.84
NOD2	rs2066845	G/C	2.3	3.4	1.5	(0.62-3.41)	0.34	1.51	(0.63-3.45)	0.35	0.75
IL23R	rs11209026	G/A	3.8	3.4	0.92	(0.40-1.93)	0.81	0.91	(0.39-1.93)	0.81	0.94
IBD5	rs1050152	T/C	45.6	43.1	0.9	(0.68-1.20)	0.48	0.91	(0.69-1.20)	0.50	0.50
IBD5	rs2631367	C/G	46.2	48.5	1.24	(0.90-1.70)	0.19	1.21	(0.89-1.64)	0.22	0.33
TNFα	rs361525	A/G	6.1	6.4	1.05	(0.56-1.87)	0.89	1.04	(0.56-1.85)	0.89	0.89

MAF: Minor Allele Frequency. OR: odds ratio. CI: 95% Confidence Interval for OR. P: P value.

3.5 Discussion

Recent evidence indicates that perianal CD represents a distinct disease phenotype and the results of this study do suggest specific associations with perianal CD patients compared with those patients without perianal involvement. Of the clinical factors investigated, this study demonstrated associations with perianal CD and young age at diagnosis, ileal disease location and complicated disease behaviour. In addition, an association with the *NCF4* gene was demonstrated.

One of the more notable findings of this study was that perianal involvement was associated with ileal disease in this cohort. Traditionally it has been believed that perianal CD is associated with distal intestinal involvement and this contention is supported by a number of referral centre based cohorts [44, 49-51, 53, 55, 57]. A possible explanation for the different association of perianal disease with intestinal disease location in this study is the broader inclusion of other perianal lesions in addition to fistulae. Several of the aforementioned cohorts included only perianal fistulae, potentially altering the relationship to intestinal disease. The relationship may also differ in this study compared to the aforementioned referral centre cohorts due to the population based nature of the data. The evidence for the association of perianal disease with colonic disease is less consistent in previously reported population based data; one study supporting an association with colonic disease [7], another showing the association only with ileocolonic location [47] and a third, the Swedish study, failing to demonstrate a relationship [48]. The population based data presented here likely represents a true relationship between ileal disease and perianal CD in this cohort. The makeup of this population based cohort highlights this relationship, as it contains a high proportion of mild colonic CD patients, who may not be as susceptible to perianal disease. The rigorous methodology employed in the present study to identify all CD patients combined with the long median follow up period of nine years and high rate of colonoscopy in this cohort giving accurate documentation of the extent of colonic disease [201] all support the contention of a genuine relationship between ileal disease and perianal disease.

The finding that young age at diagnosis is associated with perianal disease is consistent with the majority of previous studies from both referral centres [54, 58, 71]

and population based cohorts [7, 48]. However, two small studies found no relationship with age and perianal CD [45, 55]. The present study also demonstrated that perianal disease was associated with longer disease duration, consistent with the data presented in chapter 2 showing that perianal disease can occur at any time, often well after diagnosis of luminal CD. Those diagnosed at a young age had longer duration of follow up, which may have contributed to the association of young age at diagnosis. However, the fact the perianal patients were significantly younger at follow up also supports the contention that perianal disease is more likely in those diagnosed at a young age independent of disease duration.

The relationship of perianal disease with intestinal disease behaviour in the present study is also interesting. Penetrating and stricturing intestinal disease were both associated with perianal CD. Previous studies of disease behaviour and perianal disease have yielded varying results. Smith *et al* [8] and Veloso *et al* [57] found that perianal disease was not associated with penetrating intestinal disease. However, Tang *et al* did find a strong relationship between perianal and luminal fistulas but also observed that the two fistula types often occurred exclusively [7]. Of note, in the present study, while perianal disease was associated with luminal fistulising disease, 85% of patients with perianal disease did not have penetrating intestinal disease. This is consistent with Tang *et al's* data and reinforces the theory that perianal disease represents a different disease phenotype to intestinal penetrating disease.

Environmental factors undoubtedly play a role in CD pathogenesis; however their influence on perianal disease has not been well documented. Prior to the present study, no others had directly compared smoking rates in perianal and CD patients. In this cohort there was no relationship between smoking and perianal disease. The epidemiologic evidence for being breast fed is less consistent but it is likely to have a protective effect against developing CD [65]. This study did not show any effect of breast feeding on perianal disease. Any effect of these environmental factors is likely to be modest, hence the possibility of type II error would need to be considered in a study of this size.

Increasing evidence suggests that CD results from a dysregulated immune response to as yet unidentified environmental factors in genetically susceptible hosts.

Genotype not only influences susceptibility for CD overall but also the expression of certain CD phenotypes. Given that perianal disease appears to represent a separate CD phenotype, one may expect different genetic susceptibility factors to be present in these patients. After comparing the genotypes at a number of loci previously associated with CD, this study found a statistically significant association with the minor allele of neutrophil cytosolic factor 4 (*NCF4*) in patients with perianal disease. This association was apparent when perianal disease patients were compared both with healthy controls and with other CD patients without perianal involvement. In contrast, the effects of the other loci significantly associated with perianal disease (particularly *IL21* and *ATG16L1*) did not persist when compared with CD patients without perianal disease.

NCF4 is located on chromosome 22 and was first associated with CD in a genome wide association study in 2007 [207]. However, the association of NCF4 with CD has not been universally replicated and two recently published studies in large European and Canadian cohorts failed to demonstrate an association [208, 209]. While the association with CD hasn't been consistent, the *NCF4* gene does offer biological plausibility as it has a role in phagocytosis and the antimicrobial response. It encodes a protein known as p40phox which is one of the subunits of the nicotinamide adenine dinucleotide (NADPH)-oxidase enzyme system [210]. This system produces reactive oxygen species upon phagocytosis which are important for mounting an effective antimicrobial response [211]. This provides an appealing link to CD pathogenesis, with ineffective bacterial killing of phagocytised microbes producing a prolonged immune activation. The association of *NCF4* with ileal CD had previously been demonstrated in this cohort of patients [212]. The present study has now demonstrated a further association with perianal disease which maintained statistical significance after adjusting for potential confounding factors including intestinal disease location, behaviour, age and smoking.

This is the first study to demonstrate an association between the *NCF4* locus and perianal CD. As discussed in chapter 1.3.4.4, three other genetic loci had previously been shown to be associated with perianal CD; *IBD5, IRGM* and *TNFα*, none of which demonstrated a significant association in this cohort.

Of these, the *IBD5* susceptibility locus on chromosome 5q31 has been most investigated. Associations with this locus and CD have been widely replicated and possible functional variants have been identified in the carnitine/organic cation transporter genes *OCTN1* and *OCTN2* (*SLC22A4* and *SLC22A5*). Of the six studies to date investigating the *IBD5* region for a relationship with perianal disease, four have suggested an association [110, 114, 115, 117], while two others have not [74, 116]. No association was shown in this study and doubt remains as to the role of *IBD5* in perianal CD and to the possible causative variants.

IRGM was also investigated in this cohort and did not demonstrate a significant association with perianal disease when compared with the non-perianal CD group. . As discussed in section 1.3.4.4.2, *IRGM* has previously been associated with CD susceptibility overall in a large genome-wide association study [118] and the fact that it is an important autophagy gene also provides a plausible biological link to CD pathogenesis. While *IRGM* did not show an association in the present study, the potential pathogenic mechanism described and the association of *NCF4* warrant further investigation into the role of the autophagy genes in perianal disease and CD overall.

The only other gene previously associated with perianal CD is *TNFα*. Section 1.3.4.4.3 highlighted that, while the proinflammatory cytokine TNFα has an established role in CD pathogenesis, results of association studies for CD overall have been varied [122]. In addition only one small study has shown an association with perianal CD [213]. The present study failed to replicate this and further studies are required to assess this association.

The variation in this and other previously reported studies of the factors influencing perianal CD may result from differences in definitions and inclusion criteria. This study included all perianal lesions classified according to AGA guidelines whereas several previous studies considered only perianal fistulas. In addition, given that the previous study of *IRGM* demonstrated only modest odds ratios and it has a low MAF, the present study was inadequately powered to detect such a modest difference. The present data is drawn from an ethnically homogenous

population made up of 97.8 per cent Caucasians and ethnic differences are another important factor which may account for variations in study results.

There is now substantial evidence that perianal CD is associated with a more severe luminal disease course [214] and this study supports that contention, demonstrating a greater requirement for surgery and immunomodulator use in the perianal disease group. Defining risk factors for CD behaviour and attempting to predict the future course of the disease has taken on greater significance in the era of increasingly effective medical therapies, in particular anti-TNFα agents. Many physicians now advocate a "top-down" treatment strategy with early aggressive use of medical therapy in an attempt to alter the natural history of CD[215]. The data from this study suggests that patients diagnosed at a young age, those with ileal disease and those with the minor allele of *NCF4* SNP rs4821544 are more likely to develop perianal disease and could be candidates for such "top-down" therapy.

3.6 Conclusion

This study, from a large, well characterised, population based CD cohort, supports the hypothesis that perianal CD represents a distinct disease phenotype. It has demonstrated associations with perianal disease and younger age at diagnosis, complicated disease behaviour and ileal disease location. In addition, this paper represents the first report of an association of the *NCF4* gene with perianal disease. Perianal CD patients endure a more severe disease course and further studies that continue to clarify these risk factors will allow better targeting of therapy in the future.

Chapter 4

MRI in Perianal CD

Abstract

Background

In the setting of perianal CD, MRI is often used to establish the presence and anatomy of fistulas and undrained sepsis prior to examination under anaesthetic (EUA). Despite its inclusion in management guidelines, there is conflicting literature defining the accuracy and utility of MRI. The objectives of this study were to determine the concordance of MRI and EUA findings and to assess the impact of MRI on intra-operative management.

Method

All patients undergoing EUA for perianal CD at Christchurch Hospital were eligible for the study. EUA was performed by a colorectal surgeon blinded to the pre-operative MRI pelvis result. Blinded findings were documented, after which the MRI result was revealed intra-operatively. Subsequent alterations to findings or management were recorded. Discordance was resolved and a gold standard for comparison developed for each case by combining MRI, EUA and clinical outcome.

Results

Twenty patients completed the study protocol and were followed up for a median of 12 months (range 1-22 months). Unblinded EUA findings were abscess (7 patients), fistula (12), ulcer or fissure (6), haemorrhoids (1) and carcinoma (1). EUA findings were discordant with MRI in 7 patients (35%). These included 5 false positive and 2 additional true positive findings on MRI involving minor fistula classification changes. The sensitivity and specificity of MRI was 100% and 50% respectively for identification of fistulas and/or abscesses. Surgical management included abscess drainage (7 patients), loose seton (8), and defunctioning ileostomy (2). In no case did the MRI findings alter intra-operative management.

Conclusion

MRI is widely used prior to EUA for perianal CD. However, this study demonstrated minimal impact of MRI on intra-operative decision making, despite moderate discordance between MRI and EUA findings. The high sensitivity of MRI for fistulas

and abscesses suggests it is useful in excluding occult perianal sepsis and avoiding unnecessary EUA.

4.1 Introduction

Due to the complex nature of perianal CD, simple clinical examination is often inadequate or impossible and further more detailed assessment is required to accurately define the anatomy of fistulas, the presence of undrained sepsis and to plan appropriate treatment. The literature review presented in section 1.4 concluded that MRI and EAUSS have emerged as the imaging techniques of choice to complement EUA in the assessment of perianal CD. This is reflected in current management guidelines which recommend MRI or EAUSS in all perianal CD patients where surgery is the initial treatment strategy or in the presence of pain, fluctuation or stricture [24]. Much of the available literature assessing the accuracy of MRI pertains to cryptoglandular fistulas and it appears the accuracy of MRI decreases in the more complex lesions often present in CD [133]. Furthermore, the effect of MRI on management of perianal CD has not been well studied. Chapter 5 will demonstrate that many perianal CD patients require multiple surgical interventions and therefore have the potential to require multiple MRI scans. MRI is an expensive test with restricted availability in some areas hence a full assessment of its accuracy and impact on management is important.

4.2 Aims

The aims of this study were to determine the concordance of MRI and EUA findings in perianal CD and to assess the impact of MRI on intra-operative management.

4.3 Methods

Patients were recruited for the study between 1st September 2009 and 31st October 2010. All adult patients with perianal CD presenting to Christchurch hospital over this period in whom the treating surgeon considered EUA necessary were eligible for the study. The diagnosis of CD was confirmed using established diagnostic criteria[203]. Exclusion criteria were contraindications to MRI (including intra-orbital foreign body, implantable metal devices and a personal history of claustrophobia), pregnancy and the inability to provide informed consent.

Following informed consent, patients enrolled in the trial underwent preoperative clinical assessment which included classification of CD using the Montreal system, recording of medications, C reactive protein level and white blood cell count. Detailed perineal examination was performed, the presence of any perianal lesions documented and the perianal disease activity index (PDAI) recorded. Routine preoperative pelvic MRI was then performed on all patients. The MRI was performed using a 1.5T Signa Excite HD, machine (GE Medical Systems, Milwaukee, USA) with a surface phased array 8 channel coil. High resolution axial and coronal T2 weighted and T2 Fat Saturation sequences angled to the plane of the anal sphincter were used. The resulting MR images were reported by radiologists experienced in the technique.

Patients then underwent EUA performed by either a specialist colorectal surgeon or colorectal fellow. The surgeon initially performed the EUA without knowledge of the MRI findings. The blinded EUA findings and proposed management were documented on a proforma, but no therapeutic manoeuvres were undertaken at that stage. With the patient still under anaesthetic, the MR images and radiologist report were revealed to the operating surgeon. Any discordance between the blinded EUA and the MRI was documented. The EUA was then repeated and any therapeutic intervention undertaken. Changes to the proposed management recorded at the initial blinded EUA were documented. The case report form used to document findings and operative management is provided in Appendix 2.

Patients were followed up at six weeks, six months and 1 year post EUA. At each of these time points clinical data identical to that in the preoperative work up was recorded.

Those cases where discordance between EUA and MRI existed and was not resolved at EUA were reviewed by the study investigators. By combining the outcome from clinical follow up, EUA and MRI findings, an outcome based gold standard was derived using methodology employed in similar previous studies [135, 136].

The data was entered into a Microsoft Access database and statistical analysis performed using SPSS v19.0® statistical software (SPSS Inc. Chicago, Illinois). The Mann-Whitney U test was used to test for differences in PDAI between the discordant and concordant groups. The study received ethical approval from the Upper South Island Ethics Committee.

4.4 Results

Twenty-one patients gave informed consent for entry into the study. One patient did not have the MRI prior to EUA as planned and was excluded; leaving 20 patients that underwent MRI followed by EUA as per the study protocol.

Of the 20 patients, 14 were female (70 per cent) and the median age was 32 years (range 17 to 59 years). All patients had CD confirmed by Lennard Jones criteria and the Montreal classification at initial assessment is given in table 4.1. At the time of initial EUA, seven patients were taking a 5-ASA preparation, 15 immunomodulators (Azathioprine, 6-MP or methotrexate), four oral steroids and four anti-TNFα medication. The median PDAI was 11 (range 5 to 16). Median follow up after EUA was 12 months (range 1-22 months).

Table 4.1 Montreal classification of included patients

Category	n
Age	
A1	2
A2	13
A3	5
Behaviour	
B1	18
B2	2
B3	0
Location	
L1	3
L2	9
L3	8
L4	0

Table 4.2 Findings at EUA, MRI and final diagnosis using the outcome based gold standard

	EUA	MRI	Gold Standard
Abscess	**7**	**8**	**7**
Superficial	7	6	5
Supralevator	0	2	2
Fistula	**12**	**14**	**12**
Simple	1	3	1
Complex	11	11	11
Other	**6**	-	**6**
Fissure/Ulcer	4	-	4
Cancer	1	-	1
Haemorrhoids	1	-	1

Table 4.3 Discordant findings at EUA.

Patient Number	**EUA Finding**			**MRI Finding**		**Outcome**
	Abscess	Fistula	Other	Abscess	Fistula	
2	Superficial	Complex		Superficial	Complex (2)	FP MRI
4		Complex (2)		Superficial	Complex (2)	FP MRI
6	Superficial	Complex		Superficial	Complex (2)	FP MRI
11			Carcinoma		Simple	FP MRI
17	Superficial	Complex		Supralevator	Complex	TP MRI
18			Ulcer		Simple	FP MRI
20	Superficial	Complex		Supralevator	Complex	TP MRI

FP; False positive, TP; True Positive

The findings at EUA, MRI and the eventual outcome based gold standard are summarised in table 4.2. The EUA was discordant with MRI in 7 patients (35%). There were 5 false positive MRI findings and in 2 patients additional true positive findings were documented on MRI. For the identification of fistulas and/or abscesses in any patient, the sensitivity of MRI was 100% and specificity 50%. In those patients with discordance on MRI the median PDAI was 14 compared with 8 in the group with concordant MRI and EUA (p=0.248, Mann-Whitney U test).

The reasons for the discordance are detailed in table 4.3. MRI in two patients (patients 2 and 6) suggested additional separate fistula tracts which could not be identified at EUA. Patient 2 had a seton ligature placed in the fistula that was identified and a defunctioning ileostomy because of severe sepsis. After follow up of 18 months, the patient was maintained on adalimumab, the seton had been removed and the fistula had ceased draining. At no stage did the area of the second fistula suggested on the MRI cause any clinical symptoms hence this was deemed a false positive MRI finding. Similarly, patient 6 had a loose seton placed in the identified fistula and at 17 months follow up had commenced adalimumab and the seton had been removed. As the site of the other suggested fistula on MRI had not produced symptoms, this was also deemed a false positive MRI finding. Two further MRI scans were deemed false positive for the presence of fistulas. In patient 11, the MRI was reported as a complex fistula however EUA revealed an unexpected low rectal carcinoma without the presence of a fistula. In patient 18 an intersphincteric fistula was reported however only an ulcer was evident at EUA. The ulcer was biopsied and curreted. The patient was on maintenance adalimumab for ileocolic disease and at 7 months follow up the ulcer remained healed. The other false positive MRI (patient 4) suggested a peri-coccygeal abscess however despite probing, curetting and laying open sinus tracts, only a granulation tissue lined cavity was apparent and no pus was drained. Two patients had false negative EUAs. In both patients 17 and 20, abscesses were classified as superficial but the MRIs clearly showed supralevator extension. Despite the anatomic misclassification by the operating surgeon, the planned drainage manoeuvres with a combination of loose setons and malekot catheters were considered adequate and further management was not instituted as a result of the MRI. With follow up of 7 and 5 months respectively, the abscesses had clinically resolved leaving draining fistulas in both patients.

Initial surgical management included abscess drainage in seven patients, placement of loose seton ligatures in eight patients and defunctioning ileostomy in two patients. In no patient did the MRI alter the intraoperative management. One patient (patient 4) had an MRI demonstrating sacral osteomyelitis as a result of the severe perianal sepsis which was not evident at EUA and resulted in institution of long term antibiotic therapy. The patient is currently awaiting proctectomy and distal sacrectomy.

4.5 Discussion

MRI is commonly used in perianal CD and included in management guidelines as a mandatory part of the preoperative work up for many patients. This study has demonstrated a moderate level of discordance between findings at MRI and EUA that is within the previously reported range of 59-91% in studies including perianal CD patients [133-136, 138].

The discordant findings included four false positive MRI findings of fistulas, one false positive abscess and two additional true positive MRI findings where abscesses were recognised at EUA but misclassified. Discordance between these modalities can arise due to the operator dependence of both EUA and MRI. The MRIs were reported by radiologists with over ten years' experience in the technique. However, previous studies have documented that there is a level of both intra-observer and inter-observer variation in MRI interpretation even amongst experienced practitioners [138]. All reporting radiologists were aware of their participation in the study at the time of reporting and the fact that the patient was to undergo surgical exploration. This does have the potential to introduce a tendency to over reporting as recognised in previous studies [138]. The fact that the major discordance was produced by false positive MRI findings of fistulas suggests this phenomenon could have contributed to the high sensitivity but relatively low specificity documented here.

While all EUAs in the present study were performed by either specialist colorectal surgeons or fellows, it is recognised that, even in experienced hands, EUA is not completely accurate. In fact previous studies have suggested a superior accuracy of MRI to EUA [132]. The lack of a perfect gold standard can make the interpretation of discordance and sensitivity and specificity calculations troublesome. This problem was mitigated against in the present study by including clinical follow up and using the clinical outcome to determine a reference based gold standard. This methodology has previously been successfully employed in cryptoglandular fistulas [136], however, this is the first report using it exclusively in perianal CD.

Previous reports have suggested the level of disagreement between MRI and EUA increases in the presence of more complex sepsis. Schaefer *et al* only found

discordance in CD patients with complex sepsis and not in those with cryptoglandular sepsis [133]. Beets-Tan *et al* found a statistically significantly greater level of beneficial information provided by MRI in CD patients compared with non CD and a lower level in those with simple fistulas ($p<0.05$). The population in this study contained a spectrum of perianal disease ranging from no sepsis to complex sepsis, as reflected by the PDAI range at initial assessment of five to 16. The patients with discordance had a higher median PDAI than those without although this difference was not statistically significant. This trend does support the findings of these previous studies that discordance is found in more complex disease.

The most surprising finding of this study was the fact that, despite the discordance identified, intraoperative management was not altered by the MRI findings. Two previous studies have considered management change using similar methodology to that employed here. The first, involving only cryptoglandular fistulas, found intraoperative management was changed in 10% [137] and the other which included 15 CD patients found MRI altered management in 21% overall [138]. The lack of management change is partly explained by the fact that two thirds of the discordant findings were false positive MRIs. The two additional true positive MRI findings were abscesses that were classified incorrectly by the operating surgeon as superficial rather than supralevator. Adequate drainage was performed, despite this anatomic misconception, as was evidenced by resolution in both cases at clinical follow up.

The low therapeutic impact of MRI may also be explained by the institutional approach to the management of perianal CD. Of interest, no definitive fistula treatments (laying open or mucosal advancement flap) were performed in this study population, rather patients simply had abscess drainage with or without loose seton placement. With sepsis controlled, patients were then considered for anti-TNFα therapy with removal of seton following two induction doses. The key to surgical treatment in this approach then becomes the identification and adequate drainage of all sepsis, rather than its exact anatomic classification. While MRI may not always directly alter management it may increase operator confidence that sepsis has been adequately drained.

This study focused on the role of MRI in altering the immediate intra-operative management. It should be noted that in this study in one patient, MRI did provide additional information diagnosing sacral osteomyelitis and therefore altering the subsequent medical and surgical approach. In addition, there are other situations where MRI is useful in perianal CD. MRI is often used in the selection of patients for possible surgical treatment and an MRI negative for sepsis in a patient with perianal symptoms can avoid the need for an unnecessary general anaesthetic. The high sensitivity of MRI for fistulas and abscesses demonstrated in this study supports its utility for this purpose. In addition MRI is being increasingly used to monitor response to anti-TNFα therapy and its role in this setting continues to evolve [139, 140].

The small numbers in this study limit the validity of its results. Using an outcome derived gold standard demands adequate follow up to allow time for undrained infection which may have been suggested by the MRI to declare itself clinically. While the follow up in the study was relatively short with a median of 12 months, if reinfection from undrained sepsis was to occur, one would expect it to do so within this time period. Recurrence beyond this time period could well be the result of further CD activation or failure of deep tissue healing rather than inadequate sepsis drainage.

4.6 Conclusion

Preoperative MRI in perianal CD did produce a number of findings discordant with subsequent EUA. However, the nature of these findings did not produce significant management changes at EUA performed by colorectal surgeons in this study. Further larger studies of MRI in perianal CD are indicated to determine the necessity of the mandatory use of imaging prior to planned operative treatment of perianal CD as recommended by current guidelines. The high sensitivity for the presence of fistulas or abscesses confirms the utility of MRI in ruling out occult perianal sepsis and therefore avoiding unnecessary EUA.

Chapter 5

Perianal Surgical Intervention in CD

Abstract

Background

The aim of this study was describe the frequency and characteristics of perianal surgical intervention (PSI) for Crohn's disease in a population-based cohort of patients with inflammatory bowel disease (IBD).

Methods

A total of 1421 patients with IBD were recruited, representing approximately 91 per cent of people with IBD in Canterbury, New Zealand. The clinical notes were screened to confirm the diagnosis and extract clinical data, including details of PSIs.

Results

Some 649 patients with Crohn's disease were included in the analysis, of whom 119 (18·3 per cent) had at least one PSI. Of these, 61 (51·3 per cent) required further procedures. Operations for perianal abscess and fistula accounted for 72·4 per cent of interventions. PSI rates did not differ between the sexes (P = 0·218). Age less than 17 years (adjusted odds ratio (OR) 1·89 (95 per cent confidence interval 1·08 to 3·28)) and ileal disease (OR 1·76 (1·06 to 2·92)) were identified as predictors of PSI. As disease duration increased, so did the proportion of patients with complicated intestinal disease among those who had undergone PSI. The median time to first PSI from diagnosis of Crohn's disease was 28 (interquartile range 7–82) months. Sex, age at diagnosis and disease location did not influence the time to first PSI.

Conclusion

PSIs are frequent in patients with Crohn's disease, particularly those with ileal disease and those diagnosed at a young age.

5.1 Introduction

The preceding chapters of this thesis support the notion that perianal disease is a common and debilitating manifestation of Crohn's disease requiring intensive medical and surgical interventions. Despite the increasing use and demonstrated efficacy of anti-TNFα agents for perianal disease, surgery remains integral to the treatment algorithm. Surgical Interventions include simple examination under anaesthetic, abscess drainage, procedures for fissures and fistula and previous reports suggest that 12-20% of patients will go on to require proctectomy [34].

As reports of the frequency of perianal involvement overall in CD vary due to the populations studied and the classification criteria employed (see discussion chapter 2), so too does the reported frequency of perianal surgical intervention. The few previous studies concerned with rates of perianal surgical interventions (PSI) in patients with CD report rates from 4 to 42% [18, 55, 56]. As with overall incidence data, these studies evaluating PSI may have reported an inaccurate incidence of PSI in perianal CD patients overall by using single-centre, hospital-based data rather than population-based data [55, 56, 216]. As discussed in chapter 2, hospital-based studies come from referral centres, which are non-representative of the general population because of greater numbers of complicated patients. In addition, the small amount of available data regarding PSI has generally been from studies conducted in the 1980s and 1990s. Thus, new medical treatments and surgical techniques are not well represented in the literature [18, 55, 56, 216-218].

Chapter three discussed the inconsistency in the reported patient and disease characteristics associated with perianal CD in previous literature. There is little published regarding the association of these factors directly with PSI but age, gender and the location and behaviour of the disease have all been associated variably with perianal CD [219]. In particular, a number of studies report that perianal CD increases in association with more distal intestinal involvement [7, 18, 45, 55, 72] however this finding has not been universal [48] and data from this cohort presented in chapter three suggested ileal disease, not colonic disease was associated with perianal manifestations. Added to this local experience anecdotally has suggested a high rate of PSI requirement in patients with ileal disease.

A better understanding of the true rate of PSI, the operations performed and the patient factors associated with PSI is necessary to aid in refinement of the treatment algorithm, particularly in the era of biological therapy.

5.2 Aims

This study aims to describe the frequency and types of PSI and also the patient and phenotypic CD characteristics associated with PSI in a validated population-based Crohn's disease cohort in Canterbury, New Zealand.

5.3 Methods

This study was performed as part of the on-going Canterbury Inflammatory Bowel Disease Project. The study population and methods for recruitment for this project were described in section 2.3.

Over a three-year period (2003-2005), 1421 IBD patients were recruited. This number represents an estimated 91% of patients with IBD in the Canterbury region. Demographic information and clinical data, together with IBD phenotype and IBD-related surgical procedures (including PSI), were extracted retrospectively from patient medical records. This information was then entered into a custom built Microsoft Access database. IBD cases were confirmed using standard diagnostic criteria [203].

The term 'perianal disease', as employed in this study, encompasses patients with CD who experienced one or more anal manifestations included in the AGA guidelines classification described in section 1.2.1.8. Cases of CD were reviewed to identify those with perianal disease and subsequently, patients who had undergone one or more local PSI were selected from this group of patients. The type of operation performed was recorded and grouped into one of five categories; abscess drainage, procedure for anal fistula, examination under anaesthetic, procedure for anal fissure or anal dilatation.

Patients were categorized according to their age, disease location, and disease behaviour using the Montreal Classification System [220]. The data were analysed using SPSS v17.0® statistical software (SPSS Inc. Chicago, Illinois). Rates of perianal surgery between Montreal sub-categories were compared using Chi-squared testing and binary logistic regression. Kaplan-Meier Curves were constructed to demonstrate the timing of PSI relative to diagnosis. The study received ethical approval from the Upper South Island Ethics Committee and all patients gave written informed consent before taking part in the study.

5.4 Results

Of the one thousand four hundred twenty-one IBD patients recruited into the Canterbury IBD project, 715 had CD; however 66 patients were excluded from this analysis because of diagnostic uncertainty early in the course of their illness leaving 649 patients (figure 5.1).

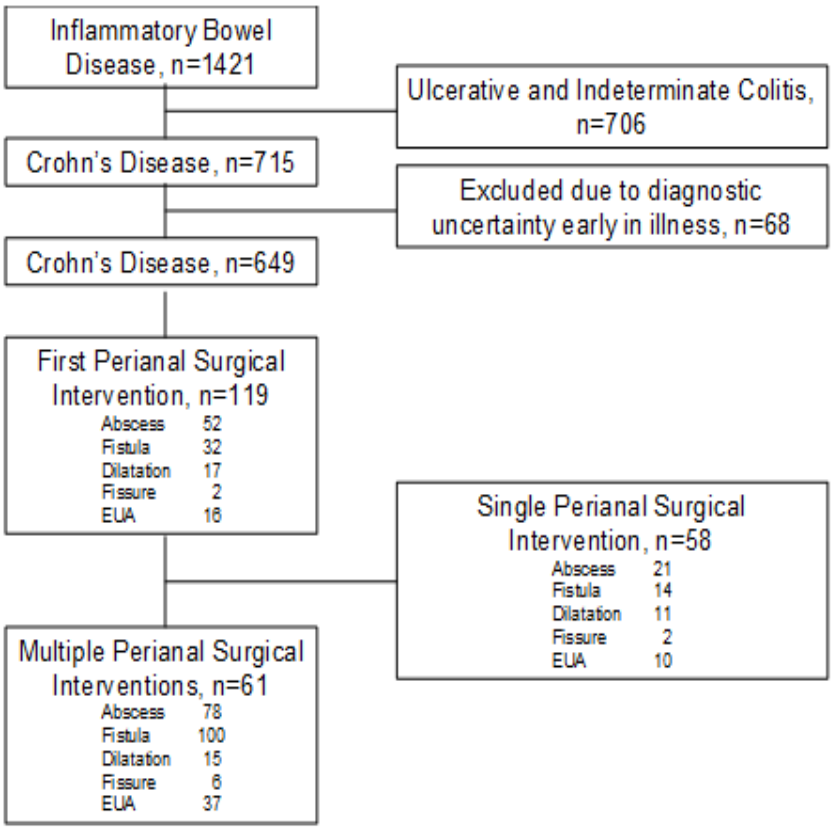


Figure 5.1 Study flow diagram

The median age was 29.4 years and there were 382 (58.9%) female patients and 267 (41.1%) male. One hundred and seventy-six (27.1%) of 649 patients had perianal CD and 119 (18.3%) had undergone at least one PSI (figure 5.1). Fifty-eight (48.7%), 26 (21.8%) and 35 (29.5%) patients had undergone one, two or greater than two PSI, respectively (figure 5.2). The median follow up from the time of diagnosis of CD was 9 years (inter-quartile range 3 to 14 years).

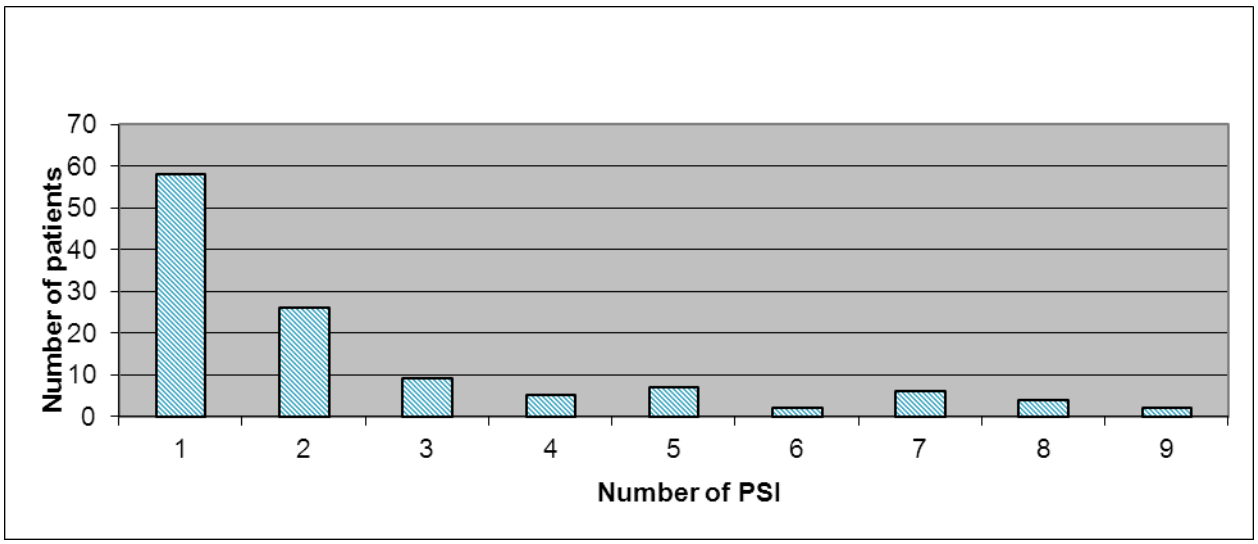


Figure 5.2 Frequency of PSI in 119 CD patients

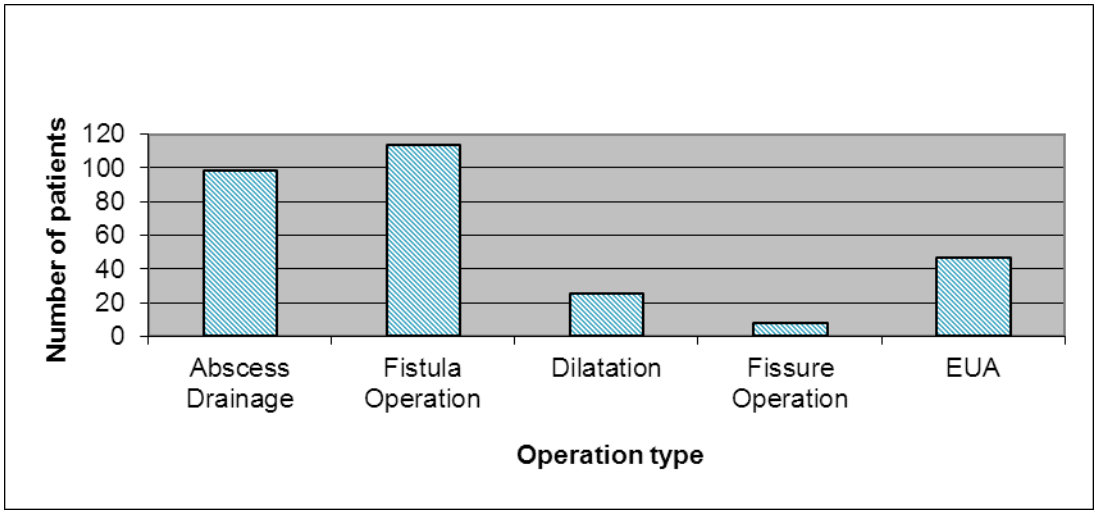


Figure 5.3 Type of PSI in 119 patients with CD

Overall, there were 294 procedures performed in the 119 patients. Drainage of perianal abscess and operations for anal fistula accounted for the majority of PSI with 99 (34%) and 114 (39%) procedures, respectively. The remaining perianal procedures performed included 47 (16%) examinations under anaesthesia, 26 (9%) anal dilatations and eight (3%) operations for anal fissure (figure 5.3). Table 5.1 shows the frequency of operation type according to whether single or multiple procedures were required. Fistula operations accounted for a higher percentage of procedures in the multiple operation group (47% versus 24%, p=0.011) while dilatation was more common in the single procedure group (18% versus 6%, p=0.002).

Table 5.1 Frequency of operation type for the group with a single perianal procedure compared with those with multiple procedures

Operation Type	**Single Procedure (%)**	**Multiple Procedures (%)**	**P value**
Abscess	21 (36)	78 (33)	0.649
Fistula Operation	14 (24)	100 (42)	0.011
Anal Dilatation	11 (18)	15 (6)	0.002
Anal fissure Operation	2 (3)	6 (3)	0.704
EUA	10 (17)	37 (16)	0.771

EUA: Examination Under Anaesthetic

Table 5.2 Perianal surgical interventions according to the Montreal Classification

Age at diagnosis	No PSI	PSI
A1	53 (71)	22 (29)
A2	301 (80.7)	72 (19.3)
A3	176 (87.6)	25 (12.4)
Location		
L1	172 (77.5)	50 (22.5)
L2	220 (88.0)	30 (12.0)
L3	133 (77.3)	39 (22.7)
L4	5 (100)	0 (0)
Behaviour		
B1	312 (85.7)	52 (14.3)
B2	162 (78.3)	45 (21.7)
B3	56 (72)	22 (28)

Values in parentheses are percentages. PSI, perineal surgical intervention

Fifty-five males (46.2%) and 64 (53.8%) females underwent at least one PSI (p=0.22). The age of the patients at diagnosis ranged from 11-86 years with a median age of 25 years. The frequency of PSI in patients classified according to the Montreal system is given in table 5.2 and the percentage of patients undergoing PSI in each category represented graphically in figure 5.4.

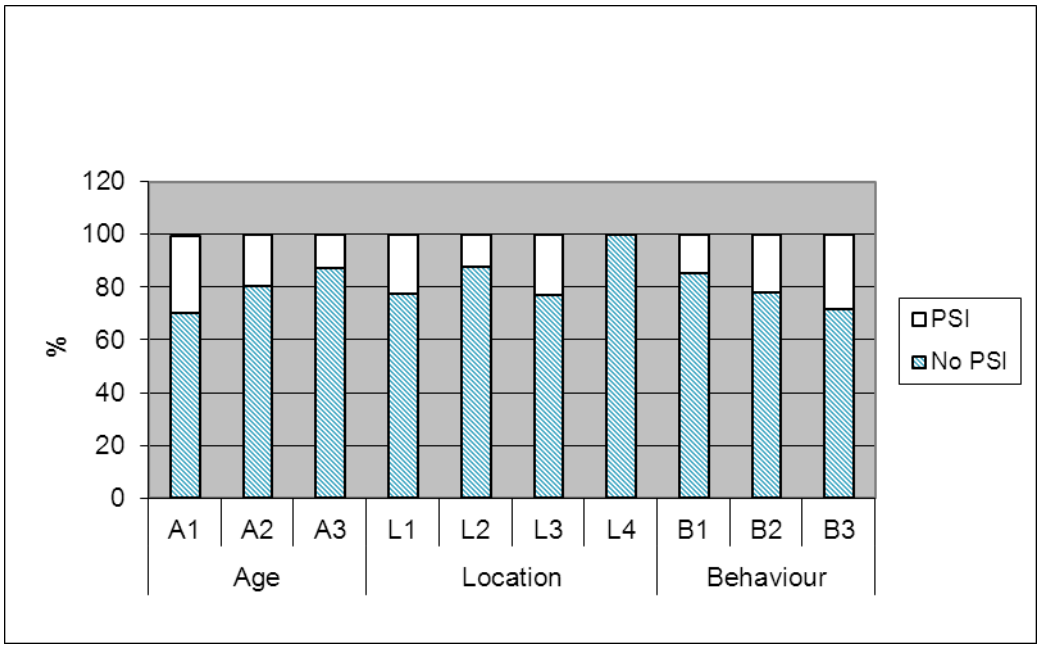


Figure 5.4 The percentage of patients with PSI according to the Montreal Classification

Univariate analysis revealed that those diagnosed at <17 years of age (Montreal A1) were 2.0 (95% CI 1.2-3.5) times more likely to require a PSI than those diagnosed over the age of 17 years, even following correction for duration of disease (p<0.009).Univariate analysis also revealed that patients with complicated intestinal disease (stricturing or penetrating) were 1.8 (95% CI 1.2-2.8) times more likely than those with uncomplicated inflammatory disease to undergo a PSI (p=0.003). Those with ileal disease location were 2.2 (95% CI 1.4-3.4) times more likely to undergo a PSI than patients with only colonic disease location (p=0.001). When adjustment for potential confounding variables was performed using binary logistic regression, ileal disease location and age less than 17 at diagnosis remained significant predictors of PSI (table 5.3).

Table 5.3 Unadjusted and adjusted odds ratios for factors associated with PSI

Risk Factor	Exposed % with PSI	Non-Exposed % with PSI	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	P Value
Ileal Disease	22.6	11.8	2.2 (1.4-3.4)	1.8 (1.1-2.9)	0.028
Complicated Disease	23.5	14.3	1.8 (1.2-2.8)	1.4 (0.9-2.2)	0.136
Age <17	29.3	16.9	2.0 (1.2-3.5)	1.9 (1.1-3.3)	0.024

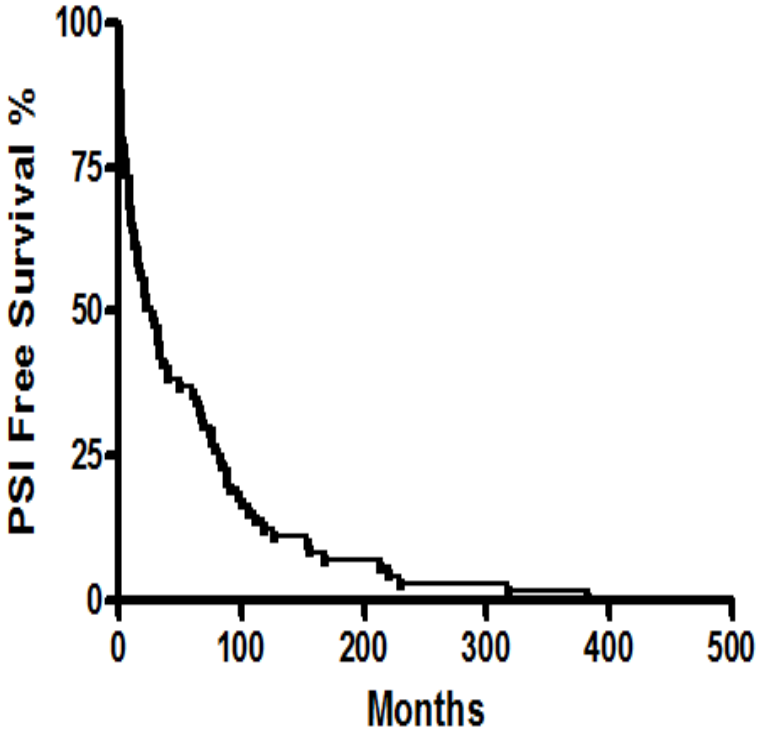


Figure 5.5 Time to first PSI in 119 patients with CD.

The time to first PSI is represented in figure 5.5. Seventeen patients (14%) underwent PSI prior to diagnosis of CD. In the remaining patients, the median time to first PSI from diagnosis of CD was 28 months (95% CI 7-82 months). This time to first PSI was not influenced by sex, age at diagnosis or disease location.

CD behaviour progressed over time from an uncomplicated to complicated phenotype in many of this cohort of CD patients with perianal disease. As the length of follow up increased, the proportion of patients with PSI and complicated disease

increased such that within 5 years of diagnosis, 30% of the patients who underwent a PSI had complicated disease. However, in the group that were greater than 10 years post diagnosis, 80% of patients who had undergone a PSI had complicated disease (p=0.0006). No evidence was found for a difference in the relationship between PSI and age or disease location as the length of follow-up increased.

Thirty-five (29.4%) of the 119 patients who underwent PSI had more than 2 surgical interventions. Males were 2.8 (95% CI 1.3-6.1) times more likely to have greater than 2 PSI than females (p=0.0085). Age, behaviour, or location of disease did not influence the rate of recurrent PSI.

5.5 Discussion

This population-based study confirms that PSI is frequent in CD patients, with 18% of CD patients having had at least one PSI and of those, more than one half required further procedures after a median of 9 years follow up.

Few studies have described the rate of PSI in CD overall, but the results from this study are within the previously reported range of 4-42% [18, 55, 56]. Unlike the population-based data presented here, previous studies have drawn data from clinic-based cohorts from specialist centres, which may have a higher proportion of complicated CD patients and therefore not represent the true rate of PSI in the CD population. In addition, the overall rate of perianal disease, whether requiring surgical intervention or not, varies widely from 10-80% due principally to the definitions used and the types of lesions included. Diagnostic uncertainty may also alter the incidence of perianal disease, with the inadvertent inclusion of ulcerative colitis patients as Crohn's colitis potentially lowering reported incidence [16]. The rigorous methodology employed in this study to classify the inflammatory bowel disease population should minimise this effect [201].

Patients in this series diagnosed before 17 years of age were over two times more likely to require a PSI than those diagnosed after age of 17 years. While no other study has reported a higher rate of PSI in younger patients, a number have suggested that perianal disease itself may be more frequent in younger patients.

Halme et al reported a series of 112 patients and found the average age of those with perianal disease was 27.7 years compared 37.4 years in those without ($p<0.01$) [54]. Roberts et al described a cohort of CD patients over 50 years of age and concluded they were less likely to present with perianal complications [71] and Cosnes et al found fistulizing complications, including perianal disease, were more common in those younger than 40 years [58]. Data presented in chapter 3 of this thesis, from this cohort also suggests an association between the occurrence of perianal disease and young age at diagnosis. However, the relationship of age to perianal disease is not consistent across studies as both Platell et al [55] and Hellers et al [45] found no difference in age in patients with or without perianal disease.

In this study, there was no significant difference in the proportion of females undergoing at least one PSI compared with males. Males, however, were significantly more likely than females to require multiple PSI. Once again, no previous studies relate gender to risk of PSI directly and considering perianal disease itself, the results concerning the impact of gender are conflicting. Sangwan et al and Hellers et al both report that significantly more males than females in their studies had perianal CD [18, 45]. On the contrary, Bell [25] noted a slight female predominance for perianal fistulae and Fry [72] reported more than twice as many females as males had perianal CD. One of the primary concerns in surgical therapy of perianal CD is the preservation of continence. As females have a higher risk of incontinence from factors unrelated to CD, such as childbirth, surgeons may adopt a less aggressive surgical approach to female CD patients which could contribute to the lower rate of multiple interventions in females in this study.

As perianal disease itself was found to be associated with ileal disease in this cohort (see chapter 3), PSI was more likely to be required in patients with ileal or ileocolonic disease than those with colonic disease. Once again, no studies relate disease location directly to the risk of PSI, however, as discussed in chapter 3, traditionally it has been thought that the risk of perianal disease increases with more distal intestinal involvement. Several clinic-based cohort studies that have considered surgical intervention have supported this contention and reported increased perianal complications with distal intestinal disease, in particular, rectal involvement [18, 45, 55, 72]. Presumably this would translate into an increase in PSI the more distal the

disease in these studies, although no data was given to confirm this. The reason for increased PSI in the group with isolated ileal disease in the present study has a number of possible explanations. The broader inclusion of other perianal lesions in addition to fistulae in this study could alter the relationship between PSI and intestinal disease location, however this seems unlikely because the majority of operations performed were still for fistulising perianal disease. Using PSI as the endpoint rather than perianal CD itself, may produce a higher rate amongst ileal CD patients if their perianal CD produced more surgically significant lesions requiring intervention. However the data presented in chapter three from this cohort demonstrates an association between ileal disease and all perianal CD, not just those requiring surgical intervention. A tempting explanation for this is the fact this data is from a population-based cohort as compared with the aforementioned referral centre cohorts. Therefore, this may represent a true relationship between ileal disease and perianal CD that is highlighted in this cohort, as it contains a high proportion of mild colonic CD patients, who may not be as susceptible to perianal disease. As discussed in sections 1.4 and 3.5, the evidence from population based cohorts is less consistent for an association of perianal disease with colonic disease location and PD overall [7, 47, 48]. The rigorous methodology employed in the present study to identify all CD patients combined with the long median follow up period of nine years and high rate of colonoscopy in this cohort giving accurate documentation of the extent of colonic disease [201] all support the contention of a genuine relationship between ileal disease and PSI.

As described in section 1.3.4 and chapter 3, genetic factors have an influence on the development of perianal CD, with a number of studies describing susceptibility loci on chromosome 5 [110, 117, 121]. In addition an association with the *NCF4* locus was identified in the present cohort (see chapter 3). The present study cohort has a homogeneous ethnicity with Caucasians making up 97.8% [221]. Underlying genetic differences in the populations studied may contribute to the reported differences in incidence and the disease characteristics of those with perianal CD.

In addition to disease location, the relationship between disease behaviour and PSI was considered. Univariable analysis based on most recent disease behaviour classification suggested an association, however, the multivariable analysis showed complicated intestinal disease behaviour did not predict the need for PSI overall. This

finding is consistent with recent studies suggesting that perianal and intestinal fistulae are distinct phenotypic entities [8, 9]. These studies contributed to the Montreal modification of the Vienna Classification to separate perianal disease from penetrating intestinal disease by addition of a perianal modifier [70]. Of particular note in one of these studies of 5491 patients, it was found that in colonic, but not ileal disease, intestinal fistulae were associated with perianal disease [9]. Hence, it is not surprising, given the aforementioned high proportion of PSI patients with ileal disease in this cohort, that overall, complicated intestinal disease was not associated with PSI.

While disease behaviour was not significantly associated with PSI, the evolution of disease behaviour over time following diagnosis in relation to PSI was of interest. At diagnosis, 70% of PSI patients had uncomplicated (B1) intestinal disease. However, later in the disease course, 80% of patients who had undergone a PSI had complicated intestinal disease. It has previously been documented that CD behaviour evolves over time with inflammatory disease, progressing to penetrating and stricturing phenotypes [58], while the location of disease remains relatively stable [222]. A previous report from this cohort of patients has demonstrated that perianal disease itself is an independent predictor for progression to complicated intestinal disease [5], hence it follows that a large proportion of those with a PSI will develop complicated intestinal disease over time.

While this study was population-based, with careful phenotyping, this was performed retrospectively and is dependent on the accuracy of the clinical records. Another weakness is the possibility of asymptomatic disease progression affecting the accuracy of disease classification.

5.5 Conclusion

Using a large, validated, population-based cohort, this study has confirmed that PSI is frequent in CD patients. The requirement for PSI was associated with ileal disease and young age at diagnosis. Over time post diagnosis, the proportion of PSI patients with complicated intestinal disease increased. The bulk of the existing literature on the subject contains studies published 10-20 years ago. While this more recent data includes patients managed in the era of new medical treatments such as anti-Tumour Necrosis Factor drugs, continuing evolution of medical management and

surgical techniques are likely to alter the frequency and characteristics of PSI in the future.

Chapter 6

The Cost of Perianal CD

Abstract

Aims

The aim of this study was to determine the direct and indirect costs of perianal Crohn's disease (CD) in patients in Canterbury in one year.

Methods

A retrospective cross-sectional analysis was performed. Perianal CD patients were recruited over a three month period. Interviews were conducted to obtain information regarding demographic, socioeconomic factors, and indirect costs. Hospital clinical notes were reviewed to determine direct health care utilization and costs.

Results

Twenty-five patients were enrolled in the study. In one year the total costs per patient for perianal CD were $20,366 with direct and indirect costs comprising $18,261 and $2,105, respectively. Extrapolating these data across New Zealand, the total cost of perianal CD in one year is approximately $49.5 million.

Conclusions

Perianal CD is a high cost disease with significant costs borne by patients and their families. Expensive pharmaceuticals comprise a significant proportion of the costs: increased access to these drugs might decrease hospital admissions and prevent work absenteeism and loss of carer productivity.

6.1 Introduction

Section 1.1 noted that the incidence of CD is increasing worldwide [3] and the peak age of onset is between 15 and 35 years [4]. CD remains incurable and a proportion of patients will endure recurrent and prolonged periods of illness requiring extensive medical and surgical interventions during what would otherwise be a highly productive time of life. Hence CD presents an increasingly significant health problem not only in terms of morbidity, but also in cost to the individual patient and society.

Previous studies have indicated that the majority of the total cost associated with this disease relates to "extensive interventions required by a small proportion of severely affected individuals" [189]. Several clinical markers of disease severity have been documented including diagnosis at a young age and the presence of perianal disease. In addition to being an independent marker of a more severe disease course, perianal disease produces local perianal complications requiring frequent intervention (see chapter 5) hence, this patient group is more likely to use significant health resources and incur the largest costs as a result of the disease. A number of international studies have considered the cost of inflammatory bowel disease overall [190-199], however, to the authors' knowledge, there are no data documenting the average patient cost of IBD in New Zealand or Australia and no previous work specifically investigating the perianal CD group.

Evidence is accumulating that newer therapies, such as anti-tumour necrosis factor alpha (anti-TNFα) antibodies, have significant efficacy in inducing and maintaining remission and have particular roles in complicated CD, such as perianal CD [150, 223]. However, these agents are expensive and predicting which patients will benefit from their early use remains a challenge [224]. The introduction of these modern biological treatments has created a need for government agencies to consider the economic impact of therapeutic alternatives. To achieve this, the cost of CD and its societal burden requires further study.

This is a cross-sectional retrospective study, which aimed to estimate the direct and indirect costs of perianal CD in one year in Canterbury using patient-based data. The costs of CD are borne not only by the taxpayer through government funded

healthcare but the patient and their family, therefore the cost perspective is approached from a societal point of view.

6.2 Methods

This study was performed in Christchurch Hospital, a tertiary university hospital serving a population of around 500,000. Ethical approval for the study was obtained from the regional ethics committee. All patients with CD according to previously documented diagnostic criteria [203] presenting to the institution during the period November 2009 to February 2010 were eligible for entry into the study. Perianal disease was defined as any symptomatic perianal lesion included in the American Gastroenterological Association classification [24]. Patients were recruited through Gastroenterology, Colorectal surgical clinics and hospital admissions.

After giving written informed consent, patients were submitted to a structured interview to obtain information regarding demographic and socioeconomic factors, work and school absenteeism, alternative health resource use and other related data for the preceding twelve month period. Participants were also offered the opportunity to nominate other costs that were not mentioned in the interview. Following the structured interview, hospital clinical notes were reviewed to determine direct health care utilization. This included hospital inpatient and outpatient visits and prescription drug use.

For the purposes of this analysis the costs were classified as direct or indirect. Direct costs included hospital (Emergency Department visits, laboratory tests, radiological investigations, endoscopy, pharmaceuticals, inpatient care and operating theatre costs) and outpatient (General Practice visits, specialist clinic visits, alternative health professional visits, non-prescription medications, pharmaceuticals, laboratory tests, District Nurse and Social Work services) associated costs. Indirect costs included; lost productivity, travel, carers, tutors and additional phone or internet requirements.

The costs of hospital resources were determined through the Costing Department of the Canterbury District Health Board (CDHB) and the Ministry of

Health. Hospital costs were calculated using DRG codes assigned to the patient each time they visited the hospital. A different code is given for each service required during each visit. Based on the quantity used during the visit the cost is calculated for each service used in a given visit. The cost department for the CDHB supplied the authors with all the codes and costs accrued by the patients during the study period. For primary care cost calculation, it was assumed that all patients were enrolled in a primary health organisation (PHO). The New Zealand Government provides subsidies to lower the cost of general practitioner (GP) visits for eligible people enrolled in a PHO. The cost of GP services was estimated using the average cost of an appointment by age as obtained from Pegasus Health PHO and the 2010 yearly capitation rates provided by the Government. The capitation rates took into account whether or not the patient had a high user health card (HUHC).

Pharmaceutical costs were calculated from the cost to the Pharmaceutical Management Agency in New Zealand (PHARMAC) provided by their pharmaceutical schedule accessed December 1st 2009. Additionally a 4% mark-up was added to pharmaceutical costs plus a $5.80 dispensing fee attributable to pharmacists. The co-payments paid by the patient are a transfer not a cost therefore these were not included.

The human capital method as described by Drummond *et al* [225] was employed in calculating indirect costs. Patients were asked the number of days they had off work as either unpaid or annual leave related to CD. This was transferred into hours off work and was multiplied by their gross hourly wage. For those patients not in work their indirect costs are discussed descriptively as monetary values were not able to be estimated. Government welfare payments received by patients not in work were not included as they represent a transfer rather than a cost.

It is conservatively estimated that there are approximately 9000 individuals with IBD in New Zealand, with perianal patients consisting of 27% based on data produced in chapter 2 of this thesis. This estimation was used to extrapolate the data to obtain values for the cost to society in New Zealand of perianal CD in one year.

6.3 Results

Some 25 patients with perianal CD (mean age 33 years, range17 to 73 years) were entered into the study. There were 15 males and 21 of the patients declared New Zealand European ethnicity. Eighteen patients were in some form of employment, three were in the education system, three were not participating in any work or education activities and one was retired.

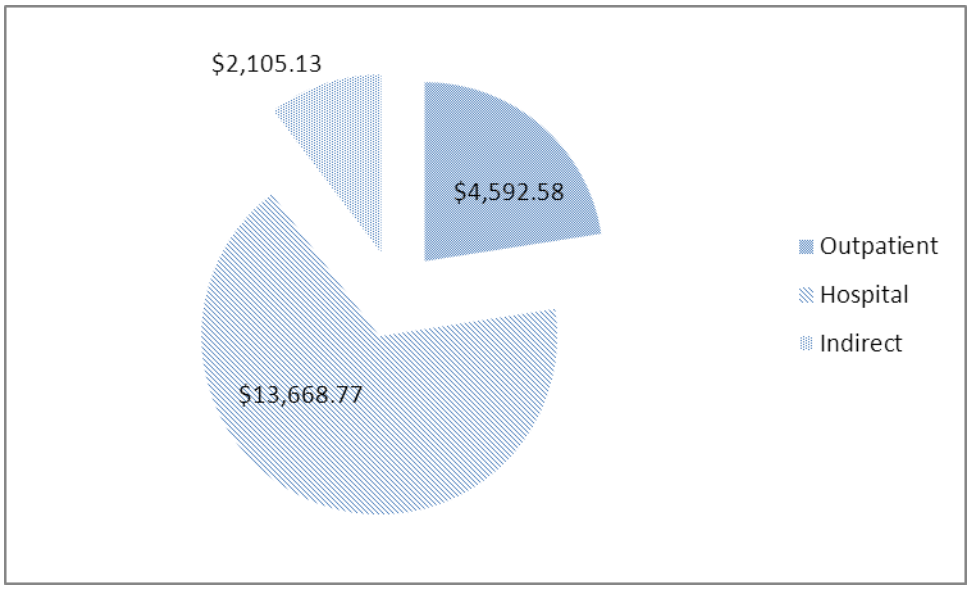


Figure 6.1 Overall cost of perianal CD divided into outpatient, hospital and indirect cost (mean cost per patient ($)).

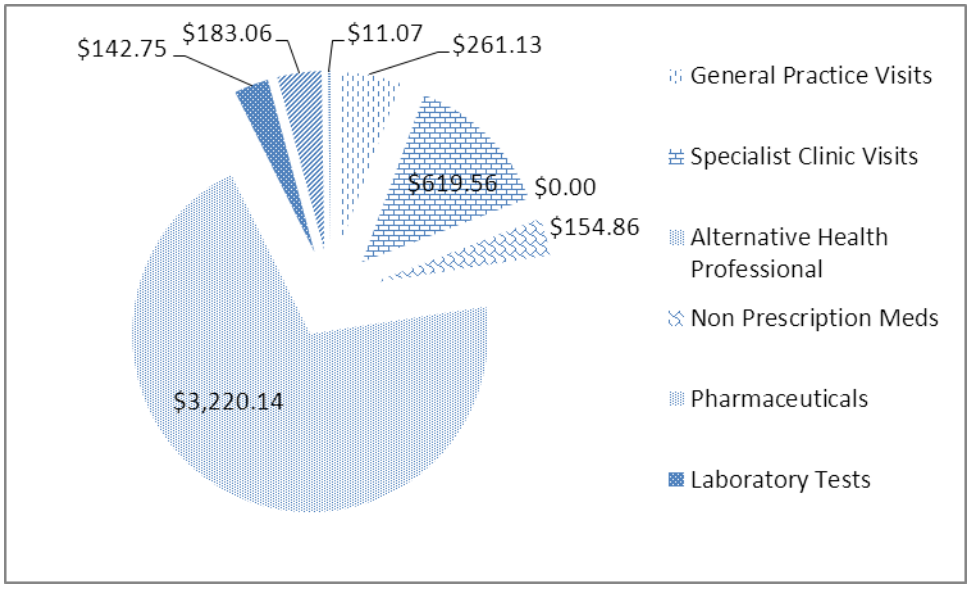


Figure 6.2 Outpatient cost of perianal CD showing the components that made up the total cost (mean cost per patient ($)).

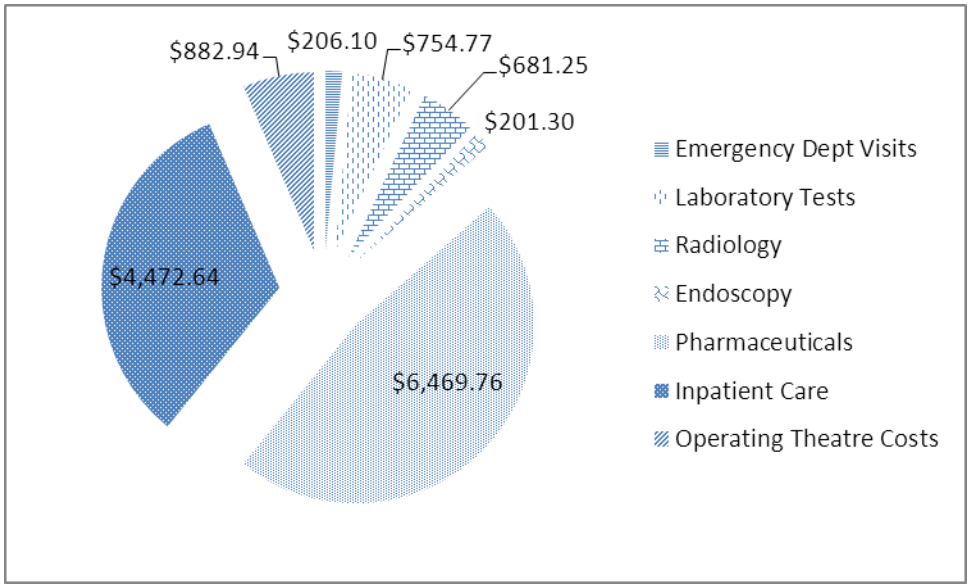


Figure 6.3 Hospital cost of perianal CD showing the components that made up the total cost (mean cost per patient ($)).

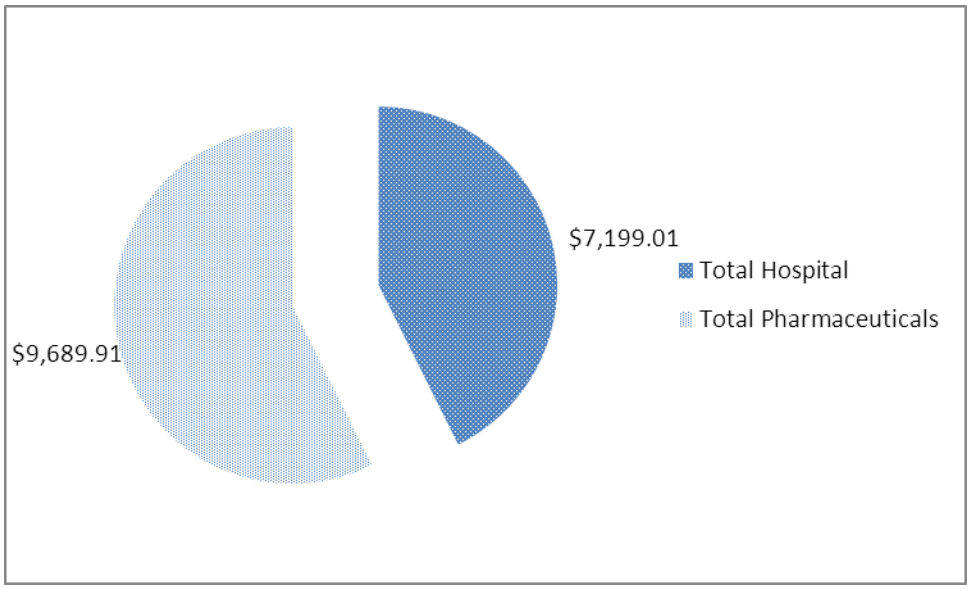


Figure 6.4 Comparison of hospital and pharmaceutical cost for perianal CD (mean cost per patient ($)).

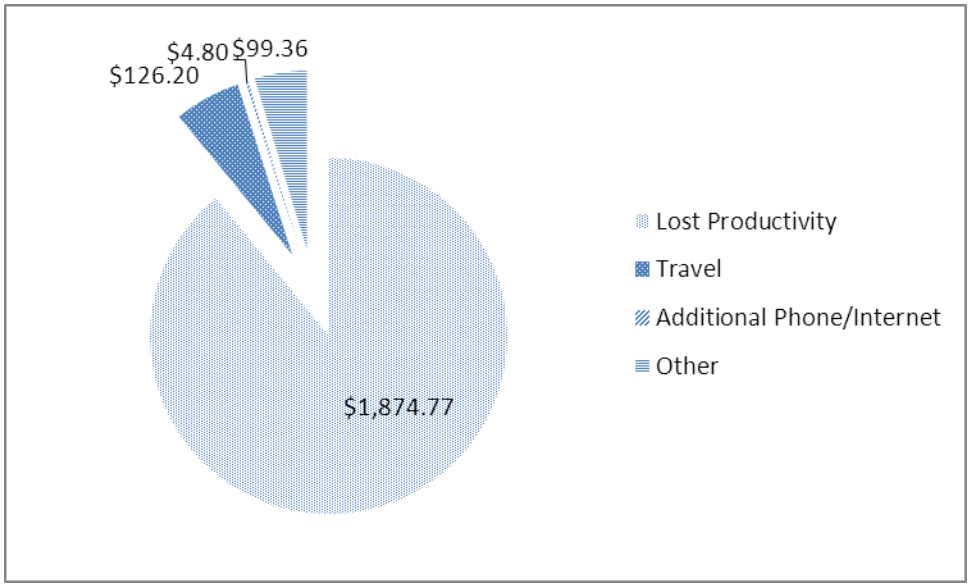


Figure 6.5 Indirect cost of perianal CD showing the components that made up the total cost (mean cost per patient ($)).

The average total costs per patient for perianal CD were $20,366 with direct and indirect costs comprising $18,261 and $2,105, respectively (Figure 1). The direct costs were further sub-divided into hospital and outpatient costs and the categories responsible for these costs are given in figures 2 and 3. The most significant costs were pharmaceuticals, followed by inpatient costs. This was highlighted when the total pharmaceutical bill was compared to other hospital costs (Figure 4). Eight of the 25 perianal CD patients (32 per cent) received anti-TNF therapy during the study period. Anti-TNFα medication made up 61 per cent of the pharmaceutical costs for perianal patients. The greatest indirect cost was patient and immediate family absenteeism from work (Figure 5). Extrapolating these data across New Zealand, the total cost of perianal CD in one year is estimated to be at least $49.5 million.

6.4 Discussion

These results confirm that perianal Crohn's disease is a high cost disorder. No previous studies in New Zealand or internationally have documented the costs created specifically by this group of patients. Several studies that considered IBD overall found that CD was associated with greater cost than ulcerative colitis [190, 197, 198]. A number of studies have also looked specifically at the cost of CD overall [192, 193, 195, 196, 199]. The heterogeneous nature of these studies in terms of methodology,

varying costs in different health care systems and the differential effects of inflation since the period of the study makes direct comparison with the present data difficult.

Juan *et al* [196] reported the annual cost per patient in a Spanish cohort in 2003 was €6,808 with €2,104 from direct costs and €4,704 from indirect costs [196]. Extrapolating data reported in 2009 by Mesterton *et al* the average annual cost of CD in Swedish patients was €9,400 [199] (approximately 16,240 New Zealand dollars at current exchange rates). Hence this group found comparable figures and also noted that increased cost was predicted by increased severity as measured by the Harvey-Bradshaw index. While the patients in the present study were not stratified for severity, perianal disease itself is a predictor of severe disease and this may explain the slightly higher direct costs reported here. This is supported by the previous finding that the presence of fistulae doubled the costs of care [199]. Patients recruited from tertiary referral centres, as in the present study, also tend to have more severe disease and this too could have contributed to the higher direct costs reported in this study. This fact and the small sample size in the present study mean some caution should be exercised in interpreting the results of this study when extrapolated to the perianal and population across New Zealand.

In contrast to the present analysis, both the previous European studies determined a higher relative contribution from indirect compared with direct costs. This may reflect the method of estimation of indirect costs. As the patients (or carers) in the present study were asked to estimate the absenteeism over the previous year, an element of recall bias may be present. In addition, no allowance for lost productivity while at work due to the disease (presenteeism) was made in this study. The patients were not asked if they were working part-time as a result of their disease. This potentially could have increase the value of their lost productivity. There was an economic recession for the majority of 2009 that could have contributed to the reduced work hours; therefore it does not seem appropriate to assume working part-time was a result of their illness. Altogether, these factors likely underestimate the indirect costs associated with CD in the current study.

These issues highlight the fact that there is some controversy in the literature in regards to the best approach to use when estimating indirect costs. The human capital-

cost method is recommended over other approaches by Liljas [226] and Johannesson [227] for cost studies from a societal perspective. These authors propose it is the most consistent with economic theory, therefore, indirect costs were estimated using the human capital method in this study, consistent with the approach taken in other recent studies [198, 199]. Despite this, the lower proportion of indirect costs found here could indicate the total cost has been underestimated in the present study hence these cost figures should be considered as a minimum.

Studies of this nature do not capture other effects of a chronic disease on productivity because the estimation of indirect costs is calculated based on the patients current gross wage rate. However, this does not take into account the wage rate the patient could have realised had they not been diagnosed with CD. Given that many patients with CD are diagnosed before or during the second or third decades of life, their disease may have potential life-long impact on educational achievement, career prospects and earning potential consequent to disrupted education and work. Previous studies have considered the effect of CD on educational achievement [228, 229]. Of note while the cases in these two studies showed significantly greater absenteeism than controls, decreased ability to present for exams and some degree of discrimination from teachers, there was no difference in level of educational achievement between cases and controls. Despite this, 47 per cent of respondents to a survey conducted in Germany felt that CD had interfered with their career prospects [198]. A separate study revealed 30 per cent of CD patients concealed their diagnosis from their employers [228]. Hence more subtle loss of productivity costs are likely to exist which have not been measured in this study.

In addition to both the direct and indirect costs discussed above, this study has not included intangible elements associated with the burden of disease, or loss of wellbeing, associated with CD. A more global assessment of the economic impact of CD would include such costs. Recent economic theory has allowed integration of these concepts into cost calculations. Using willingness to pay measures of mortality and morbidity associated with disease, economists have developed estimates of the Value of a Statistical Life. This can be used to attach a monetary value to the non-financially derived Disability Adjusted Life Year concept and thereby derive a financial cost associated with the burden of disease [230]. This was not attempted in

the present study where the aim was to produce estimates for future cost-benefit analysis that will utilise the direct costs associated with CD.

The principal direct costs incurred were for inpatient care and pharmaceuticals. In the adult group with perianal disease, the total pharmaceutical bill was greater than the other hospital associated costs (Figure 4). In several early studies, hospital costs were found to make up a higher proportion of the total cost of care for IBD than pharmaceutical costs [191, 193, 231]. However, these three studies were performed prior to the widespread use of anti-TNFα agents. As the present study was performed in the era of biologic treatments, the proportionally increased pharmaceutical costs are likely to represent a genuine finding and are consistent with the other most recent cost study from Sweden [199]. Anti-TNFα therapy is expensive but it may prove cost-effective if it leads to a reduction in hospitalisation and the high costs associated with this. This study was not designed to assess this. However, Hay and Hay [232] have previously created a model for the cost-effectiveness of expensive drug therapy in CD. Their model demonstrated that if a new drug reduced other costs such as hospitalisation by 20% then, despite a doubling of the pharmaceutical bill, the overall cost of care would reduce by 13% [232].

Surgery was another significant direct cost associated with CD in the present study. As patients undergoing surgery tend to more severely affected, direct comparisons of efficacy with medical treatments for similar clinical states are limited. However, Silverstein *et al* [192] found in a Markov analysis that despite higher costs for surgery, post-surgical remission was longer than for patients treated medically. These authors concluded that surgery may therefore be a more cost-effective option in selected cases [192]. Laparoscopic surgery is now increasingly used in the treatment of IBD. Short term advantages of laparoscopic surgery include improved pulmonary function, decreased ileus, shorter hospital stay and improved cosmesis [233]. In the longer term, there does not appear to be any difference in recurrence rates compared with open surgery [234]. While operative time and intraoperative expenses are increased, total hospital costs are reduced with the decreased length of stay [233]. Hence, any future cost-benefit analysis will need to allow for the impact of laparoscopic surgery.

6.5 Conclusion

This study has confirmed that perianal CD patients consume significant health resources. Prior to this, no studies in New Zealand have estimated the cost of IBD and no international studies have estimated the indirect costs of perianal Crohn's disease. With the advent of increasingly costly and effective medical therapies and evolving surgical treatment, this research will provide valuable information for future cost-effectiveness studies.

Chapter 7

Discussion

7.1 Overview

CD represents a significant health problem with an increasing incidence worldwide [3] and a documented high incidence in New Zealand [201]. It is a protean disorder with a range of different phenotypes included under the banner of CD. Of these, perianal manifestations are common and a source of significant morbidity. Despite the fact it is now more than 70 years since the description of a perianal fistula in a patient with regional enteritis by Penner and Crohn [11], significant gaps exist in our understanding of the epidemiology, aetiology and management of this phenotype of CD.

This thesis has addressed a number of these aspects of perianal CD which are summarised here in the context of existing knowledge and proposed directions for future research. Consistent data on fundamental features of the disease such as the incidence and type of perianal involvement in CD are lacking. While advances have been made in the aetiology of CD overall, how these aetiological factors relate to the perianal phenotype is poorly understood. The first part of this thesis (chapters 2 and 3) addressed these issues. While CD remains incurable, advances in technology in both diagnosis and management have led to a more complex and sophisticated treatment algorithm. The second part of this thesis addressed aspects of diagnosis (chapter 4) and management (chapter 5) and finally the health economic implications of this chronic disorder were dealt with in chapter 6.

7.2 The spectrum of perianal CD

In studying perianal CD, the first hurdle is to assess and describe the extent of the problem. Section 1.2 highlighted that existing data on the incidence of perianal CD is highly variable due to the definitions of perianal disease employed and the sources from which the study populations have been drawn. Due to the fact that perianal disease is a predictor of severe luminal disease behaviour, its occurrence is

over represented in referral centre cohorts compared with population based data. Despite the publication of several classification systems for perianal disease, a lack of clinical utility meant none have gained widespread clinical use. This has resulted in varying definitions used in previous studies and subsequent difficulty in comparing results amongst institutions. The classification from the AGA technical review of perianal CD was adopted for use in this thesis as it is clinically based and provides clear definitions of the various perianal manifestations of CD [24].

Using this definition, chapter 2 provided the results of a descriptive study of perianal CD in the Canterbury population, which found the rate of perianal involvement was 26.6 per cent. To the author's knowledge, the sub-phenotypic classification provided (see table 2.1) is the only existing classification of perianal disease drawn from a population based cohort. This study also assessed the temporal relationship of the onset of perianal manifestations to the diagnosis of CD and supported existing data that it is highly variable.

Future research should employ consistent definitions of perianal CD with clear sub-phenotypic classification. This is crucial to allow comparisons of cohorts when considering the results of both descriptive studies assessing aetiological factors for perianal disease, such as that presented in chapter 3, and interventional studies of modern medical therapy.

7.3 Aetiology

The results from chapter 2 raise the questions as to why only a subset of patients suffer perianal CD and at time points so distant to the onset of the luminal manifestations of the disease. Section 1.2.9 highlighted the fact that perianal fistulas occur independently of luminal fistulas and that perianal disease is now considered a separate disease phenotype as recognised in the Montreal Classification of CD [70]. If perianal CD does indeed represent a different disease phenotype, then it may be expected that different aetiological factors contribute in this group of patients. Several genetic and environmental factors have been recognised to contribute to the pathogenesis of CD overall. Chapter 3 documented the results of a study investigating whether any of these factors were specifically associated with perianal CD.

The study considered whether the phenotypic factors included in the Montreal classification were associated with perianal disease. The findings of an association with young age at diagnosis and complicated disease behaviour were consistent with previous studies. Notably the finding that perianal disease was associated with ileal disease involvement was contrary to a number of previous studies from referral centre cohorts and likely reflects the different spectrum of disease represented in these tertiary centre studies compared to the population based data collected in this study.

The strongest environmental associations with CD overall include smoking and a history of being breast fed, however this study did not demonstrate any specific association of these factors with perianal CD when compared to CD overall. To the author's knowledge, this is the first study to investigate the relationship of environmental risk factors to perianal disease. While no associations were found, the issue deserves further study in larger cohorts particularly given the possibility of under-powering discussed in section 3.5.

Heritability plays a significant role in CD aetiology as evidenced by the most recent meta-analysis of GWAs which documented 71 distinct susceptibility loci for CD [107]. Genotype-phenotype interaction studies indicate many of these genes are associated with susceptibility to different phenotypes of CD. The perianal phenotype has previously been associated with *IBD5, IRGM* and *TNFα,* all with varying strength and consistency. This study investigated 13 SNPs previously associated with CD and of these found a previously unreported association of perianal disease with the *NCF4* genotype. In many of the loci associated with CD, functional variants are yet to be identified however the product the *NCF4* gene has a biologically plausible role in the pathogenesis of CD. *NCF4* encodes a crucial protein mediating the oxidative burst in phagocytosis and alteration in its function may contribute to the abnormal innate immune response to commensal bacteria hypothesised to underlie CD pathogenesis. The other genes previously associated with perianal CD investigated in this study did not show an association. However, it should be noted that the study was underpowered to detect an association with IRGM and TNFα (see section 3.2). The finding of a distinct susceptibility locus for perianal CD supports the hypothesis that perianal CD is a separate phenotype with distinct risk factors and these associations

warrant further research. It is important such studies are powered to detect differences in genotype frequencies which may involve combining cohorts from different regions. This highlights the need for consistency in perianal CD classification discussed above. Further defining the cohort at risk of perianal disease not only contributes to our understanding of the pathogenesis of perianal CD but has substantial clinical relevance. Given that perianal CD patients endure a severe disease course from both the perianal and luminal manifestations, early identification of these patients allows for a "top-down" treatment strategy in an attempt to alter the natural history of the CD.

Other potential contributors to the pathogenesis of perianal CD exist that were not investigated in this thesis. The commensal gut microbiological flora likely plays a role in CD pathogenesis. Few studies have considered the role of microbiological flora in perianal CD and the only study which cultured aspirates from CD perianal abscesses found a predominance of gram positive organisms [235] in contrast to studies which have investigated cryptoglandular sepsis and found predominantly gram negative enteric organisms [236, 237]. Further research comparing cultures from these different forms of perianal sepsis may shed more light on the role bacteria play in perianal CD. In addition to microbiological factors, immunological factors have also been implicated, in particular abnormal myofibroblast function has been documented in perianal CD fistulas [238]. A case report finding that human autologous fibroblasts seeded onto biocompatible scaffolds healed a perianal CD fistula [239] also supports a role for abnormal fibroblast function and suggests further research in this area is warranted.

7.4 Assessment

Treatment of the perianal CD depends on the nature of the lesion and in the modern era may involve expensive medical therapy or complex and potentially morbid surgical intervention. Simple clinical examination is often insufficient to allow treatment decisions to be made, hence further imaging and/or EUA are often necessary. Modern imaging techniques such as EAUSS or MRI are now often used as an adjunct to EUA and while there accuracy is relatively well documented in cryptoglandular fistulas, there is a paucity of evidence attesting to their efficacy in the more complex perianal sepsis of CD. Despite this lack of evidence, guidelines now

suggest the use of MRI or EAUSS in perianal CD patients undergoing surgery. The results presented in chapter 4, which is the only study using this methodology exclusively in CD patients, do not support the routine use of MRI with EUA. While a relatively high rate of discordance was found between MRI and EUA results, this was mainly related to minor fistula classification changes and did not produce management changes at EUA. In addition to the other limitations discussed in section 4.5, this was a small study designed to fit within the time constraints of this thesis and a larger study using similar methodology would be of value.

7.5 Management

Evidence suggests that perianal CD patients require intensive medical and surgical intervention. Even in the era of anti-TNFα agents for perianal disease, surgery remains integral to the treatment algorithm and 12-20% will go on to require proctectomy. A lack of data exists documenting the rate of perianal surgical intervention and the risk factors for this. The data that does exist comes from referral centre cohorts so likely overestimates PSIs and distorts the risk factors. Hence, the study presented in chapter 5 set out to determine the rate of PSI in the CD population and to identify risk factors associated with this. The data confirmed that PSI remains a frequent occurrence in the era of biologic treatments and multiple interventions are often required. The risk factors identified here mirrored those of perianal disease itself. Further study into risk factors (be they phenotypic, genetic, serologic or others) for patients likely to undergo a complicated course requiring surgery is warranted particularly as these patients may be then be identified for early escalation of medical therapy.

7.6 Cost

To this point it has been demonstrated that CD has an increasing incidence, relapsing nature and requirement for intensive, prolonged medical and surgical management. These facts combined with the knowledge that perianal CD predicts a severe disease course; all suggest that perianal CD is likely to be a high cost disorder. With the advent of increasingly expensive therapies, an estimate of the cost of perianal CD from the societal viewpoint is essential to aid in future decisions in

treatment funding. Chapter 6 provides the results of the first study looking specifically at the costs associated with perianal CD patients and confirmed that these are significant. The strength of the methodology was that it included both direct and indirect treatment costs which both need to be considered for a disease such as CD which affects patients during the productive years of life. This study provides valuable baseline data for consideration when funding decisions are made in the future.

7.7 Summary

Perianal CD represents a significant health problem in terms of morbidity and cost but many unanswered questions remain with respect to its aetiology, pathogenesis and optimal management. This thesis has provided the first detailed classification of the disease in a population based cohort. It has supported the hypothesis that perianal CD represents a distinct disease phenotype by documenting distinct clinical and genetic associations with perianal disease. With regard to investigation of perianal disease it has suggested that MRI is not an essential tool in dictating operative management. Finally it has confirmed that perianal disease requires frequent and costly interventions. Further research into the clinical and genetic factors associated with perianal disease building on that presented here not only has the potential to contribute to a greater understanding of the pathogenesis of CD but also has significant potential therapeutic implications in the future.

Appendix A

Table A1 Association results and *in silico* analyses for 32 previously confirmed Crohn's disease risk loci (Reproduced from reference [107]).

dbSNP ID	Chr.	Left–right (Mb)	Risk allele	Allele frequency in controls	P_{meta}	OR (95% CI)	Association reported with other phenotypes	Positional candidate genes of interest
rs11209026	1p31	67.13–67.54	G	0.932	1.00×10^{-64}	2.66 (2.36–3.00)	UC, AS, Ps, PBC, GC, BD	***IL23R***
rs2476601	1p13	113.66–114.42	G	0.907	4.47×10^{-9}	1.26 (1.17–1.37)	T1D[a], RA[a], SLE, Ps, Vitiligo[a], AITD	***PTPN22***
rs4656940	1q23	158.96–159.20	A	0.801	6.17×10^{-7}	1.15 (1.09–1.21)	SLE, RA	***CD244***, *ITLN1*
rs7517810	1q24	170.92–171.21	T	0.246	1.51×10^{-15}	1.22 (1.16–1.28)	HepC, SLE, SSc, T2D	***TNFSF18, TNFSF4, FASLG***
rs7554511	1q32	199.11–199.32	C	0.726	1.58×10^{-7}	1.14 (1.08–1.19)	UC, celiac, MS	***C1orf106***, *KIF21B*
rs3792109	2q37	233.81–234.23	A	0.529	6.76×10^{-41}	1.34 (1.29–1.40)	UC	***ATG16L1***
rs3197999	3p21	48.16–51.73	A	0.297	6.17×10^{-17}	1.22 (1.16–1.27)	UC	***MST1, GPX1, BSN***
rs11742570	5p13	39.88–41.00	C	0.606	7.08×10^{-36}	1.33 (1.27–1.39)	MS	***PTGER4***
rs12521868	5q31	129.41–132.05	T	0.422	1.41×10^{-20}	1.23 (1.18–1.28)	Ps, fibrinogen, asthma, TB, UC	***SLC22A4, SLC22A5, IRF1, IL3***
rs7714584	5q33	150.01–150.38	G	0.088	7.76×10^{-19}	1.37 (1.28–1.47)	TB	***IRGM***
rs6556412	5q33	158.43–158.88	A	0.332	5.37×10^{-14}	1.18 (1.13–1.24)	Ps, SLE, malaria, asthma	***IL12B***
rs6908425	6p22	20.60–21.25	C	0.784	1.41×10^{-8}	1.17 (1.11–1.23)	T2D, Ps, UC	*CDKAL1*
rs1799964	6p21	31.49–32.98	C	0.209	3.98×10^{-11}	1.19 (1.13–1.25)	Multiple including UC	***LTA***, *HLA-DQA2*, ***TNF, LST1, LTB***
rs6568421	6q21	106.50–106.67	G	0.301	4.37×10^{-8}	1.13 (1.07–1.18)[b]	SLE, RA	***PRDM1***
rs415890	6q27	167.26–167.47	C	0.522	2.51×10^{-12}	1.17 (1.12–1.22)	RA, Graves	***CCR6***
rs1456896	7p12	50.22–50.34	T	0.690	1.20×10^{-8}	1.14 (1.09–1.20)	AD, SLE, MCV, ALL	***IKZF1, ZPBP, FIGNL1***
rs4871611	8q24	126.54–126.65	A	0.609	1.51×10^{-12}	1.17 (1.12–1.23)		
rs10758669	9p24	4.93–5.29	C	0.349	1.00×10^{-13}	1.18 (1.13–1.23)	UC, MPD	*JAK2*
rs3810936	9q32	116.47–116.74	C	0.682	1.00×10^{-15}	1.21 (1.15–1.27)	UC, leprosy, SpA	***TNFSF15, TNFSF8***
rs12242110	10p11	35.22–35.94	G	0.315	1.10×10^{-9}	1.15 (1.10–1.20)	UC	***CREM***
rs10761659	10q21	63.97–64.43	G	0.538	4.37×10^{-22}	1.23 (1.18–1.29)	BC	*ZNF365*
rs4409764	10q24	101.26–101.33	T	0.492	2.29×10^{-20}	1.22 (1.17–1.27)	UC	*NKX2-3*
rs7927997	11q13	75.70–76.04	T	0.389	5.62×10^{-13}	1.17 (1.12–1.22)	Atopy[a]	*C11orf30*
rs11564258	12q12	38.42–39.31	A	0.025	6.17×10^{-21}	1.74 (1.55–1.95)	PD, leprosy	***MUC19***, *LRRK2*
rs3764147	13q14	43.13–43.54	G	0.245	1.41×10^{-10}	1.17 (1.12–1.23)	Leprosy	***C13orf31***
rs2076756	16q12	49.02–49.41	G	0.260	3.98×10^{-69}	1.53 (1.46–1.60)	Leprosy, atopy, Blau, GvHD	***NOD2***
rs2872507	17q21	34.62–35.51	A	0.458	1.51×10^{-9}	1.14 (1.09–1.19)	Asthma, UC, PBC, T1D, RA, WBC	***GSDML, ZPBP2, ORMDL3, IKZF3***
rs11871801	17q21	37.57–38.25	A	0.756	2.51×10^{-8}	1.15 (1.10–1.21)	MS[a], obesity, HIES	***MLX***, *STAT3*
rs1893217	18p11	12.73–12.92	G	0.153	1.29×10^{-14}	1.25 (1.18–1.32)	T1D[a], celiac	*PTPN2*
rs740495	19p13	1.04–1.13	G	0.247	8.13×10^{-12}	1.16 (1.10–1.21)		*GPX4, SBNO2*
rs1736020	21q21	15.62–15.77	C	0.579	9.33×10^{-12}	1.16 (1.11–1.21)	UC	
rs2838519	21q22	44.41–44.52	G	0.391	2.09×10^{-14}	1.18 (1.13–1.23)	Celiac, UC	***ICOSLG***

The table reports new data for loci confirmed in the earlier meta-analysis[1]. Left–right association boundaries are given for each index SNP (NCBI's dbSNP build v130; see Online Methods). Associations with other relevant traits were identified by a literature search using the US National Institutes of Health catalog of genome-wide association studies and the HUGE database (version 1.4)[43,44]. Candidate genes of interest are listed. Those in bold were highlighted by *in silico* analyses (GRAIL connectivity, presence of an eQTL effect with LOD ≥ 5.0 or implicated coding SNP; see main text and **Supplementary Table 6** for more details). Loci tagged by rs4656940 and rs7554511 previously replicated strongly (at 0.00048 and 2.3×10^{-6}, respectively[1]) and still pass genome-wide significance on combined analysis. UC, ulcerative colitis; AS, ankylosing spondylitis; Ps, psoriasis; PBC, primary biliary cirrhosis; T1D, type 1 diabetes; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T2D, type 2 diabetes; MS, multiple sclerosis; AD, Alzheimer's disease; MCV, mean corpuscular volume; ALL, acute lymphocytic leukemia; SpA, spondyloarthritis; PD, Parkinson's disease; AITD, autoimmune thyroid disease; BC, breast cancer; BD, Behcet's disease; GC, gastric cancer; HepC, hepatitis C susceptibility; SSc, systemic sclerosis; MPD, myeloproliferative disease; TB, tuberculosis; GvHD, graft versus host disease; WBC, white blood cell count; HIES, hyper immunoglobulin E syndrome.
[a]Association in the opposite direction in different traits. [b]Loci with more than one independent association.

Table A2 Association results and in silico analyses for 39 newly confirmed Crohn's disease risk loci (reproduced from reference [107]).

dbSNP ID	Chr.	Left–right (Mb)	Risk allele	Allele frequency in controls	P_{meta}	$P_{repl.}$	$P_{comb.}$	OR (95% CI)	Association reported with other phenotypes	Positional candidate genes of interest
rs2797685	1p36	7.66–7.89	A	0.190	2.69×10^{-10}	1.40×10^{-2}	7.10×10^{-9}	1.05 (1.01–1.10)	Celiac	*VAMP3*
rs3180018	1q22	153.24–154.39	A	0.250	1.29×10^{-9}	2.70×10^{-5}	2.30×10^{-13}	1.13 (1.06–1.19)[a]	T2D, asthma, PD	*SCAMP3, MUC1*
rs1998598	1q31	195.58–196.21	G	0.302	4.90×10^{-9}	1.60×10^{-2}	8.70×10^{-9}	1.04 (1.00–1.09)	Asthma	*DENND1B*
rs3024505	1q32	204.87–205.10	T	0.157	8.32×10^{-9}	1.50×10^{-7}	1.60×10^{-14}	1.12 (1.07–1.17)	T1D, UC, SLE, BD, HepC	***IL10, IL19***
rs13428812	2p23	25.30–25.46	G	0.326	1.41×10^{-8}	5.90×10^{-4}	8.50×10^{-10}	1.06 (1.03–1.10)		*DNMT3A*
rs780093	2p23	27.24–27.71	T	0.418	1.10×10^{-4}	3.30×10^{-8}	4.70×10^{-11}	1.15 (1.10–1.21)	CRP, glucose, TGs	***GCKR***
rs10495903	2p21	43.30–43.80	T	0.129	7.70×10^{-8}	2.90×10^{-8}	1.60×10^{-14}	1.14 (1.09–1.20)[a]	T2D, PC	***THADA***
rs10181042	2p16[b]	60.77–61.74	T	0.420	6.61×10^{-9}	N/A	N/A	1.14 (1.09–1.19)	RA, UC, celiac	***C2orf74,*** *REL*
rs2058660	2q12[c]	102.17–102.67	G	0.231	1.58×10^{-12}	N/A	N/A	1.19 (1.14–1.26)	Celiac, asthma, T1D, HSV	***IL18RAP, IL12RL2, IL18R1, IL1RL1***
rs6738825	2q33	197.85–198.67	A	0.473	1.82×10^{-7}	1.60×10^{-3}	3.50×10^{-9}	1.06 (1.02–1.11)	CAD	***PLCL1***
rs7423615	2q37	230.76–230.94	T	0.187	4.57×10^{-9}	7.40×10^{-6}	3.10×10^{-13}	1.12 (1.07–1.18)	CLL	***SP140***
rs13073817	3p24	18.58–18.86	A	0.322	8.20×10^{-7}	1.00×10^{-3}	6.70×10^{-9}	1.08 (1.03–1.13)		
rs7702331	5q13	72.49–72.62	A	0.600	2.00×10^{-6}	6.40×10^{-7}	5.90×10^{-12}	1.12 (1.07–1.17)		
rs2549794	5q15	96.11–96.45	C	0.409	4.47×10^{-11}	2.00×10^{-3}	1.10×10^{-10}	1.05 (1.02–1.09)	AS, PD, T1D, PET	***ERAP2, LRAP***
rs11167764	5q31	141.39–141.62	C	0.796	1.10×10^{-9}	4.20×10^{-3}	2.00×10^{-9}	1.06 (1.02–1.11)		*NDFIP1*
rs359457	5q35	173.15–173.47	T	0.571	5.25×10^{-8}	3.30×10^{-6}	2.50×10^{-12}	1.08 (1.04–1.12)		***CPEB4***
rs17309827	6p25	3.35–3.41	T	0.639	6.16×10^{-7}	3.10×10^{-4}	6.70×10^{-9}	1.10 (1.05–1.16)		
rs1847472	6q15	90.86–91.14	G	0.658	3.63×10^{-6}	1.40×10^{-4}	5.10×10^{-9}	1.07 (1.03–1.11)	T1D, celiac	***BACH2***
rs212388	6q25	159.26–159.46	G	0.393	1.41×10^{-7}	2.40×10^{-5}	2.30×10^{-11}	1.10 (1.05–1.14)	RA, celiac, T1D[d]	*TAGAP*
rs6651252	8q24	129.56–129.67	T	0.865	2.29×10^{-6}	2.40×10^{-13}	3.90×10^{-18}	1.23 (1.17–1.30)		
rs4077515	9q34[c]	138.27–138.54	T	0.411	4.37×10^{-19}	1.50×10^{-19}	1.30×10^{-36}	1.18 (1.13–1.22)	UC, AS	***CARD9, SNAPC4***
rs12722489	10p15	6.07–6.21	C	0.852	8.51×10^{-6}	5.20×10^{-5}	2.90×10^{-9}	1.11 (1.05–1.16)	MS, T1D, vitiligo, RA, AA, asthma, AITD	***IL2RA***
rs1819658	10q21	59.50–59.81	C	0.774	1.41×10^{-7}	1.10×10^{-10}	9.10×10^{-17}	1.19 (1.13–1.25)	AD	*UBE2D1*
rs1250550	10q22[e]	80.67–80.77	G	0.669	2.00×10^{-10}	7.30×10^{-22}	1.10×10^{-30}	1.19 (1.15–1.23)	Celiac, MS, vitiligo, BC	*ZMIZ1*
rs102275	11q12	61.28–61.44	C	0.341	7.24×10^{-8}	1.70×10^{-5}	2.30×10^{-11}	1.08 (1.04–1.12)	CAD, dyslipidemia	***FADS1***
rs694739	11q13	63.58–64.05	A	0.626	3.38×10^{-7}	3.50×10^{-4}	6.00×10^{-10}	1.10 (1.05–1.16)	AA	*PRDX5, ESRRA*
rs2062305	13q14	41.72–42.00	G	0.346	2.00×10^{-6}	5.70×10^{-5}	4.90×10^{-10}	1.10 (1.05–1.15)	BMD, RA	***TNFSF11***
rs4902642	14q24	68.23–68.39	G	0.584	2.00×10^{-7}	4.50×10^{-5}	1.60×10^{-10}	1.07 (1.11–1.04)[a]	Celiac, T1D	*ZFP36L1*
rs8005161	14q35	87.28–87.71	T	0.119	1.29×10^{-8}	5.90×10^{-11}	4.20×10^{-18}	1.23 (1.16–1.31)[a]		***GALC, GPR65***
rs17293632	15q22	65.20–65.27	T	0.233	1.41×10^{-13}	2.00×10^{-8}	2.70×10^{-19}	1.12 (1.07–1.16)	CAD, T2D	*SMAD3*
rs151181	16p11[e]	28.20–28.94	G	0.386	1.10×10^{-10}	1.20×10^{-3}	1.50×10^{-11}	1.07 (1.03–1.12)	T1D, obesity, asthma, CRC, SLE, RA, IBD	***IL27, SH2B1, EIF3C, LAT, CD19***
rs3091315	17q12[b]	29.51–29.70	A	0.723	1.70×10^{-13}	N/A	N/A	1.20 (1.14–1.26)	HIV resistance	***CCL2, CCL7***
rs12720356	19p13	10.26–10.50	G	0.084	9.20×10^{-10}	1.90×10^{-5}	1.40×10^{-12}	1.12 (1.06–1.19)[a]	T1D, SLE, MS, HIES	***TYK2, ICAM1, ICAM3***
rs736289	19q13[e]	38.42–38.47	T	0.612	2.69×10^{-7}	2.00×10^{-3}	8.70×10^{-9}	1.06 (1.02–1.11)		
rs281379	19q13[e]	53.78–53.97	A	0.487	8.60×10^{-10}	5.20×10^{-5}	7.40×10^{-12}	1.07 (1.04–1.11)	B12, norovirus, HP	***FUT2, RASIP1***
rs4809330	20q13	61.65–61.95	G	0.709	2.51×10^{-12}	4.60×10^{-5}	2.70×10^{-15}	1.12 (1.06–1.18)	Glioma	***RTEL1, TNFRS-F6B, SLC2A4RG***
rs181359	22q11	20.14–20.39	T	0.203	6.31×10^{-13}	2.30×10^{-6}	4.80×10^{-16}	1.10 (1.06–1.15)	RA, celiac, SLE, MCV	***YDJC***
rs713875	22q12[e]	28.23–29.00	C	0.471	5.70×10^{-9}	8.30×10^{-5}	7.30×10^{-12}	1.08 (1.04–1.13)	IBD, T1D	*MTMR3*
rs2413583	22q13	38.00–38.14	C	0.830	1.70×10^{-10}	9.50×10^{-18}	1.10×10^{-26}	1.23 (1.17–1.29)		*MAP3K7IP1*

The table lists Crohn's disease susceptibility loci newly identified since the first meta-analysis[1] with $P < 5 \times 10^{-8}$ in the combined analysis (discovery plus replication sample) and $P < 0.05$ in the replication stage. Seven of these loci have previously been reported (see footnotes b and e below). Column headings and abbreviations are as described in **Table 1**. Additional abbreviations are as follows: CRC, colorectal cancer; CRP, C-reactive protein; TGs, triglycerides; PC, prostate cancer; HSV, human simplex virus; CAD, coronary artery disease; CLL, chronic lymphocytic leukemia; BMD, bone mineral density; B12, serum vitamin B12 levels; HP, *Helicobacter pylori*; AA, alopecia areata.
[a]Loci with more than one independent association. [b]Loci that previously showed suggestive association and replication[1] but not at genome-wide significance. [c]*IL18RAP* and *CARD9* associations were reported[7], but not previously at genome-wide significance. [d]Association in the opposite direction in different traits. [e]Loci previously reported at genome-wide significance in GWAS published subsequent to design of the current replication experiment[5,6].

Appendix B

Case Report Form 3 for study investigating the role of MRI in perianal CD (see chapter 4).

MRI in Perianal Crohn's Disease Case Report Form 3
Operative Assessment and Treatment

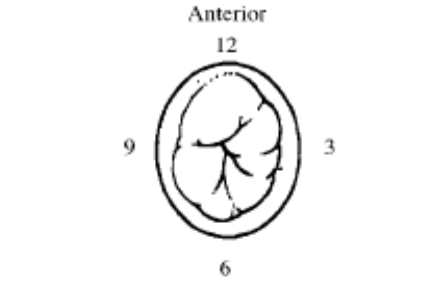
Patient ID: _____

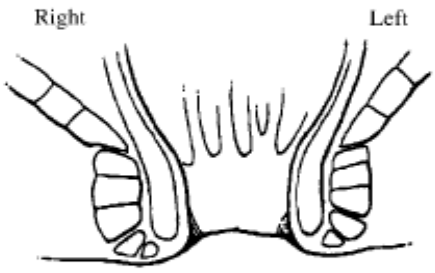
Date: _____

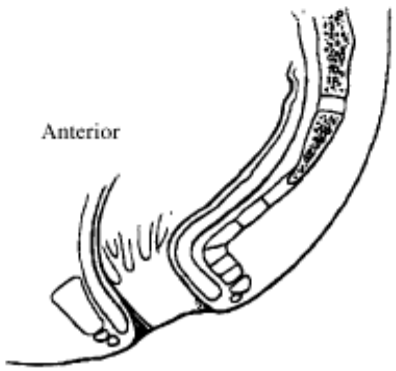
Assessor: _____

3.1 Findings (Blinded to MRI):

#1 Draw and number each fistula







#2 Classify each numbered fistula

		1	2	3	4	5	6	7	8	9	10
Primary Track											
Superficial											
Intersphincteric											
Transsphincteric											
Suprasphincteric											
Extrasphinteric											
Internal Opening (Patient lying on back)											
Site											
Level (*With respect to the rectum*)	below										
	at										
	above										
External Opening(s)											
Number											
Sites (o'clock)											
Horse-shoeing											
Intersphincteric											
Infralevator											
Supralevator											
Abscess											
Superficial											
Supralevator											

MCxxxx

3.2 Proposed Management

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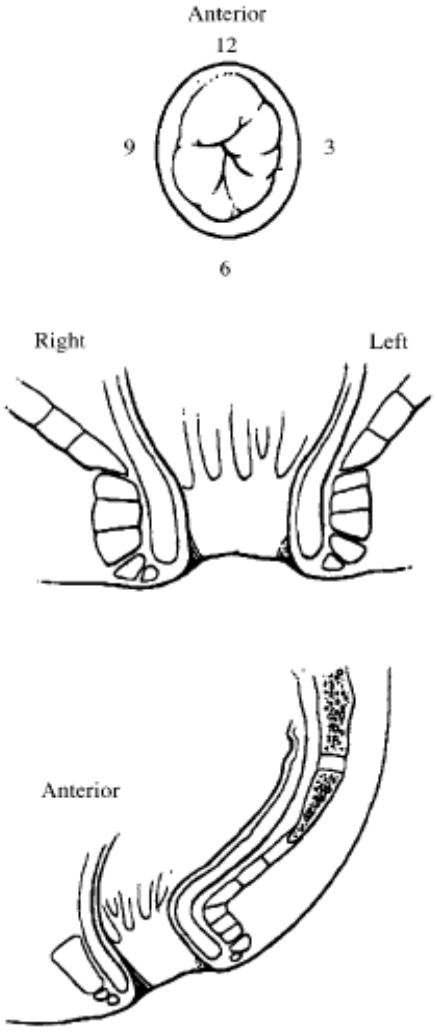
_____

3.3 Findings (Unblinded to MRI):

#1 Draw and number each fistula



#2 Classify each numbered fistula

	1	2	3	4	5	6	7	8	9	10
Primary Track										
Superficial	–	–	–	–	–	–	–	–	–	–
Intersphincteric	–	–	–	–	–	–	–	–	–	–
Transsphincteric	–	–	–	–	–	–	–	–	–	–
Suprasphincteric	–	–	–	–	–	–	–	–	–	–
Extrasphinteric	–	–	–	–	–	–	–	–	–	–

Internal Opening (Patient lying on back)

		1	2	3	4	5	6	7	8	9	10
Site		–	–	–	–	–	–	–	–	–	–
Level *(With respect to the rectum)*	below	–	–	–	–	–	–	–	–	–	–
	at	–	–	–	–	–	–	–	–	–	–
	above	–	–	–	–	–	–	–	–	–	–

External Opening(s)

	1	2	3	4	5	6	7	8	9	10
Number	–	–	–	–	–	–	–	–	–	–
Sites (o'clock)	–	–	–	–	–	–	–	–	–	–

Horse-shoeing

	1	2	3	4	5	6	7	8	9	10
Intersphincteric	–	–	–	–	–	–	–	–	–	–
Infralevator	–	–	–	–	–	–	–	–	–	–
Supralevator	–	–	–	–	–	–	–	–	–	–

Abscess

	1	2	3	4	5	6	7	8	9	10
Superficial	–	–	–	–	–	–	–	–	–	–
Supralevator	–	–	–	–	–	–	–	–	–	–

MCxxxx

3.4 Actual Management

_____

_____

_____

3.5 Discordance and Management Change

Discordance between blinded EUA and MRI?	**Y**	**N**
Discordance resolved on unblinded EUA?	**Y**	**N**
Change in Management produced by MRI?	**Y**	**N**

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