

**RADIOLOGICAL, CLINICAL AND LABORATORY BASED
STUDIES IN THE PATHOGENESIS OF DESMOID TUMOURS
IN FAMILIAL ADENOMATOUS POLYPOSIS**

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Declaration of Originality

I hereby declare that this thesis has been composed by myself, and has not been previously submitted to any other university. The work of this thesis is a record of my own work, and I have acknowledged all contributors and collaborators (please see appendix A1).

The work described herein was carried out between August 2010 and August 2012 during my appointment as a Clinical Research Fellow at the Polyposis Registry, St Mark's Hospital, Harrow, UK.

Santosh Bhandari

March 2013

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Abstract

Around 10% of desmoid tumours (DT) in familial adenomatous polyposis (FAP) patients grow relentlessly, causing major morbidity and mortality. Radiological, clinical or cellular predictors of aggressive DT are yet to be identified. The work described in this dissertation aimed to identify such predictors by radiological studies, and to develop management guidelines and identify possible targets for therapy.

Nine FAP patients with DT underwent 64 multi detector computed tomography (64-MDCT), 1.5 Tesla magnetic resonance imaging (1.5T MRI), Diffusion Tensor Imaging (DTI), Dynamic Contrast Enhanced MRI (DCE-MRI) and Fluorine-18 Flurodeoxyglucose Positron Emission Tomography (^{18}F -FDG PET CT). Direct comparison between 1.5T MRI and 64-MDCT found that MRI was at least equivalent to MDCT in the assessment of DT. DTI was feasible and detected an anisotropic diffusion, which was less directional than in muscle. Metabolic (FDG PET) and vascular imaging (DCE-MRI) of DT demonstrated a spectrum of findings and found that DT were generally hypovascular and poorly glucose avid.

A review of nine FAP patients with intra-abdominal desmoid (IAD) and air-fluid level (AFL) on cross sectional imaging found that either a direct communication or translocation of microorganism from bowel led to the development of AFL. Some of these patients could be treated successfully with antibiotics and/or a percutaneous drain; however, the majority ultimately required complex surgery.

Full sequence *APC* mutation analysis performed in three primary DT cell lines detected 'second hit' somatic mutation in only one, confirming the establishment of a true DT cell line. A study of choline metabolism using choline kinase alpha (*CHKA*) as a marker for activation, however, failed to demonstrate up regulation of choline metabolism in desmoid tumour cells. Studies of the Wnt signalling pathways confirmed the activation of canonical Wnt signalling in DT. Interestingly Wnt11, a non-canonical Wnt, was also up regulated in DT.

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List of Abbreviations

AARs	amino acid repeats
ADC	apparent diffusion coefficient
AEBS	antiestrogen binding sites
AFL	air-fluid level
AIF	arterial input function
AMP	adenosine monophosphate
APC	adenomatous polyposis coli protein
<i>APC</i>	the human adenomatous polyposis coli gene
<i>Apc</i>	the murine homologue of <i>APC</i>
APS	ammonium persulphate
AWD	abdominal wall desmoid
bp	base pair
bh	breath holding
BSA	bovine serum albumin
CC	cubic centimeter
CDP	cytidine 5'diphosphocholine
cDNA	complementary DNA
ChoK	choline kinase
CHRPE	congenital hypertrophy of the retinal pigment epithelium
COX-2	cyclo-oxygenase 2
CT	computed tomography
DAB	3,3'-diaminobenzidine
DAG	diacylglycerol
DAPI	4',6-diamidino-2-phenylindole
DCE	dynamic contrast enhanced
ddH ₂ O	double distilled water
DMEM	dulbecco's modified eagle medium
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DPL	desmoid precursor lesion

DT	desmoid tumour
DTI	diffusion tensor imaging
DWI	diffusion weighted imaging
EAD	extra-abdominal desmoids
ECF	entero cutaneous fistula
ECL	enhanced chemiluminescence
EDTA	ethylenediaminetetraacetic acid
EES	extra vascular extra cellular space
ER	oestrogen receptors
EtOH	ethanol
FA	flip angle
<i>FA</i>	fractional anisotrophy
FAP	familial adenomatous polyposis
FBS	foetal bovine serum
FCS	fetal calf serum
FDG	fluorodeoxyglucose (2-deoxy-2- ⁽¹⁸⁾ F)fluoro-D-glucose)
FoV	field of view
GSK	glycogen synthase kinase
HIFU	high intensity focused ultrasound
H ₂ O ₂	hydrogen peroxide
HRP	horseradish peroxidase
HSP	heat shock protein
IAD	intra-abdominal desmoid
IRA	ileo-rectal anastomosis
IAUGC	integrated area under gadolinium curve
IC	immunocytochemistry
IH	immunohistochemistry
JNK	jun N- terminal kinase
Kb	kilobase
Kv	kilovoltage

LOH	loss of heterozygosity
mA	milliampere
MAb	monoclonal antibody
MBq	megabecquerel
MCR	mutation cluster region
MDCT	multi-detector computed tomography
MeOH	methanol
mM	millimolar
MRI	magnetic resonance imaging
MSC	mesenchymal stem cells
mSv	millisieverts
NaOAc	sodium acetate
NEX	number of excitations
NMR	nuclear magnetic resonance
NSAID	non-steroidal anti-inflammatory drug
OD	optical density
PA	phosphatidic acid
PAb	polyclonal antibody
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PC	phosphatidylcholine
PCho	phosphocholine
PE	phosphoethanolamine
PET	positron emission tomography
PIC	protein inhibitor complex
PLD	phospholipase D
PPIA	peptidylprolyl isomerase A gene
qPCR	quantitative RT-PCR
RA	relative anisotropy
REC	research and ethics committee

RFA	radiofrequency ablation
RIPA	radio-immunoprecipitation assay
RNA	ribonucleic acid
ROI	region of interest
RPC	restorative proctocolectomy
rpm	revolutions per minute
RT-PCR	real time polymerase chain reaction
SBM	small bowel mesentery
sd	standard deviation
SDS	sodium dodecyl sulfate
SI	signal intensity
SMA	smooth muscle actin
ST	slice thickness
STIR	short tau inversion recovery
SUV	standardized uptake value
TBST	tris-buffered saline with tween
TC	tissue culture
TCF	t cell factor
TE	time to echo
TI	time to inversion
TPC	total proctocolectomy
TPN	total parenteral nutrition
TR	time to repetition
USS	ultrasonography
VOI	volume of interest
VRE	vancomycin resistant E coli
VIBE	volumetric interpolated breath-hold examination
WB	western blot
Wg	wingless

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PART 1 - BACKGROUND

Chapter 1

Introduction

1.1 Familial Adenomatous Polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant colorectal cancer predisposition syndrome caused by inheritance of or a new mutation in the adenomatous polyposis coli (*APC*) tumour suppressor gene. Population prevalence of FAP is between 1 in 7,500 and 1 in 13,000 and the disease penetrance nearly reaches 100 percent by the age of 40 years (Bisgaard et al, 1994).

FAP predisposes to development of numerous adenomatous polyps in the colorectum, which inevitably progress to carcinoma if left untreated. However, other parts of gastrointestinal tract such as the stomach and duodenum are also involved in FAP. Gastric lesions seen in FAP are usually fundic gland polyps without malignant potential (Wu et al, 1998). Adenomatosis of duodenum, particularly involving the ampullary region is seen in nearly ninety percent of FAP patients, however, only ten percent of these progress to periampullary cancer (Clark et al, 2009).

1.1.1 The *APC* gene and APC Protein

In 1982 Herrera et al first described an interstitial deletion of 5q chromosome in a mentally retarded patient with multiple developmental abnormalities and colorectal polyposis. Bodmer et al later localized the gene to chromosome 5 near bands 5q21-5q22 in FAP patients (Bodmer et al, 1987). Two different groups working independently subsequently identified the mutation in the Adenomatous Polyposis Coli (*APC*) gene as the cause of FAP (Grodin et al, 1991; Kinzler et al, 1991).

APC is a large gene and consists of 8535 bp spanning 21 exons (Fernhead et al, 2001). Over 75 percent of the coding sequence of the *APC* gene is contributed by exon 15 which is the also the region in which the majority of germline and somatic mutations occur (Beroud et al, 1996).

APC encodes a 2843 amino acid protein, which is broadly expressed throughout the human body. APC protein is implicated in wide variety of cellular processes including cell migration, cell adhesion, chromosome segregation, spindle assembly, apoptosis and neuronal differentiation (Hanson et al, 2005). Despite these many roles, the classic function of APC in neoplasia is inhibition of Wnt signalling pathway. Details of the Wnt signaling pathway are described in the section on desmoid tumour (1.2.3).

APC primarily works as a negative regulator of neoplasia. A germline mutation in the *APC* gene predisposes patients with FAP to develop colorectal polyposis. A second hit somatic mutation in the other allele of *APC*

results in the intestinal tumourigenesis and desmoid development, a feature consistent with Knudson's two-hit hypothesis (Miyoshi et al 1992; Miyaki et al, 1994). The likelihood and form of somatic mutation is determined by the precise location of germline mutation of *APC* (Lamlum et al, 1999).

1.1.2 Genotype-Phenotype correlation in FAP

A genotype-phenotype correlation in FAP is well documented. An *APC* mutation around codon 1300 is shown to be associated with severe phenotype, the patient having a high polyp burden and increased risk of colorectal cancer at early age (Groves et al, 2002). Clustering of somatic mutations has also been demonstrated in the *APC* gene, with more than 60 per cent clustered in an area corresponding to less than ten percent of the coding region, also called the mutation cluster region (MCR) (Miyoshi et al, 1992). This region corresponds to area between codons 1250 and 1450 on exon 15 of the *APC* gene, and is also a common site of germline mutations. Germline mutations 5' of codon 160 and 3' of codon 1450 are associated with an attenuated colorectal phenotype, with later onset of more scanty adenomas, usually less than 100 in numbers (Will et al, 2008).

Similar genotype-phenotype correlation has been described in some extracolonic manifestations of FAP. A far 3' *APC* mutation predisposes to desmoid development and is associated with a higher incidence of sebaceous cyst, osteoma and more severe upper gastrointestinal polyposis (Nieuwenhuis et al, 2008; Wallis et al, 1999). Figure 1.1 demonstrates some of the common genotype - phenotype correlations in FAP.

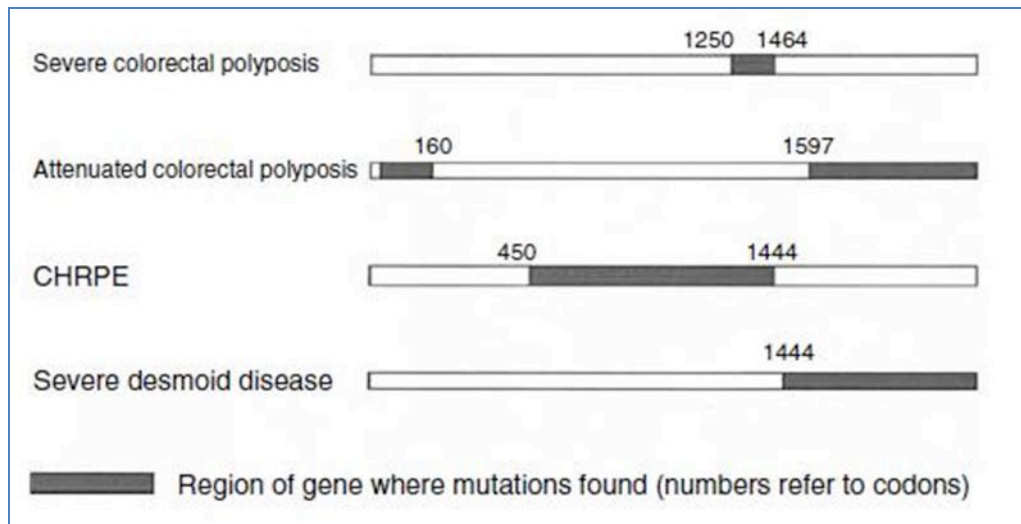


Fig 1.1 – *APC* genotype-phenotype correlations in FAP (Adapted from Church J. 'Hereditary colorectal cancer' Chapter in The ASCRS Textbook of Colon and Rectal Surgery)

1.1.3 Extra-colonic manifestations of FAP

The broad expression of *APC* gene throughout human body and its multiple functions makes FAP truly a multi-system disorder. Increased incidence of hepatoblastoma and medulloblastoma have been reported in FAP patients (Kingston et al, 1983; Kropilak et al, 1989). Similarly, extracolonic cancers such as of gall bladder, thyroid, pancreas and adrenal gland have also been described (Bombi et al, 1984; Giardiello et al, 1993; Painter et al, 1985). Benign extracolonic manifestations associated with FAP include isolated or multiple osteomas of the mandible and skull, epidermoid cysts of the skin, dental abnormalities including accessory teeth, and congenital hypertrophy of retinal pigment epithelium (CHRPE). Desmoid tumours are benign but locally invasive tumours associated with FAP and are discussed in detail in section 1.2.

1.1.4 Clinical management of FAP

FAP predisposes to the development of multiple colorectal adenomas with inevitable progression to cancer. Therefore the current management of FAP aims to offer prophylactic surgery. Majority of the FAP patients are currently managed in institutions with dedicated polyposis registries. Studies have shown such management approach improves the prognosis of patients while compared to those who are treated outside the registry (Bulow et al, 1996). Registering individuals and families in the dedicated registries allows for accurate tracing and better screening of non-index cases.

Members of known FAP family are first offered genetic screening test to detect *APC* mutation. Such screen detected FAP patients undergo prophylactic surgery, either colectomy with ileo-rectal anastomosis (IRA) or restorative proctocolectomy (RPC), during their late teens or early twenties. Patients with a MCR mutation (codon 1250 to 1450), 500 or more colonic polyps or twenty or more rectal polyps at diagnosis are usually offered RPC. Patients with milder phenotype of FAP (germline mutation 5' of codon 160 or 3' of codon 1450) and low rectal polyp burden are usually offered IRA. However, it has been suggested that the penetrance for colorectal cancer is less than 100 per cent in the latter group of patients and some of these patients may be managed endoscopically, without the need for prophylactic surgery (Sinha et al, 2010).

Following surgery, patients are regularly checked for polyp development in the rectum (IRA) or ileal pouch (RPC) with six monthly and yearly flexible

endoscopy respectively. Polyps detected during surveillance are usually treated with endoscopic ablation therapy. Pharmacological agents, particularly NSAIDs (Celecoxib, Sulindac) have also been shown to have a role in delaying polyp progression. Surgery is primarily reserved for increased polyp burden and unfavorable histology findings.

Gastric polyps found in FAP are usually non pre-malignant and do not pose clinical issue. However, management of duodenal adenomas, which can progress to cancer, is challenging and based on the Spigelman scoring system (Spigelman et al, 1989). This system classifies duodenal polyposis into four stages based on polyp number, maximum polyp size, degree of dysplasia and polyp histology. Stage I and II disease is managed with endoscopic surveillance alone while stage III disease is usually managed with therapeutic endoscopy. Duodenectomy is generally considered for stage IV disease.

1.2 Desmoid Tumour

1.2.1 Nomenclature and epidemiology

Desmoid tumours (DT) are mesenchymal tumours originating from the musculoaponeurotic structures throughout the body. They can occur sporadically or in association with FAP. The term 'desmoid' was coined from Greek word 'desmos', which means tendon like. Various other terminologies such as aggressive fibromatosis, deep fibromatosis and musculoaponeurotic fibromatosis have been used to describe these tumours in the past.

Desmoid tumours are rare and account for only 0.03% of all neoplasm (Caspari et al, 1995). The overall estimated annual incidence of desmoid is 2-5 per million population (Reitamo et al, 1986). However FAP patients are about 1000 time more likely to develop these tumours compared to the general population (Gurbuz et al, 1994) and the lifetime incidence of desmoid tumour in FAP patients is between 10 to 25 percent (Bertario et al, 2001).

1.2.2 Desmoid in FAP

Desmoids are locally infiltrative tumours but, unlike cancer, do not metastasize distantly. In post-colectomy FAP patients, desmoids are the leading cause of morbidity and mortality (Clark et al, 1999). In a series of studies published from St Mark's Hospital, an Italian registry and the Cleveland Clinic, desmoids have ranged from the second to the third commonest cause of death in FAP patients (Nugent et al, 1993; Arvanitis et al, 1990; Bertario et al, 1994). Additionally these tumours affect patients at a young age, with an average age of death reported to be 35 years in these studies. Figure 1.2 shows a large desmoid tumour in a patient with FAP.

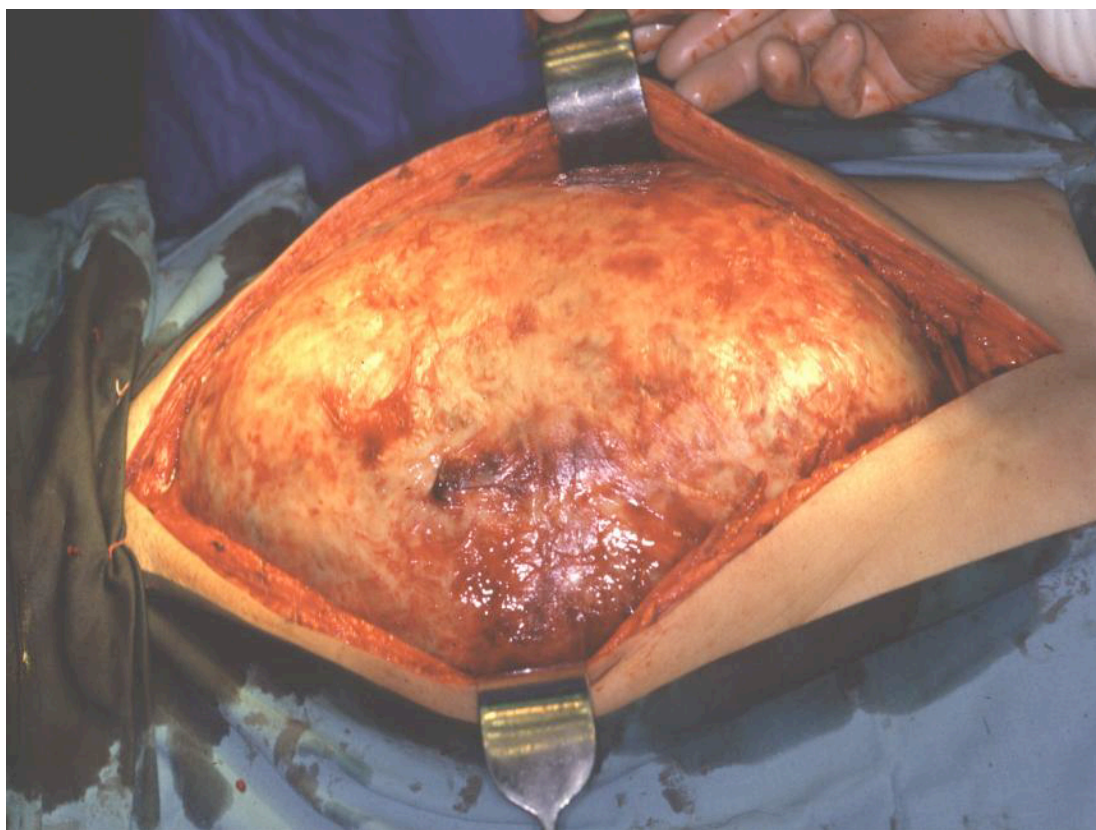


Fig 1.2 – Intra-operative image of desmoid tumour in FAP (Image courtesy of Professor Robin Phillips, St Mark's Hospital)

FAP associated desmoids are genetically and biologically different from sporadic desmoids; FAP desmoids have mutation in the *APC* gene but the majority of sporadic desmoids have mutation in the gene encoding β -catenin (CTNNB1) (Lazar et al, 2008). Sporadic desmoids frequently occur in extremities and along the axial skeleton whereas FAP associated desmoids show propensity for intra-abdominal sites, especially the small bowel mesentery, and abdominal wall. About 70% of FAP associated desmoids are intra-abdominal.

The clinical course of desmoid tumours is variable: 50% show a stable course, 30% exhibit cycles of growth and regression, 10% resolve spontaneously and

10% progress rapidly (Church J, 1995). The rarity of the tumour coupled with its variable course makes meaningful research into desmoid tumour vary difficult. Many studies described in the literature have combined both sporadic and FAP associated desmoids to increase the sample size. However, all the studies described in this report are purely based on FAP related desmoid.

1.2.3 The Wnt signalling pathway and desmoid tumour

The principle role of *APC* as tumour suppressor is the regulation of the Wnt signalling pathway. Wnts are a family of secreted glycoproteins that modulate cell growth, differentiation and morphogenesis during development. However, abnormal activation of Wnt signalling also leads to cancer development (Sugimura and Li 2010).

The Canonical Wnt signalling pathway has been extensively studied. Its primary role is stabilisation of cytosolic β -catenin. In the absence of Wnt signalling, normal APC forms a protein complex with AXIN and GSK-3b and promotes degradation of β -catenin (Brenz 2002). Binding of Wnt on the cell surface disrupts the β -catenin degradation complex resulting in the accumulation of β -catenin in the cytoplasm. The β -catenin molecules subsequently move to the nucleus and activate Wnt pathway transcription factors (Moon et al, 2004).

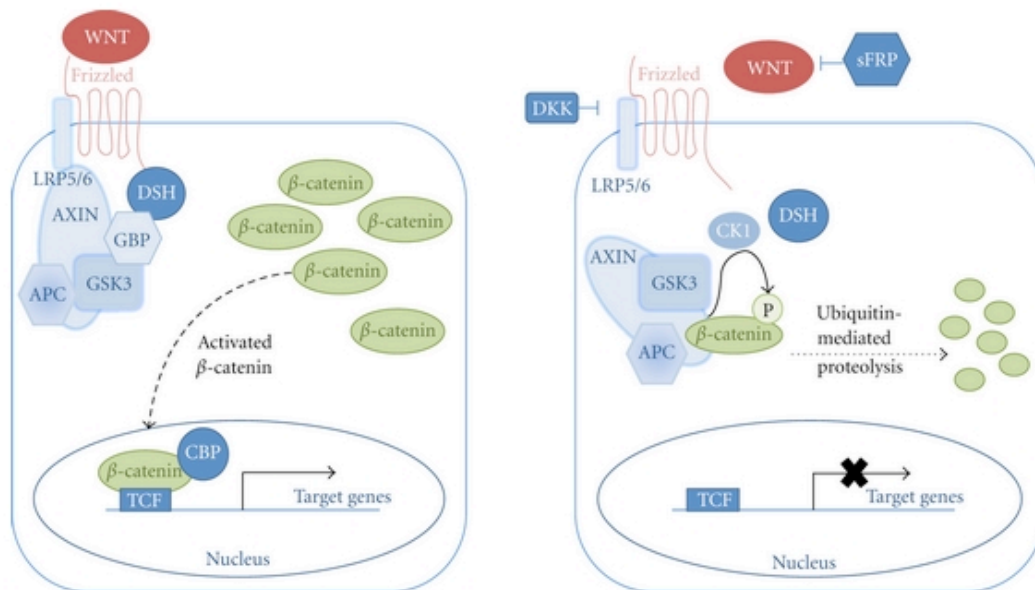


Fig 1.3 - Diagram depicting the canonical Wnt signalling pathway

APC and β -catenin mutations (*CTNNB1*) have been analysed in both sporadic and FAP associated desmoid tumours. Most FAP associated desmoid tumour show a second hit somatic mutation of the *APC* gene. Prior studies of the secondary somatic mutations that occur in colonic adenomas from FAP patients revealed that the type and location of the somatic mutation were nonrandom and at least partially determined by the location of the germ-line mutation (Lamlum et al, 1999). The *APC* gene product contains seven 20 amino acid β -catenin degradation repeats (AARs). These repeat segments permit binding of β -catenin leading to its ultimate degradation. The 'just right' model of FAP tumorigenesis proposes that there is an ideal level of β -catenin binding suitable for adenoma progression to colon cancer, and selective pressure results in nonrandom selection of somatic mutations resulting the appropriate number of AARs (Albuquerque et al, 2002). Analysis of FAP associated desmoid tumours by Latchford et al revealed that

87% (26/30) of tumours had one allele with no AARs and preferentially retained a total of two AARs 57% (17/30) (Latchford et al, 2007). These authors suggested that specific levels of β -catenin activity are required by the different tumour types, with desmoids preferentially requiring two AAR segments. A large Japanese study (86 colorectal tumours, 40 extracolonic tumours) identified similar associations between AARs and phenotype. With respect to FAP associated desmoid tumours, 5 of 6 were found to have two AARs in the Japanese study (Miyaki et al, 2008).

The majority of sporadic desmoid tumours harbour β -catenin mutation producing stabilized product not degradable by the APC, AXIN and GSK-3 β complex. Due to abnormal APC or β -catenin products resulting from these mutations, canonical Wnt signalling is thought to be constitutively activated in desmoid tumours. Increased β -catenin protein and β -catenin binding to TCF-3 have been described in sporadic desmoid tumours (Tejpar et al, 2001).

Non-canonical Wnt signalling pathway is mediated by specific Wnt ligands and is independent of β -catenin. Apart from its role of antagonising canonical Wnt signalling, the non-canonical Wnt pathway is thought to regulate cellular motility and polarity. Further description of these pathways and laboratory based studies to establish activation of these pathways in a cell line from FAP associated desmoid tumour is detailed in chapter 10.

1.2.4 Cell of origin

Considerable debate existed for long time regarding whether desmoids represented simply a reactive process similar to wound healing or whether they were true neoplasms. Two studies in late 1990's have firmly confirmed that desmoid is a clonal proliferation and hence represents a true neoplasm (Li et al, 1996; Middleton et al, 2000).

Although now agreed as a neoplastic process, the cell of origin of desmoids still remains unidentified. Using an animal model Wu et al recently demonstrated that mesenchymal stem cells (MSC) and desmoids had similar gene expression profiles, and mice deficient in MSCs but prone to desmoids (mice with *APC* mutation and deficient MSC production) developed fewer desmoid tumours while gastrointestinal adenoma rates were unaffected. In fact, desmoid development in the mouse was directly proportional to the number of MSCs present. Additionally, MSCs with the *APC* mutation from heterozygote *Ap^c^{wt/1638N}* mice produced DTs when transplanted to immunodeficient mice, but MSCs without mutation did not. Furthermore they found that MSCs from mice with inducible expression of β -catenin (*Catnb^{tm2kem}* mice) could also induce desmoid-like tumours when transplanted to immunodeficient mice. Finally, they showed that these tumours were clonally derived from the donor MSCs with use of a green fluorescent protein tag (Wu et al, 2010).

In another study, Carothers et al analysed 16 human desmoid tumours (8 FAP associated desmoids and 8 sporadic desmoids) using immunohistochemistry

and found that desmoid tissue expressed MSC markers but surrounding normal tissue did not. They next developed a primary desmoid cell line from the human desmoid tissue (FAP associated desmoid) and showed that the cells had an immunohistochemical profile consistent with MSC, and were able to differentiate into chondrocytes, osteocytes, and adipocytes, confirming that they were MSCs. These human desmoid derived MSCs were found to have elevated β -catenin in the nuclei (similar to desmoid tissue) and demonstrated up-regulation of Notch and Hedgehog pathways (Carothers et al, 2012). Although these studies do not definitely prove that the MSCs are the cell of origin of desmoid tumour in FAP, they do demonstrate the importance of MSCs in desmoid development.

Many other studies have shown that majority of the cells in desmoid tumours are myofibroblasts (Kiryu et al, 1985; Gabbiani, 1996). Using primary cell lines established from desmoid tumours from FAP patients, Sinha et al demonstrated ubiquitous expression of cytoplasmic α -SMA in these cell lines, further suggesting myofibroblastic nature of these cells (Sinha et al, 2010).

1.2.5 A model for desmoid development

In 1998 Clark et al proposed a model for intra-abdominal desmoid tumour development and progression, which is analogous to the adenoma-carcinoma sequence in colorectal cancer. According to this model, a plaque like area of peritoneal thickening, 'Desmoid Precursor Lesion (DPL)' which is histologically compatible with desmoid can form in FAP patients due to abnormal fibroblast function. These DPL may progress to 'mesenteric

fibromatosis' (perhaps in some cases by surgical trauma) causing puckering and tethering of small bowel. In some patients this fibromatosis subsequently progresses to solid mesenteric desmoid tumour (Clark et al, 1998). This model was subsequently validated by Middleton et al using a CT scoring system in asymptomatic patients with FAP. This study demonstrated higher rate of mesenteric fibromatosis and desmoid formation in patients with previous DPL in comparison to control subjects (Middleton et al, 2003).

1.2.6 Desmoid tumour pathology

Macroscopically, desmoids tend to be large firm tumours with a coarse trabeculated surface. They may appear to be scar-like and encapsulated but frequently infiltrate adjacent tissue structures. Histologically, desmoids are characterised by mature highly differentiated fibroblast in an abundant collagen matrix containing blood vessels. The fibroblasts have a propensity to concentrate at the periphery of the lesion, and the cellularity is low. There are usually few mitotic figures and necrosis is usually absent.

1.2.7 Anatomical distribution of desmoid tumour in FAP

Desmoid tumours can arise at any site but more often arise in musculoaponeurotic structures. According to site of origin, these tumours can be divided into intra-abdominal desmoids (IAD), abdominal wall desmoids (AWD) and extra-abdominal desmoids (EAD). While sporadic desmoids occur primarily in extra-abdominal sites, the FAP desmoids show propensity to develop intra-abdominally and in abdominal wall.

1.2.7.1 Intra-abdominal desmoids

Fifty to seventy percent of FAP desmoids are intra-abdominal primarily originating from the small bowel mesentery (Clark et al, 1999). Other intra-abdominal sites described include transverse mesocolon, retroperitoneum and ligamentum teres (Burke et al, 1990). Due to their infiltrative nature, IAD are likely to cause mesenteric ischaemia, small bowel obstruction or perforation and ureteric obstruction. IAD are the major cause of morbidity and mortality in FAP patients with desmoid disease. Occasionally these tumours present with an air-fluid level on cross sectional imaging. A study into this rare presentation of IAD is detailed in chapter 6.

1.2.7.2 Abdominal wall desmoids

Abdominal wall desmoids are less problematic than IAD and are usually amenable to surgical excision, although have a high recurrence rate of over 40%. AWD account for around 15% of desmoid tumours in FAP.

1.2.7.3 Extra-abdominal desmoids

These include tumours occurring outside the abdominal wall and abdominal cavity and account for about 15% of FAP associated desmoid. Common sites include the musculoaponeurotic planes of thoracic wall, the back and paraspinal muscles. EAD developing in the breast, head and neck region and palm of hands have been described in the literature.

1.2.8 Risk factors for desmoid in FAP

The precise aetiology of FAP associated desmoids remains unknown. However various risk factors have been described in the literature. The implicated risk factors in desmoid include hormonal influences, surgical trauma, genetic predisposition and family history.

1.2.8.1 Hormonal influence

The female sex hormone oestrogen has long been implicated in pathogenesis of desmoid tumour. This stems largely from studies in sporadic desmoid tumour, where females of childbearing age were found to have the highest incidence of tumour (Reitamo et al, 1982). Other studies have demonstrated high levels of oestrogen receptors (ER) and antiestrogen binding sites (AEBS) in desmoid tumours (Sutherland et al, 1980; Lim et al, 1986).

More recent studies have found variable effects of sex steroids in FAP associated desmoid. A study by Church et al found that pregnancy ameliorated the course of IAD significantly while compared to non-pregnant female controls (Church JM and McGannon E, 2000). In contrast to this study, Nieuwenhuis et al found an increased risk of AWD formation following pregnancy (Nieuwenhuis et al, 2008). A few other studies have failed to correlate a change in desmoid status with pregnancy (Clark et al, 1999; Mulik et al, 2003).

A recent meta-analysis combining seven studies evaluating over 2500 FAP patients with 388 desmoid tumours, however, found that females were one and half times more likely to develop desmoids compared to male patients (Sinha et al, 2010).

1.2.8.2 Trauma

Excessive adhesions and uglier scars following similar surgery have long been observed in patients with FAP (Lockhart-Mummery, 1967). Similarly, desmoids have been associated with many surgical wounds, including the sites of laparotomy, thoracotomy, laparoscopy and cesarean section.

A study by Clark et al, looking for the presence of 'desmoids precursor lesions' in the mesentery of FAP patients undergoing laparotomy, detected such lesion in many patients without prior abdominal surgery. These lesions, however, were more advanced resulting in 'mesenteric fibrosis' in patients who had prior laparotomy. The author thus concluded that the trauma of surgery might have caused a precursor lesion to advance in stages to become a true tumour (Clark et al, 1998). A review of studies by same author further noted that over 70% of abdominal wall and intra-abdominal desmoids in FAP patients occurred after abdominal surgery with the majority occurring within first five years (Clark et al, 1996). In a recent meta-analysis of FAP patients with desmoid tumour, Sinha et al reported a threefold increased risk for developing desmoid tumour in FAP patients who had undergone previous abdominal surgery compared to those without previous operation (Sinha et al, 2010).

While the majority of the evidence suggests that FAP related desmoids, particularly AWD and IAD, occur following surgery, a study from the Cleveland Clinic has reported detection of IAD in four percent of patients during primary surgery for FAP (Hartley et al, 2004).

1.2.8.3 Genetic predisposition

Development of desmoids among individuals with FAP has long been correlated with specific *APC* mutations. Caspari et al first described that patients with *APC* germline mutation 3' to 1444 had an increased predisposition to desmoid tumour (Caspari et al, 1995). Further studies with small numbers of FAP patients with desmoid tumour suggested that germline mutations in these patients tended to occur at the 3' end of the gene (Dobbie et al, 1996; Wallis et al, 1999). Bertario et al from the Hereditary Colorectal Tumor Registry in Milan analysed 809 FAP patients with 107 DTs and reported a 12-fold increased risk of DT when the *APC* mutation occurred 3' of codon 1444, as compared with upstream mutations (Bertario et al, 2001). They found that genotype was the strongest predictor of desmoid development in a multivariate analysis. In a further study of 320 FAP patients with 50 DT from St Mark's Hospital, Sturt et al reported a sevenfold increase in the odds of presenting with desmoid tumours in patients harbouring germline mutation 3' of codon 1399 (Sturt et al, 2004). In a recently published meta-analysis combining all the relevant previous studies (2098 FAP patients and 300 DT), Sinha et al have reported that a germ line mutation 3' to codon 1399 increased the odds for desmoids development in FAP patients by four times (Sinha et al, 2010).

The normal *APC* gene product contains seven 20 amino acid β -catenin degradation repeats (AARs) which bind and downregulate β -catenin. The number of these AARs remaining in the truncated *APC* protein has been shown to have functional importance, with desmoid development preferentially requiring two AARs segments. A combination of 'first' and 'second' hit mutations is the important factor in providing this optimal level of β -catenin dysregulation. In *APC* gene the codon 1399 lies immediately after the second 20 amino acid β -catenin binding repeat. Thus in patients with germline mutation 3' to codon 1399 loss of heterozygosity (LOH) occurs spontaneously at a relatively high frequency, resulting in a more severe desmoid disease (Sturt et al, 2004).

1.2.8.4 Family history and the role of modifier genes

Eccles et al first described a variant of FAP, termed hereditary desmoid disease, in 1996. They reported 100% penetrance for desmoid tumour in three-generation kindred with mutation at the extreme 3' end of the *APC* gene and argued for a role for modifier gene in this family, as such total penetrance of desmoids is rarely seen in other families with 3' mutation (Eccles et al, 1996). There are also reports of families with 5' germline mutation and a high proportion of members affected with desmoids, further highlighting the role of possible modifier gene in such families (Sturt et al, 2006). Combining two separate studies (Bertario et al, 2001; Sturt et al, 2004) in a meta-analysis, Sinha et al found that a patient with positive family history of desmoids is seven times more likely to develop DT. This risk is independent of the site of

the germline mutation, suggesting a role for modifier genes in desmoid tumour development in such families (Sinha et al, 2010).

1.2.9 Clinical features of desmoid in FAP

Desmoid tumours display a variable clinical course. The majority of tumours may remain stable or even regress, with only ten percent growing relentlessly to cause major morbidity or mortality in FAP patients.

Intra-abdominal desmoid tumours may remain asymptomatic until their growth and infiltration cause visceral compression or vascular compromise. Symptoms of intestinal obstruction or small bowel ischemia due to involvement of the superior mesenteric artery may be the first manifestation of IAD. Retroperitoneal extension causing ureteric compression and hydronephrosis or neural involvement may be another presenting feature. Rarely IAD can present with sepsis with an air-fluid level detected on cross sectional imaging, either due to necrosis of tumour or fistulation into viscera. Fig 1.4 shows an example of IAD with air-fluid level on CT scan.



Fig 1.4 – Intra-abdominal desmoid with air-fluid level (arrow)

Abdominal wall desmoids and extra abdominal desmoids are less troublesome and are usually asymptomatic. They mostly present with complaints of cosmesis. However some AWD may infiltrate into the peritoneal cavity resulting in abdominal symptoms. Similarly EAD may present with functional deficits depending on their anatomical location.

1.2.10 Imaging of desmoid tumours

The majority of desmoids are clinically evident as palpable mass at presentation. Imaging is used primarily to confirm the diagnosis, to establish the relationship of tumour to surrounding structure before surgical excision, to assess tumour progression and monitor for complication.

1.2.10.1 *Computed tomography*

Computed tomography (CT) scanning is currently the standard technique for assessing suspected intra-abdominal desmoid tumours in FAP and has also been widely used to evaluate desmoids at other sites (Healey et al, 1997). At CT, desmoids are seen either as ill-defined or well-circumscribed masses. Without contrast, these tumours show variable attenuation relative to muscle. With contrast, desmoid tumours may or may not appear enhanced. Einstein et al conducted a study on 34 desmoids (17 abdominal wall desmoids, 14 mesenteric desmoids and 3 retroperitoneal desmoids) in 25 patients using contrast enhanced CT scan and reported that more than two thirds of such lesions had well defined borders with the majority showing attenuation equal to or greater than of surrounding muscle (Einstein et al, 1991). Fig 1.5 demonstrates a typical appearance of desmoid tumour on contrast enhanced CT scan.



Fig 1.5 – Contrast enhanced CT scan demonstrating three desmoid tumours (asterisks)

Computed Tomography is routinely performed nowadays using a 64-slice multidetector CT (MDCT) system. This system acquires 64 overlapping 0.6 mm slices per rotation, resulting in great improvement in spatial resolution. CT, however, incurs a high radiation exposure. A study comparing the role of modern CT and MRI in assessment of FAP desmoid is further discussed in chapter 3.

1.2.10.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a well-established imaging modality for investigating soft tissue tumours. It avoids radiation exposure and is currently considered gold standard technique for the diagnosis and prognostication of benign and malignant soft tissue tumours, as well as rectal cancer (Tuncbilek et al, 2005).

In MRI, desmoids are seen as masses of low signal intensity relative to muscle on T1 weight images while signal intensity on T2 weighted image is variable (low, intermediate or high) (Casillas et al, 1991). Fig 1.6 demonstrates typical T1 and T2 weighted images of desmoid tumours in a patient with FAP.

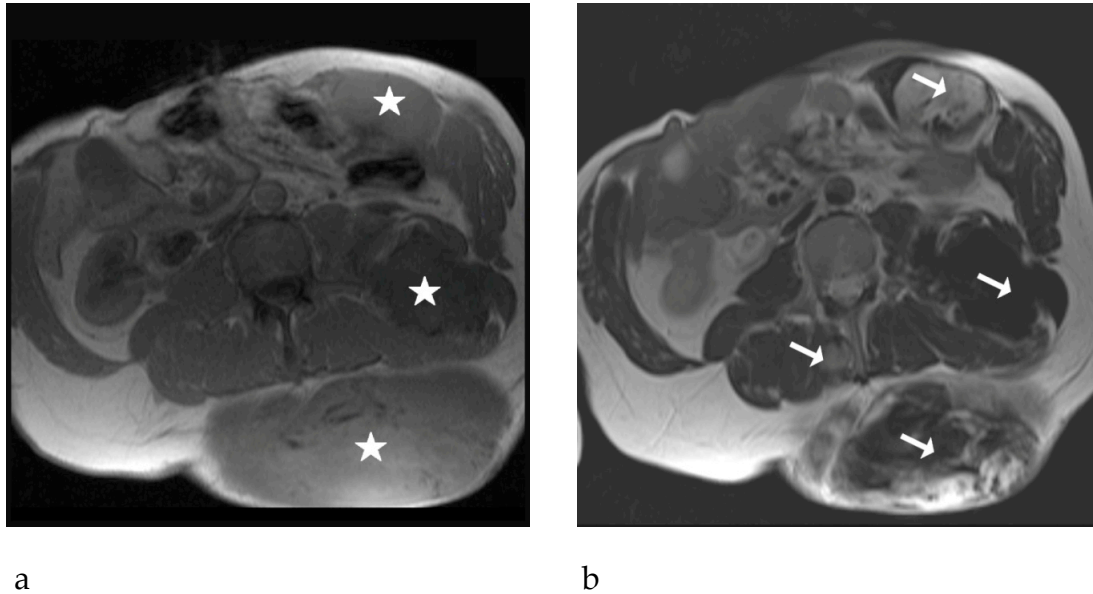


Fig 1.6 - Typical appearances of desmoid tumour in T1 weighted (stars, a) and T2 weighted (arrows, b) MRI. Variable signal intensity of these tumours is quite obvious in the T2 weighted MRI

Healey et al studied 35 FAP associated desmoids using CT and MRI and reported that CT detected more intra-abdominal desmoids compared to MRI and was also more effective in showing the relationship of IAD to the bowel. In their study eight desmoid tumours with high signal intensity on initial T2 weighted images showed significant growth on the follow up scans. Based on this finding they concluded that the high signal intensity on T2 weighted MRI was associated with aggressive desmoid behaviour (Healey et al, 1997).

MRI techniques have significantly improved since the study by Healey et al. Modern day 1.5T MRI provides better soft tissue resolution. Furthermore advanced MRI imaging techniques such as diffusion tensor imaging (DTI), which assesses diffusion of water molecule in the tissue, and dynamic contrast enhanced imaging (DCE-MRI), which assesses vascularity of the tissue, are gaining increasing use in clinical practice. These advanced MRI

techniques provide more functional information and further insight into tumour biology and behaviour. Studies using these imaging modalities to characterise desmoid tumours and assess their behaviour are described in chapters 4 and 5.

1.2.10.3 *Positron Emission Tomography*

Positron emission tomography (PET) is a functional imaging method which, in combination of traditional imaging such as CT or MRI, serves as in vivo method of evaluating metabolism and physiology in normal and diseased tissue (Aoki et al, 2003). ^{18}F 2- Deoxy -2- fluoro-D- glucose (FDG) is the most commonly used radiolabelled tracer for PET and is widely used in oncology practice to detect the recurrence or metastasis of many cancers and also to evaluate the response to therapy (Visioni et al, 2011). FDG–PET is also effective in differentiating between benign and malignant soft tissue tumours (Griffeth et al, 1992).

There are limited reports of use of FDG-PET in assessment of desmoid tumour. Souza et al described a case of chest wall desmoid tumour in 72-year-old female with previous history of esophageal cancer and breast cancer. In this study low level of tracer uptake by the tumour and its well defined appearance helped them to determine the benign nature of the mass preoperatively (Souza et al, 2010). In a study of five patients with desmoid tumours using FDG PET, Basu et al reported that the FDG uptake by these tumours was heterogeneous. In this study, high avidity for FDG uptake was found in an aggressive recurrent desmoid tumour that was subsequently

treated with chemotherapy. The treatment response to chemotherapy was reflected in decreased FDG uptake in repeat PET CT (Basu et al, 2007). Makis et al have recently described a desmoid tumour of the colon mimicking colonic malignancy due to its high FDG avidity on PET CT (Makis et al, 2012). These studies have highlighted a potential role of FDG PET in assessing desmoid tumour aggressiveness, tendency to recurrence and response to therapy. A study combining functional MRI scan and FDG PET to assess biological behavior in FAP desmoids is described later in chapter 5.

Recent years have also seen the increasing use of 18 F fluorocholine based PET in the imaging of different cancers (Mertens et al, 2010). This has stemmed from detection of increased choline metabolism in many cancer cells. Choline metabolism in desmoid tumour has not been studied. Increased choline metabolism in desmoid may provide a foundation for exploring the use of choline based PET in assessment of tumour behaviour. A study of choline metabolism using in-vitro model of desmoid tumour is detailed in chapter 9.

1.2.10.4 Ultrasound scanning in desmoid tumour

Desmoid tumours typically appear as homogenous anechoic or hypoechoic masses on ultrasound scanning. In the management of FAP patients, ultrasound is frequently used to monitor the progression of desmoid disease, particularly for the assessment of hydronephrosis caused by IAD. It is used to help guide the biopsy needle during percutaneous biopsy of AWD and IAD and also for percutaneous drainage of IAD presenting with an air-fluid level

and sepsis. Wang et al have recently described US-guided high intensity focused ultrasound (HIFU) as an effective minimally invasive therapy for local control of extra-abdominal desmoids (Wang et al, 2011).

1.2.11 Treatment of desmoid tumour

Treatment of desmoid tumour is complex and controversial. Following diagnosis, small asymptomatic desmoid tumours may simply be observed with regular clinic follow up, with or without imaging. Treatment is usually warranted for symptomatic patients or patients with rapidly growing tumours. The available options for treatment are pharmacological treatment (non-steroidal anti-inflammatory drugs (NSAIDs) and /or anti-estrogen), chemotherapy, surgical excision and/or radiotherapy. However there is lack of high quality evidence in the literature on which to base these treatments. The natural history of the disease, with some tumours showing spontaneous regression without any treatment, poses additional difficulty for evaluation of efficacy of one particular treatment.

1.2.11.1 *Pharmacologic treatment*

1.2.11.1.1 *Non-cytotoxic drugs*

NSAIDs have long been used in patients with desmoids with varying success. Sulindac, indomethacin and celecoxib are the commonly used NSAIDs. These agents are either used alone or in combination with anti-estrogen. An early case report found that indomethacin used at 100mg/day orally provided a

rapid resolution of the desmoid tumour (Waddell et al, 1980). Tsukada et al used sulindac in 14 patients with a history of FAP and recurrent abdominal desmoid tumour and reported an overall response rate of 57%, however many of these responses were typically delayed by 24 months (Tsukada et al, 1992).

Tamoxifen is the most commonly used anti-oestrogen agent to treat desmoid tumour. Other agents used include toremifene and raloxifene. Hansmann et al conducted a study using high dose tamoxifen (120mg/day) and sulindac (300mg/day) and reported no tumour growth in three out of eight patients with sporadic desmoids and 10 out of 13 patients with FAP associated desmoids (Hansmann et al, 2004). In a recently published systematic review, anti-estrogen therapy was found to be effective in about 50% of patients irrespective of whether the tumours were primary or recurrent, or sporadic or FAP related. Interestingly no added advantage was conferred by combining anti-estrogen with NSAIDs (Bocale et al, 2011).

Other drugs that have been used to treat desmoids include glucocorticoids, interferon alpha, cyclic AMP inhibitors, anti-fibrinolytic agent pirfenidone, and the tyrosine kinase inhibitor iminitab mesylate. However, the true efficacy of NSAIDs, anti-estrogen and other agents used to treat desmoid tumour is difficult to interpret due to variable course of the tumour, small number of patients included in these studies and the lack of randomised control trials.

1.2.11.1.2 *Cytotoxic chemotherapy*

Despite the lack of any randomised trials cytotoxic chemotherapy for desmoid is relatively widely reported in the literature (Patel et al, 1993; Okuno et al, 2003). Both treatments with low-dose chemotherapy using vinblastin and methotrexate and, more commonly, a regimen based on doxorubicin and dacarbazine, have been described. Azzarelli et al have reported a sixty percent stable course with minor tumour shrinkage and symptom relief in thirty patients, after treatment with low doses of vinblastin and methotrexate (Azzarelli et al, 2001). Gega et al have described use of doxorubicin and dacarbazine followed by meloxicam, a cyclooxygenase 2 (COX-2) inhibitor in seven FAP patients with symptomatic unresectable desmoid tumours. Significant tumour regression was observed clinically and radiologically in all seven patients, with three patients showing a complete response. The average progression-free survival period in this study was 74 months (range, 32.5 to 107.5 months) (Gega et al, 2006).

The current practice at our institution is to use cytotoxic chemotherapy as a last resort in patients with inoperable aggressive desmoid disease where non-cytotoxic treatment with a combination of non-steroidal and anti-estrogen has failed.

1.2.11.2 *Surgery for desmoid tumour*

Surgery for AWD and EAD is generally safe, however a high recurrence rate up to 45% has been reported (Heiskanen et al, 1996; Clark et al, 1999).

Nieuwenhuis et al recently reported a 63% progression free survival at ten years following excision of 14 AWD and EAD in FAP patients (Nieuwenhuis et al, 2011).

Surgery for IAD is more problematic. A previous study from St Mark's Hospital reported a perioperative mortality of 36% after excision of IAD mainly due to haemorrhagic complications. Nearly half of the surviving patients in this study needed extensive enterectomy resulting in the need for long-term parenteral nutrition. In addition, the tumour recurred in 71% of these patients (Clark et al, 1999).

The surgical management of desmoid tumour, however, has changed over the last decade and many centres are now adopting a less aggressive surgical approach. More recently a wait and see approach to DTs has been advocated as many DT either show a prolonged stable phase or even spontaneous regression (Calvert et al, 2012).

1.2.11.3 Newer treatment modalities in desmoid tumour

Recently there have been increasing reports of radiologically guided targeting of desmoid tumours. A percutaneous chemical ablation of two desmoids (one intra-abdominal) using acetic acid under radiological guidance was first described by Clark TW. A complete response was achieved in one patient and a partial response was achieved in the other patient; these responses were sustained during two years of follow up (Clark 2003).

Tsz-Kan et al first described the use of radio frequency ablation (RFA) in treating a 47-year-old patient with recurrent desmoid tumour in the lower back. They reported no recurrence of tumour after 28 month using serial follow up MRI (Tsz-Kan et al, 2007). More recently Ilaslan H et al described treatment of five EADs with RFA. None of the treated tumours recurred during a mean follow up of 30 months (range 4-68 months), however one patient developed cellulitis and another soft tissue necrosis following the RFA treatment (Ilaslan et al, 2010).

Kujak et al used percutaneous cryoablation in the treatment of five EAD in five patients including two FAP patients. In three tumours cryoablation was used for local control of disease following recurrence after multiple treatment with different modalities. In two tumours, cryoablation was primarily used to palliate the symptoms of pain caused by proximity or involvement of nerve or vessel by the tumours. All the patients treated for local control of disease had significant improvement in symptoms, with complete resolution of tumours in two patients and moderate decrease in tumour size in a third. Despite some reduction in the size of tumours, no significant symptom relief was achieved in palliative group using this modality of treatment (Kujak et al, 2010).

Ultrasound guided high intensity focused ultrasound treatment for extra-abdominal desmoids has been recently described by Wang et al. They have reported a significant shrinkage (>50% in volume) of all the treated tumour (n=25) during a mean follow up of 30 months (range 8-55 months) (Wang et al, 2011).

All these techniques described above, however, represent experience of individual groups treating small number of desmoid tumours. While these initial results may be promising, further research with larger patient numbers is needed to validate the potential role of any individual techniques in treating desmoids tumours.

Chapter 2

Aims of work described in this thesis

Desmoid tumours remain an outstanding challenge in the management of FAP patients. These tumours show a variable course with only ten percent growing relentlessly causing major morbidity and mortality. However no molecular, pathological or clinical marker of aggressive phenotype has yet been identified. Early identification of the desmoid that may behave aggressively could allow targeting of treatment to such patients early, preventing major morbidity and mortality.

The studies described in this thesis characterise these tumours further and also aim to identify markers of aggressive phenotype by the use of modern imaging modalities. Treatment of aggressive intra-abdominal phenotype of desmoid is reviewed and a management algorithm developed. Laboratory based studies investigate choline metabolism and Wnt signalling pathways using a cell culture model of desmoid tumour.

2.1 Imaging Studies

The first imaging study described in this thesis compares the 64-MDCT to modern day 1.5T MRI for the assessment of FAP desmoids. MRI spares the high radiation incurred by CT and is an important consideration while

imaging young patients with FAP and desmoids. Further study using diffusion tensor imaging (DTI) to evaluate its feasibility and explore its use in the assessment of desmoid tumour is described in a second study. The third study combined the metabolic (PET CT) and vascular imaging (DCE-MRI) and explores their potential role in predicting the behavior of FAP desmoid.

2.2 Clinical study

A clinical review of intra-abdominal desmoids presenting with air-fluid level on cross sectional imaging was carried out. The treatment in the tertiary care centre specialising in FAP desmoids was reviewed and a management algorithm was developed.

2.3 Laboratory studies

An in-vitro desmoid cell model was used for the laboratory studies described in this thesis. The first chapter describes the confirmation of desmoid tumour cell line by 'second hit' mutation analysis in the cells. A study to investigate the abnormal choline metabolism in desmoid tumour cells is described in the second chapter. Finally studies investigating the Wnt signalling pathways in desmoid tumour, by immunocytochemistry, immunohistochemistry, western blot, RT-PCR and qPCR, is described in the third chapter.

PART 2 – IMAGING STUDIES

Chapter 3

A prospective comparison of 64-slice multidetector computer tomography and 1.5-Tesla magnetic resonance imaging in the assessment of desmoid tumours in familial adenomatous polyposis

3.1 Background

Cross-sectional CT is currently used as standard technique for evaluating desmoids in FAP (Sturt et al, 2006). Multi-detector computed tomography (MDCT) utilizes multi-planer reformatting images to accurately localize the site of pathology; 64 overlapping 0.6mm slices per rotation are acquired during 64-MDCT. This advanced technique results in better spatial resolution and elimination of spiral artifacts encountered by helical CT and is currently widely used in practice. However CT incurs a high radiation exposure. The typical effective dose of 64-MDCT is 15.2 millisieverts (mSv), which is equivalent to 760 plain chest X-ray (Baskerville, 2008). Furthermore 0.4% of cancer diagnosed in United States has been attributed to high radiation exposure from CT (Brenner et al, 2007).

Magnetic resonance imaging (MRI), on the other hand, utilizes a strong magnetic field to acquire the images and hence avoids radiation exposure.

Due to its superior contrast to noise ratio, MRI is well established in the differential diagnosis and prognostication of many benign and malignant soft tissue tumour and cancers including rectal cancers (Tuncbilek et al, 2005). Intuitively MRI may provide better assessment of desmoids. Nevertheless, to date there have been few MRI data relating to FAP associated desmoids. Various case reports and small series have described the MRI features of desmoids (Souza et al, 2010; O'Keefe et al, 1990; Chummun et al, 2010; Castellazi et al, 2008), however, many of these combine sporadic and FAP related desmoids to maximise case numbers. Clinically this is less useful as FAP related desmoids differ from sporadic desmoids both in their clinical traits and biology.

3.2 Aims of this study

A previous study from St Mark's Hospital comparing MRI and CT for assessing desmoids in FAP demonstrated that MRI can show both IAD and AWD in FAP patients, but the anatomical imaging by CT was superior. This study also showed high signal intensity on T2-weighted images to be predictive of aggressive desmoid behaviour (Healey et al, 1997). A typical appearance of an IAD diagnosed on MRI by Healy et al in 1996 is demonstrated in figure 3.1. Poor image resolution of this subtle pathology made interpretation difficult at that time.



Fig 3.1 - Typical image of intra-abdominal desmoid diagnosed by Healy et al on MRI in 1997 (image courtesy of Miss Susan K Clark, St Mark's Hospital)

Since this study, both CT and MRI technology and protocols have improved substantially. To the best of our knowledge no studies have compared state of the art MRI and MDCT. The aim of this study was to establish whether modern 1.5T MRI techniques provide equivalent or superior assessment of FAP associated desmoids to 64-slice MDCT.

3.3 Materials and methods

3.3.1 Patient selection

The St Mark's Hospital Polyposis Registry database was searched to identify patients with a diagnosis of FAP, defined by either an identified *APC* mutation or the presence of over 100 colorectal adenomas with no evidence of *MYH* mutation. FAP patients with confirmed desmoid tumour based on previous radiological and clinical evidence were identified and prospectively recruited. All patients underwent abdominal MDCT and MRI, with scans being performed within one month of each other.

3.3.2 Regulatory approvals

Ethical approval for this study was obtained from the Harrow Research and Ethics Committee (REC reference number: 07/H0719/100). Research and development approval and study management approval at St Mark's Hospital and Paul Strickland Scanner Centre, Mount Vernon Hospital sites was obtained from the North West London Hospitals Research and Development Department (R&D reference number: RD7/090). Informed written consent was obtained from all recruited patients.

3.3.3 Image acquisition

MDCT was performed using a 64 MDCT scanner (Brilliance 64, Phillips Healthcare, Eindhoven, The Netherlands). Following IV contrast

administration (100ml at 3mls⁻¹ via a pump injector; Visipaque 270 GE Healthcare, New Jersey, USA) and a delay of 65 seconds, imaging was performed using the parameters summarised in Table 3.1.

MDCT Kv	mA	Rotation Speed	Matrix	FoV	Collimation	ST
120	280	0.75	512	350	64x0.625	2mm

Table 3.1 – Acquisition parameters for MDCT

MRI was performed using a 1.5-Tesla MR scanner (Siemens Avanto, Siemens Healthcare, Erlangen, Germany) and multi-channel multi-element coil system. T1 and T2-weighted axial, coronal and sagittal sequences of the abdomen and pelvis were supplemented by short tau inversion recovery (STIR) and T1-weighted post gadolinium axial sequences. The acquisition parameters are listed in Table 3.2. Respiratory gating was used to reduce movement artifacts caused by breathing, and 10mg of intravenous hyoscine-N-butylbromide (Buscopan; Boehringer Ingelheim, Ingelheim am Rhein, Germany) was used to reduce peristalsis.

1.5T-MRI	TR	TE	FA	TI	ST	NEX	Matrix	FoV
T1	200	4	70		5	1	256/179	70
T2	3180	97	150		5	1	256/179	150
STIR	3800	93	160	150	5	1	256/131	160
T1-post contrast	195	4	70		8	1	256/157	70

Table 3. 2 - Acquisition parameters for MRI

3.3.4 Image analysis

Two experienced gastrointestinal radiologists assessed the MDCT and MRI viewed on standard reporting workstations (Brilliance, Phillips Healthcare; Leonardo, Siemens Healthcare) on separate occasions. In case of discrepancy, consensus was reached via discussion. Abdominal MDCT was used as the gold standard for the identification of all IAD, AWD and EAD on MRI.

3.3.5 Outcomes of interest

For each modality the number of desmoids and desmoid tumour size was documented. The size of desmoids was obtained by automated region of interest analysis and the area recorded in cm². All desmoid lesions were classified by site into IAD, AWD or EAD. IAD incorporated mesenteric desmoids; AWD incorporated desmoids located in anterior and posterior abdominal wall muscles; EAD incorporated desmoids at other sites. For each lesion its margin was noted on both modalities and its relationship to bowel,

urinary tract and vessels, including the proximal superior mesenteric artery, was recorded.

3.3.5.1 *Contrast enhancement ratio*

The enhancement ratio on each modality was calculated for each lesion by dividing the desmoid enhancement by the aortic enhancement. This ratio was compared between the two modalities.

3.3.5.2 *T2 signal intensity*

The signal intensity on T2-weighted images was defined in relation to muscle and water. Low signal intensity was less than or equivalent to that of muscle, intermediate signal intensity was greater than that of muscle but less than that of water, and high signal intensity was greater than or equal to that of water. If a lesion had mixed signal intensity but had a significant proportion of high signal intensity (50% or more), it was considered of high signal intensity.

3.3.6 Statistical analysis

Statistical analysis was conducted using STATA™ SE 10.1 for Macintosh (STATA Corp, Texas, USA). Desmoid tumour size is described as median and range; these were compared using Mann-Whitney U-test. Contrast enhancement ratios obtained by the two modalities were reported as mean value and standard deviation. These proportions were compared using Student's t-test following test of normality. A p-value less than 0.05 was

considered statistically significant.

Desmoids were stratified into two groups based on signal intensity on T2-weighted images, those with high signal intensity and those with low or intermediate signal intensity. The mean MRI contrast enhancement ratio between these two groups was also compared using Student's t-test.

3.4 Results

Nine patients with FAP and desmoid disease underwent MDCT and MRI within one month of each other. The demographic details of the included patients are described in Table 3.3.

Patient	Age at desmoid diagnosis (years)	Gender	APC mutation (codon)	Surgery for FAP
1	26	F	1392	IRA
2	43	M	1465	RPC
3	50	M	1551	RPC
4	22	M	1556	IRA
5	34	M	1551	RPC
6	24	F	1509	RPC
7	32	F	1182	IRA
8	24	F	213	IRA
9	31	F	1444	IRA

Table 3.3 - Demographics of included patients

Twenty-three desmoids (9 IAD, 10 AWD and four EAD) were identified on MRI. Twenty-one desmoids (9 IAD, 9 AWD and three EAD) were identified

on MDCT. MRI identified two additional desmoids, one EAD and one AWD, which were not easily detected even on retrospective review of MDCT (Fig 3.2, Fig 3.3).

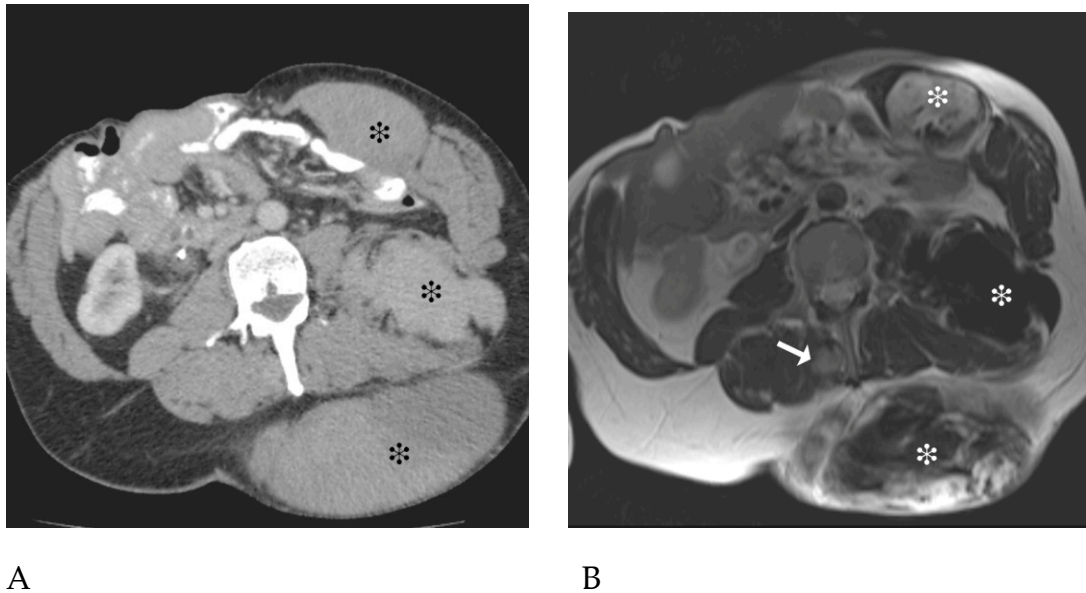


Fig 3.2 - Contrast-enhanced CT (A) and T2 weighted MRI (B) demonstrating multiple desmoid tumours (asterisks). The extra-abdominal desmoid (EAD) shown on MRI (arrow) is difficult to identify on CT

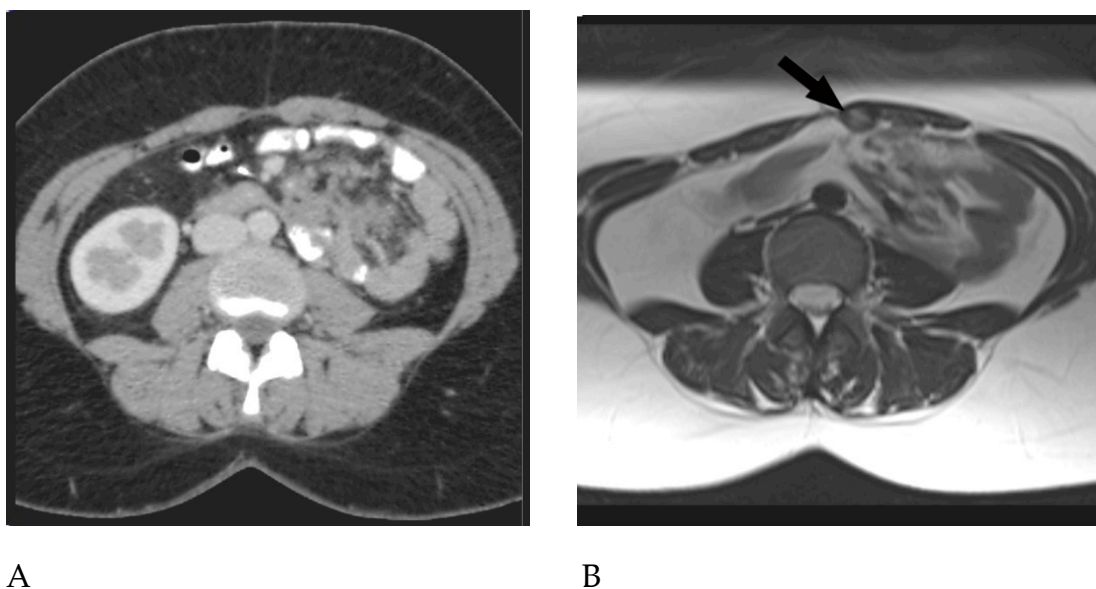


Fig 3.3 - Contrast-enhanced CT (A) and corresponding T2 weighted MRI (B) of abdominal wall desmoid (AWD). This lesion was easily detected on MRI (arrow) and missed on CT

For the 21 lesions seen on both modalities, there was concordance with regard to desmoid size, site and extent of local infiltration margin. The contrast enhancement ratio for MRI was significantly greater than for MDCT. Table 3.4 summarises the results of comparison between the two modalities.

DT	Site	Size (cm²)		Infiltration			T2-signal	Enhancement Ratio	
		CT	MRI	Bowel	Ureter	SMA origin		CT	MRI
1	IA	63	61	Yes	Yes	No	Low	0.47	0.75
2	AW	181	179				High	0.43	1.38
3	IA	79	81	Yes	Yes	No	Low	0.49	1.38
4	IA	11	10	No	Yes	No	Low	0.54	1.12
5	EA	215	215				Low	0.5	1.24
6	AW	28	27				High	0.48	1.81
7	IA	74	72	Yes	Yes	No	Low	0.49	0.75
8	EA	41	39				Low	0.79	0.33
9	AW	13	14				Low	0.69	0.99
10	AW	49	51				High	0.66	2.17
11	IA	85	84	Yes	No	No	Low	0.7	0.66
12	EA	15	14				High	0.53	0.87
13	AW	5	4				Low	0.67	1.11
14	IA	73	72	No	No	No	Low	0.43	1.66
15	AW	74	72	No	Yes	No	High	0.25	1.26
16	AW	5	7				Intermediate	0.38	1.07
17	IA	126	126	No	No	No	Low	0.37	0.88
18	IA	22	21	Yes	No	No	Low	0.25	0.91
19	AW	3	4				Intermediate	0.19	0.89
20	IA	26	27	No	No	No	Low	0.36	0.72
21	AW	2	3				High	0.38	1.55
Median		41	39				Mean	0.479	1.12
Range		2 - 215	3 - 215				Standard Deviation	0.159	0.432
P-value*		0.985					P-values	<0.0001	

* Mann-Whitney U-test; § Students t-test

Table 3.4 – Summary of results

3.4.1 Intra-abdominal desmoids

Nine IAD were identified, all localized to the small bowel mesentery. There was consistency between the two modalities in the assessment of number, site and local extent of these mesenteric desmoids. Five IAD involved the bowel; four resulted in extrinsic ureteric compression. While infiltration around mesenteric vessels was demonstrated on both scans, none compromised the proximal superior mesenteric artery. The median size of these IAD was 73 (range, 11-126) cm² on MDCT and 72(10-126) cm² on MRI (p=0.98). For these IAD, the mean contrast enhancement ratio for MRI – 0.98(standard deviation, 0.34) – was significantly greater than for MDCT – 0.46(0.13) (p=0.0005). All nine IAD had a low T2-signal intensity.

3.4.2 Abdominal wall desmoids

Nine AWD were identified, with both MDCT and MRI agreeing on desmoid number, site and local extent. The size of these AWD on MDCT – 13(2-181) cm² – was equivalent to that on MRI – 14(3-179) cm² (p=0.99). The contrast enhancement ratio on MRI – 1.36(0.42) – was significantly greater than for MDCT – 0.46(0.18) (p<0.0001). These AWD displayed variable T2-signal intensity, as described in Table 3.4. MRI identified one extra AWD that was difficult to identify even on retrospective review of MDCT due to its similar density to muscle (Fig 3.3).

3.4.3 Extra-abdominal desmoids

Three EAD were detected by MDCT. MRI identified these lesions and one additional EAD, within the erector spinae muscle (Fig 3.2). For the three EAD seen on both modalities, there was consistency regarding site and local infiltration. The size of these EAD on MDCT – 41(15-215) cm² – was equivalent to that on MRI – 39(14-215) cm² (p=0.98). The contrast enhancement ratio of these EAD on MRI – 0.81(0.46) – was not significantly different when compared to MDCT – 0.61(0.16) (p=0.50). These EAD had range of T2-signal intensity, as described in Table 3.4. The additional EAD detected by MRI displayed high T2-signal intensity and was thus conspicuous (Fig 3.2).

3.4.4 T2-signal intensity and MRI contrast enhancement ratio

The comparison between mean (standard deviation) MRI contrast enhancement ratio and signal intensity on T2-weighted images shows that the high signal intensity group had a significantly greater MRI contrast enhancement ratio compared to the low and intermediate group – 1.51(0.45) and 0.96(0.32) respectively (p=0.006). Figure 3.4 displays the comparison between T2-signal intensity and MR enhancement ratio.

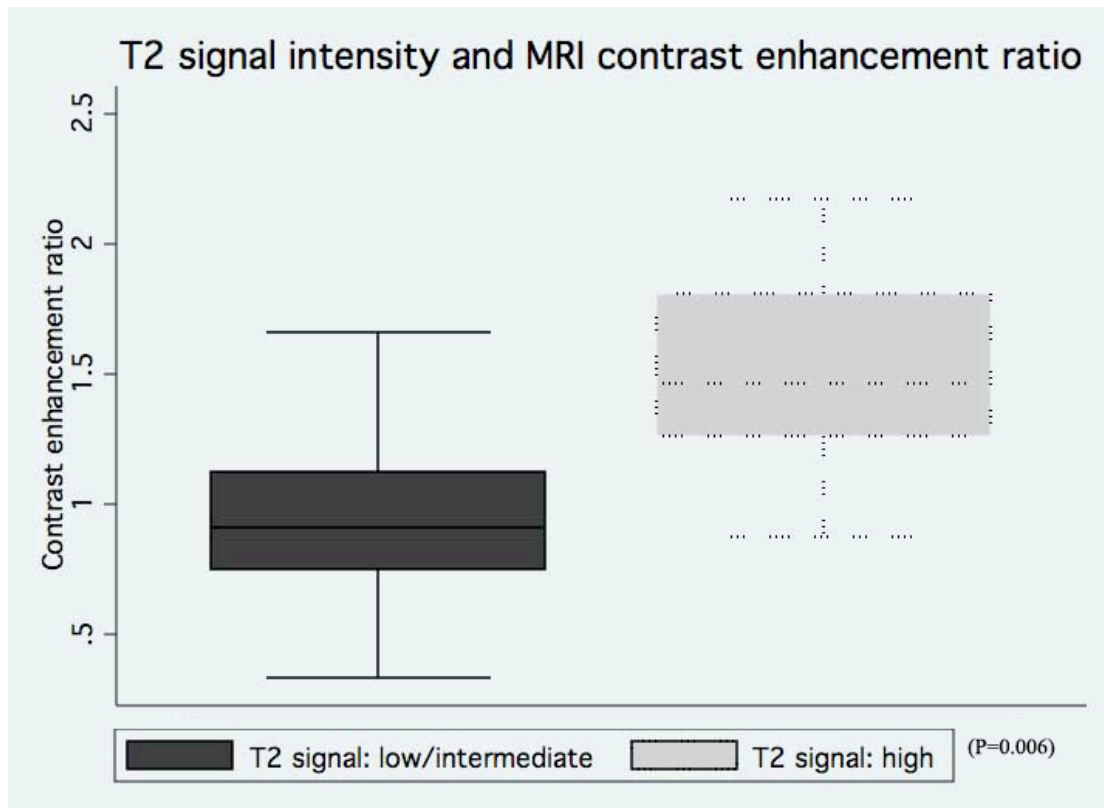


Fig 3.4 – T2 signal intensity and MRI contrast enhancement ratio

The MRI contrast enhancement ratio across the three anatomical sites for desmoids was – IAD 0.97(0.39), AWD 1.54(0.40) and EAD 0.79(0.46). No significant difference was detected between these three groups ($p>0.05$).

3.5 Conclusions

The results of this study demonstrate that MRI identified a greater number of desmoids than MDCT. MRI has been proven to be superior to CT in the assessment of soft tissue tumour heterogeneity due to its superior soft tissue contrast (Tunckbilek et al, 2005). There have been significant improvements in imaging technology since Healy et al (1997) compared CT and MRI for the assessment of desmoids in FAP. Our current study supports the use of modern day MRI for assessing desmoids in FAP patients.

3.5.1 Desmoid detection rate

Healy et al detected 35 desmoids (22 IAD and 13 AWD) on CT compared with 34 desmoids (21 IAD and 13 AWD) on MRI, giving a detection rate of 97% on MRI; no EAD were identified by either modality (Healy et al, 1997). In their study, there was one false positive diagnosis (the pancreatic head was misdiagnosed as a desmoid on MRI) and one false negative diagnosis (a desmoid was misdiagnosed as a loop of small bowel on MRI).

In the current study 21 desmoids were detected (9 IAD, 9 AWD and three EAD) on MDCT and 23 (9 IAD, 10 AWD and four EAD) on MRI, giving a detection rate of 110% on MRI. The two additional desmoids detected by MR, whilst small in size, were of high T2-signal and more conspicuous. There were no false positive or false negative diagnoses. Advancements in modern day MRI technology, including better spatial resolution, particularly when utilizing breath-hold sequences, have enabled this superior detection rate. This is consistent with another study, where MRI was superior to CT in the assessment of soft tissue tumours due to its superior soft tissue contrast (Tunckbilek et al, 2005).

Unlike the study by Healy et al where MRI was inferior to CT in delineating the extent of mesenteric desmoids, in our study both MDCT and MRI were equivalent in assessment of infiltrative extent of intra-abdominal desmoids, in particular small bowel and ureteric involvement. Both techniques were able to demonstrate disease around mesenteric vessels but the superior mesenteric

artery remained uncompromised in all patients, again reflecting improvements in MRI technology since Healy et al conducted their study.

3.5.2 T2 signal characteristics and contrast enhancement ratio

Healy et al also suggested that high signal intensity on T2-weighted images might be useful in identifying aggressive desmoids. In this study high signal intensity on T2-weighted images was associated with a significantly higher MRI enhancement ratio (in comparison to the low/ intermediate signal intensity group), suggesting that this may reflect greater cellularity and vascularisation of potentially aggressive desmoid tumours. The potential for signal intensity and MRI enhancement ratio as a surrogate for desmoid growth warrants further exploration. Nevertheless it should be borne in mind that this remains controversial as another study of aggressive fibromatosis (Castellazzi et al, 2008) has been unable to predict desmoid behaviour based on the MRI signal alone.

3.5.3 Summary

The major limitation of this study is the small number of patients available for analysis. However, FAP associated desmoid tumours are rare entities and this study does provide valuable prospective comparison between MDCT and MRI. These data suggest that MRI is clearly no worse than MDCT for the assessment of desmoids in FAP.

In addition, the interpretation of both MDCT and MRI is operator dependant. Particularly, the detection of rare pathologies, such as subtle desmoids, relies

heavily on reader expertise. Currently, greater experience exists in assessing desmoids with CT than with MRI. As the learning curve for desmoid characterisation with MRI improves, and further evidence is accrued, MRI may feasibly become the primary modality in the future for assessing desmoids. However, both readers in this study were experienced gastrointestinal radiologists at a tertiary FAP centre.

The MRI examination time was approximately 25 minutes, which is longer than CT but was well tolerated by the study patients. Generally young patients are able to manage the breath-holds required for MRI without difficulty. However, it can be a problem for some, resulting in reduced accuracy of MRI compared to CT in the assessment intra-abdominal relationships. No patient declined MRI and no one suffered claustrophobia during this study.

FAP patients with IAD require periodic imaging to assess the disease status, to evaluate symptoms and to exclude complications (in particular silent ureteric obstruction) of desmoid disease. Current practice using CT scan potentially incurs a heavy radiation burden given the effective dose of 10mSv/examination for abdominal pelvic CT. These patients are usually young. Hence, MRI is an attractive prospect due to its lack of radiation.

In summary, this study has shown that MRI is at least equivalent to and may be superior to MDCT in the detection of desmoid tumours in patients with FAP, and supports the use of modern day MRI for assessing desmoids in patients with FAP.

Chapter 4

Diffusion tensor imaging (DTI) in the assessment of desmoid tumours in familial adenomatous polyposis

4.1 Background

The imaging appearance of desmoid tumour depends on the degree of fibroblastic proliferation, fibrosis, collagen content and vascularity. In MRI most abdominal wall or extra-abdominal desmoid tumours are well-circumscribed homogeneous lesions, which appear isointense relative to muscle (O'Keefe et al, 1990). Intra-abdominal or mesenteric desmoids typically appear more infiltrative (Casillas et al, 1991).

CT is performed most commonly for tumour assessment and to monitor growth (Einstein et al, 1991; Healy et al, 1997) but has its limitations in predicting tumour aggressiveness and growth rate. The potential of MRI in FAP as a primary assessment tool has been explored previously (Healy et al, 1997): desmoid tumours may appear hypo- or isointense relative to muscle on T1-weighted sequences, while T2 signal intensity may be more variable, purported to be due the degree of fibrosis and cellularity: T2 hyperintensity has been suggested as an imaging marker of actively growing tumours (Healy et al, 1997), though data have been conflicting outside of FAP (Castellazzi et al, 2008). With improvements in MR systems and introduction of parallel

imaging technology, diffusion weighted studies within the abdomen and pelvis have become possible. Advanced MRI techniques that assess water diffusion, such as diffusion tensor imaging (DTI) may provide further information of the microstructure in particular its cellular and fibrous content (Manenti et al, 2007; Gurses et al, 2006; Takayama et al, 2008), and may potentially provide evidence of tumour aggressiveness.

4.2 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is an advanced MRI technique that allows the mapping of the diffusion process of molecules, mainly water in biological tissue, non-invasively. Molecular diffusion in tissues is not free, but reflects interactions with many obstacles, such as macromolecules, fibres, membranes, etc. Water molecule diffusion patterns can therefore reveal microscopic details about tissue architecture. In general, in tissues without a well-ordered microstructure, the water molecules flow in an isotropic way, ie, in an identical way in the different spatial directions. On the other hand, in structures with a high degree of structural order, molecular diffusion has a preferential direction or anisotropy (Le Bihan et al, 2001). The magnetic resonance measurement of an effective diffusion tensor of water in tissues can provide unique biologically and clinically relevant information that is not available from other imaging modalities.

DTI has long been successfully applied in the field of neuroradiology, particularly in the assessment of acute cerebral ischemia (Schaefer et al, 2000). Initially, DTI in other than intracranial sites did not yield sufficient image

quality due to susceptibility artefacts and motion artefacts. More recently, technical advances in MRI, such as the development of parallel imaging, high gradient amplitudes, and multichannel coils, have enabled the performance of DTI in the body. These developments have initiated the investigation of applicability of DTI for tumour characterisation, both intra- and extracranially (Vermoolen et al, 2012).

Diffusion weighted images can be assessed in two ways, qualitatively, by visual assessment of signal intensity, and quantitatively, by measurement of the apparent diffusion coefficient (ADC). The ADC value quantifies water proton motion, which in biological tissues is a combination of true water diffusion and capillary perfusion. The ADC value can theoretically be used to characterise tissues, as the degree of diffusion is correlated to cellular density and extracellular space volume (Guo et al, 2002; Sugahara et al, 1999). Malignant tumours are reported to have a high cellular density and low extracellular space volume, which is associated with impeded water proton diffusion and low ADC values. In contrast, various benign lesions are characterised by an increased amount of extracellular matrix with minimal increase of cellular density, which may result in higher ADCs (Kwee et al, 2008). Other parameters assessed in the DTI are Fractional Anisotropy (*FA*) and Relative Anisotropy (*RA*). *FA* describes the fraction of diffusivity that can be ascribed to anisotropic diffusion, and *RA*, the ratio of the anisotropy to isotropic part, may inform on the presence and degree of fibrosis, which may demonstrate higher directionality.

4.3 Aim of the study

The aim of this exploratory study was to assess the feasibility and potential of DTI for desmoid tumour assessment in FAP. Given the unpredictable growth pattern of desmoid tumours in FAP patients, an imaging technique that may provide insight into the tissue microarchitecture may help to identify potentially aggressive tumours in order to tailor treatment at an earlier stage.

4.4 Materials and methods

4.4.1 Patient selection

Nine patients with FAP and desmoid tumours (four male, five female, median age 31.5 years, range 22-50 years) were identified from the St Mark's Hospital Polyposis Registry and recruited prospectively. All patients had undergone prophylactic colectomy. Patient demographics are summarised in Table 4.1.

Patient	Age at DT diagnosis	Gender	APC mutation (codon)	Surgery for FAP	Current treatment
1	26	F	1392	IRA	Sulindac Toremifene
2	43	M	1465	RPC	-
3	50	M	1551	RPC	Chemotherapy
4	22	M	1556	IRA	Celecoxib
5	34	M	1551	RPC	-
6	24	F	1509	RPC	Observation
7	32	F	1182	IRA	Sulindac Toremifene
8	24	F	213	IRA	Sulindac Raloxifene
9	31	F	1444	IRA	Sulindac

Table 4.1 - Patient demographics

The inclusion criteria were the presence of a desmoid tumour; the exclusion criteria were standard exclusion criteria for MRI including presence of metalwork or pacemaker, inability to lie flat due to desmoid disease and claustrophobia.

4.4.2 Regulatory approvals

Regional ethics committee approval (Harrow Research and Ethics Committee Ref number: 07/H0719/100) was obtained for this study. Informed consent was obtained from all enrolled patients.

4.4.3 Image acquisition

MRI was performed at 1.5-Tesla (Siemens Avanto, Siemens Healthcare, Erlangen, Germany) using a multi-coil multi-channel system. Patients underwent respiratory gated 3D T1-weighted, T2-weighted and STIR sequences encompassing the abdomen and pelvis in axial, coronal and sagittal planes (for MRI acquisition parameters refer to Table 3.2) prior to DTI.

DTI encompassing the desmoid tumour, or the largest tumour if multiple tumours were present, in the axial plane with a 12cm z-axis coverage was performed using the following parameters: free breathing single shot echo planar sequence with parallel acquisition, parallel imaging factor 2; TR 3000ms, TE 90ms, FOV 260mm, matrix 99x128, NEX 3, section thickness/coverage 5mm/120mm, b values: 0 and 800 s/mm²; 20 diffusion gradient orientations; pixel bandwidth 1600Hz; acquisition time 3 minutes 26 seconds.

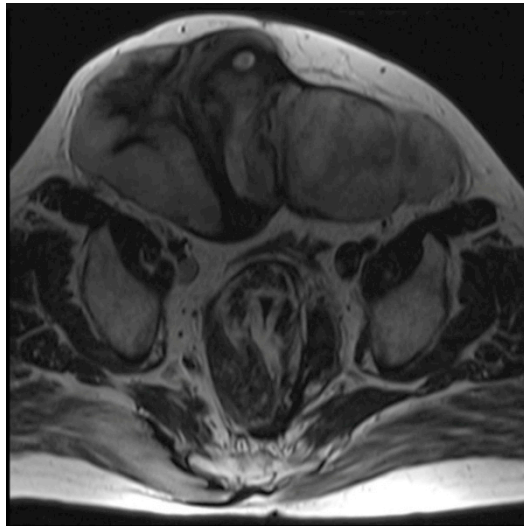
4.4.4 Image analysis

Images were viewed on a standard workstation (Leonardo, Siemens Healthcare, Erlangen, Germany) by two radiologists (with a specialist interest in gastrointestinal and oncological imaging) in consensus. The morphological sequences were viewed as a single contiguous volume of the abdomen and pelvis using the 3D taskcard. The site (IA, AW and EA), size, and T2 signal characteristics (signal intensity relative to muscle (hypo, iso or hyperintensity)); and pattern of signal intensity (homogeneous,

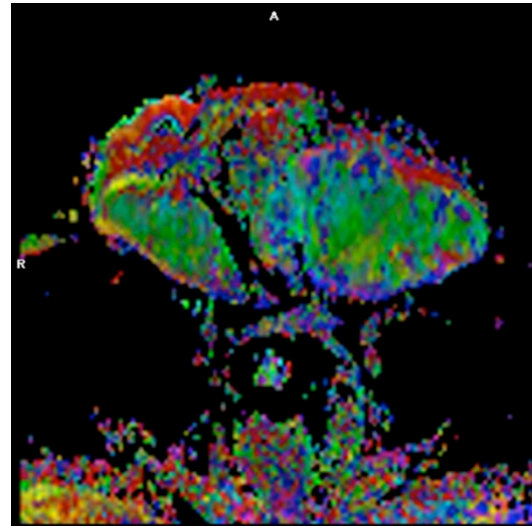
heterogeneous)) were recorded for each desmoid tumour encompassed by the DTI sequence.

The diffusion tensor images were analyzed using commercial tensor software (Neuro3D, Siemens Healthcare), which automatically generated and displayed the following pixel maps: b_0 , fractional anisotropy (FA), relative anisotropy (RA) and diffusivity (apparent diffusion coefficient; ADC). FA and RA parametric maps provided complementary information. FA measures the fraction of diffusivity that can be ascribed to anisotropic diffusion (i.e., directionality; values range from 0 to 1, where 1 = highly directional) while RA represents the ratio of the anisotropic part to its isotropic part, or a normalized standard deviation (values range from 0 to $\sqrt{2}$).

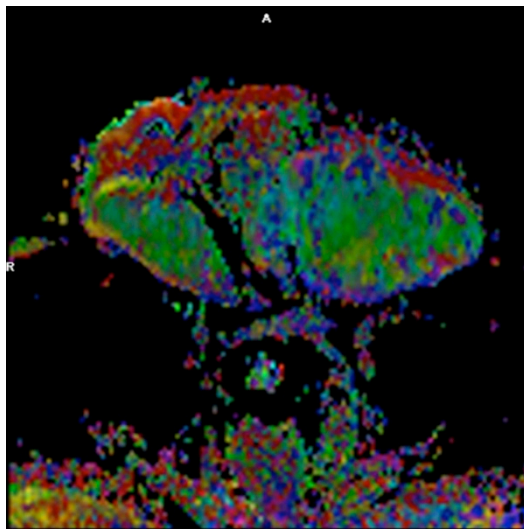
Pixel interpolation and a 40% threshold for display of each parameter map were employed. Regions of interest (ROI) were delineated around the tumour boundary on the b_0 images, which had been previously registered and fused to corresponding T2-weighted images. These were replicated automatically by the software for the corresponding displayed FA , RA and ADC maps (Fig. 4.1).



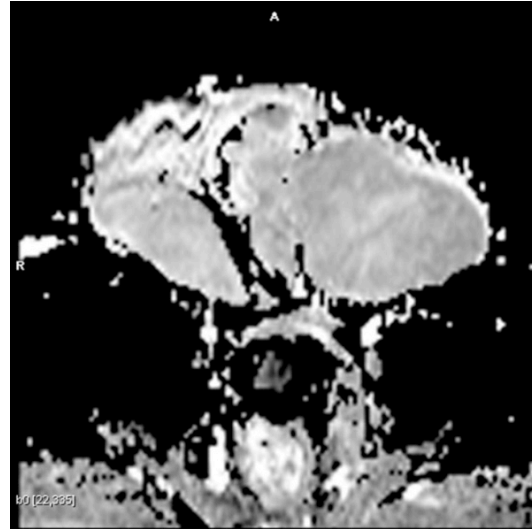
A



B



C



D

Fig 4.1 – Figure demonstrating T2-weighted (A), and corresponding fractional anisotropy (B), relative anisotropy (C) and apparent diffusion coefficient (D) maps of an extra-abdominal desmoid tumour

The parameter values from all axial images inclusive of tumour were averaged to provide an overall tumour parameter value. The directionality of anisotropy was colour coded on the parameter maps as follows: antero-posterior (green), medio-lateral (red) and cranio-caudal (blue). The pattern of directionality was recorded for each desmoid tumour as follows: heterogeneous or homogeneous.

For the control muscle (erector spinae or gluteus maximus), a circular region of interest (112mm²) was placed centrally within the muscle, and the mean value for each parameter *FA*, *RA*, and *ADC* ($\times 10^{-3}$), were recorded.

4.4.5 Clinical treatment and follow up

Clinical treatment in summarised in Table 4.1. Patients were followed up as per standard clinical practice with clinical assessment six monthly post ileorectal anastomosis (including flexible sigmoidoscopy) or one yearly post restorative proctocolectomy (including pouchoscopy). Imaging was repeated one year following initial imaging assessment.

4.4.6 Statistical analysis

Following testing for data normality, median values and range were calculated for the *FA*, *RA* and *ADC* values for all desmoid tumours and control muscles, and were compared using Mann-Whitney U test. *FA*, *RA* and *ADC* values were also compared for each site (IA, AW or EA) and for different T2 signal tumours (hypo, iso or hyperintense) using ANOVA and for different T2 pattern tumours (homogenous, heterogeneous) using Mann-Whitney U test. A p-value less than 0.05 was considered statistically significant.

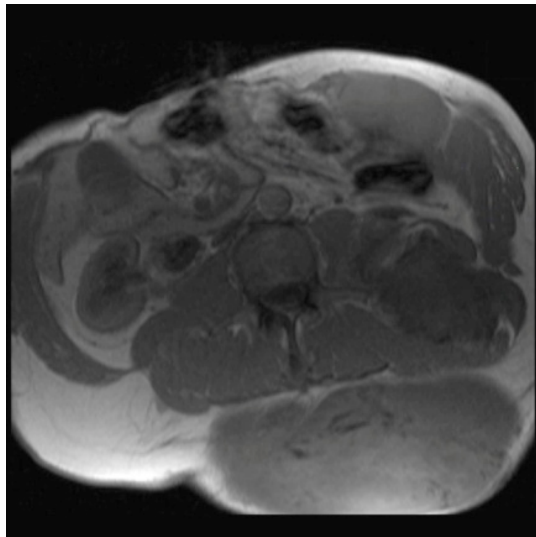
4.5 Results

Fifteen desmoid tumours were evaluated: six IA, six AW and three EA, ranging in size from 1.6cm to 22.9 cm. The tumour characteristics (site, size, signal characteristics and pattern) on morphological and DTI sequences are summarised in Table 4.2.

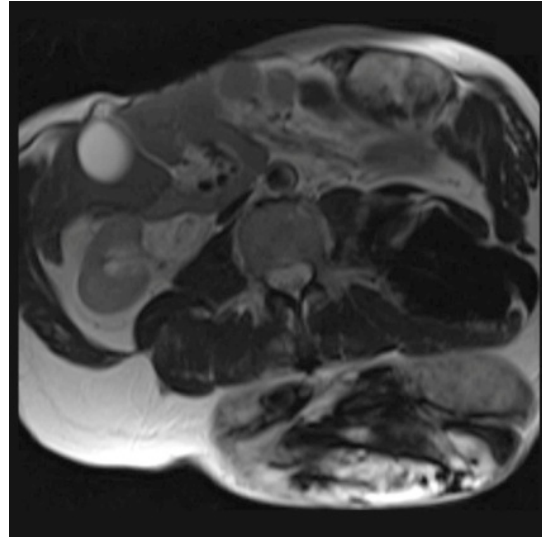
Morphology					T2 characteristics				DTI Signal pattern	
Tumour	Site	SI	Size (cm)		Baseline		1 year post		Baseline	1 year post
			Baseline	1 year post	Signal Intensity	Pattern	Signal Intensity	Pattern		
1	IA	Infiltrative	6.8	5.5	Hypo	Homo	Iso	Homo	No signal	No signal
2	IA	Mixed	11.7	10.5	Iso	Hetero	Iso	Homo	Hetero	No signal
3	AW	Solid	9.5	6.9	Hyper	Hetero	Hyper	Hetero	Hetero	Hetero
4	EA	Solid	10.4	7.4	Hypo	Homo	Hypo	Homo	No signal	No signal
5	EA	Solid	23.0	22.9	Hyper	Hetero	Hyper	Hetero	Hetero	Hetero
6	EA	Solid	1.9	3.1	Hyper	Hetero	Hyper	Hetero	Homo	Homo
7	AW	Solid	2.1	1.6	Hyper	Homo	Hyper	Hetero	Homo	Homo
8	IA	Infiltrative	9.0	8.2	Iso	Homo	Iso	Homo	No signal	No signal
9	AW	Solid	11.0	10.1	Hyper	Hetero	Iso	Hetero	Hetero	Hetero
10	IA	Mixed	16.9	6.2	Hyper	Hetero	Hyper	Hetero	Homo	Homo
11	AW	Solid	3.4	3.7	Hyper	Hetero	Hyper	Hetero	Homo	Hetero
12	IA	Mixed	11.6	-	Hypo	Hetero	-	-	Hetero	-
13	AW	Solid	22.7	-	Hyper	Hetero	-	-	Hetero	-
14	IA	Infiltrative	11	8.7	Hypo	Homo	Hypo	Homo	Hetero	No signal
15	AW	Solid	2.9	1.3	Hyper	Homo	Hyper	Homo	Homo	Homo

Table 4.2 - Summary of the characteristics of the individual desmoid tumours in successive patients

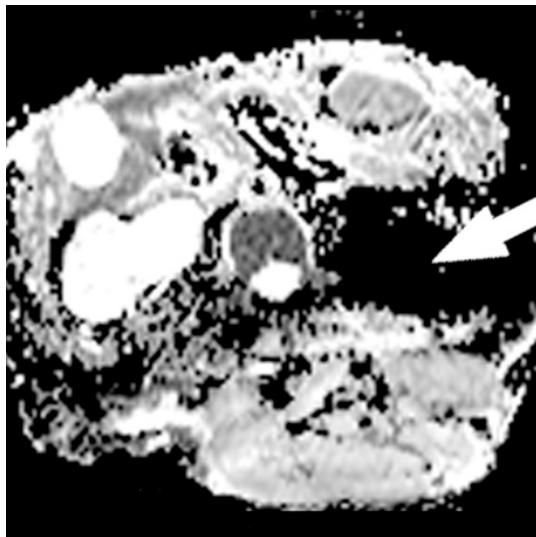
DTI was successful in 12 of 15 desmoid tumours on the baseline study. Quantification was not possible in two IA (mesenteric) and one EA desmoid tumours, which were of low signal on T1 and T2 sequences, due to dense fibrosis (Fig.4.2).



A



B



C

Fig 4.2 - T1 - weighted (A), T2 - weighted (B) and ADC (C) images of a patient with multiple desmoid tumours: No diffusion signal was obtained for this EAD (arrow) on due to the extent of fibrosis

At baseline median (range) of RA, FA and ADC were 0.23 (0.17 to 0.26), 0.27 (0.21 to 0.31) and $1.65 (1.39 \text{ to } 1.91) \times 10^{-3} \text{ mm}^2\text{s}^{-1}$ for desmoids. These were

significantly different from control muscle: 0.27 (0.23 to 0.40), 0.32 (0.28 to 0.46), and 1.45 (0.92 to 1.63) $\times 10^{-3} \text{ mm}^2\text{s}^{-1}$ ($p=0.0001$, $p=0.0001$, $p=0.0016$ respectively). There was no statistically significant difference in RA, FA or ADC between tumour sites (IA, AW or EA), or tumours of different signal intensity (hyper/iso/hypointense on T2-weighted sequences) and signal intensity patterns (homogeneous/heterogeneous) (Table 4.3).

Parameter	Tumour site				T2 signal intensity				Pattern		
	IA	AW	EA	P value	Hyper	Iso	Hypo	P value	Homo	Hetero	P value
FA	0.254	0.270	0.253	0.65	0.264	0.268	0.268	0.97	0.275	0.245	0.77
RA	0.212	0.228	0.235	0.65	0.222	0.226	0.226	0.98	0.231	0.208	0.92
ADC	1.73	1.62	1.74	0.53	1.67	1.57	1.72	0.77	1.68	1.63	0.77

Table 4.3 - Comparison of fractional anisotropy (FA), relative anisotropy (RA) and apparent diffusion coefficient (ADC) by desmoid tumour site, T2 signal intensity, and pattern

At follow up one year later, two patients had died (one from desmoid related disease; one from pulmonary embolus); leaving 13/15 tumours for analysis. Of these DTI was possible in only 8/13 tumours. The majority of tumours decreased in size (median: 10.4 to 6.2cm; $p=0.04$); tumour increased in size in only one patient (1.6 to 3.1cm; +61%). Median (range) of desmoid tumour RA, FA and ADC were 0.24 (0.19 to 0.32), 0.28 (0.23 to 0.37) and 1.85 (1.48 to 2.06) $\times 10^{-3} \text{ mm}^2\text{s}^{-1}$ respectively; this was not significantly different from baseline ($p=0.77$, 0.71 and 0.34 respectively).

4.6 Discussion

This study demonstrated that desmoid tumours show anisotropy: *FA*, *RA* and *ADC* differed from normal skeletal muscle, but did not vary significantly between sites, T2 signal intensity or pattern. In general *FA* and *RA* were lower than normal muscle (Kermarrec et al, 2010) indicating a less directional pattern than muscle fibres. This is not surprising given macroscopic and microscopic appearances at pathological evaluation. The lack of significant difference in values between tumour sites may reflect the small number of tumours in this exploratory study, however, there was a trend for AW and EA tumors to have higher *FA* and *RA* than IA tumours.

High T2 signal intensity has been suggested as a marker of disease activity (Healy et al, 1997): *FA*, *RA* and *ADC* values did not differ for hypo, iso or hyperintense tumours indicating this reflects a different aspect of tumour biology which may complement other MRI parameters. The majority of tumours showed shrinkage at one year. *ADC* increased indicating a reduction in cellularity mirrored by a decrease in T2 signal intensity in some lesions, though this was not statistically significant. *FA* and *RA* were not significantly different. These preliminary data support the hypothesis that assessment of water diffusion may potentially provide additional clinically relevant information, but requires further study.

ADC values in our study were comparable with a recently published diffusion weighted (DW) MRI study encompassing 8 sporadic tumours, which showed that sporadic desmoids had higher *ADC* values compared

with other soft tissue tumours (1.36 versus $0.88 \times 10^{-3} \text{ mm}^2\text{s}^{-1}$) indicating these are less cellular (Oka et al, 2011).

4.7 Summary

There are limitations to this study. Firstly the assessment of desmoid tumours DWI was not feasible in three out of fifteen tumours initially, increasing to five tumours at one year follow up due to the dense fibrosis. Secondly patient numbers were small, and only one patient's tumour showed interval growth; but it should be recognized that this is a very rare condition. Thirdly there was a lack of pathological correlation, however, surgery is only undertaken following careful consideration in patients with desmoid tumours in FAP and none of these patients underwent surgery subsequent to MRI.

In summary, this study has shown that DTI is feasible for the assessment of desmoid tumours in FAP. Desmoid tumours show anisotropy, but this is less directional than normal muscle. DTI parameters may reflect the tumour microstructure, however, its clinical role remains indeterminate, requiring further evaluation.

Chapter 5

Role of Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and Fluorine-18 Flurodeoxyglucose Positron Emission Tomography (¹⁸F-FDG PET) in predicting behaviour of desmoid tumours in familial adenomatous polyposis

5.1 Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI)

Conventional contrast-enhanced MRI displays a single snapshot of tumour enhancement after contrast administration. While such image can provide useful anatomical information, it lacks functional information. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), on the other hand, relies on fast MRI sequences obtained before, during and after the rapid intravenous administration of a gadolinium based contrast agent. This MRI technique is almost analogous to a movie and enables the depiction of physiologic alterations as well as morphologic changes in the tissue (Türkbey et al, 2010). DCE-MRI images can be quantified to provide information on tumour perfusion, vessel permeability and volume of extracellular extra vascular space. These quantifications serve as a non-invasive biomarker for

tumour angiogenesis, which is a key step in the pathophysiology of tumour growth and metastatic spread (Padhani et al, 2001).

DCE-MRI has been increasingly used for the initial diagnosis and staging of many tumours. Moreover, it has been used as a biomarker for monitoring response to conventional chemotherapy, antiangiogenic therapy or radiotherapy for various cancer types such as colon, prostate, breast and gynecologic malignancies (Türkbey et al, 2010)

5.2 Fluorine-18 Flurodeoxyglucose Positron Emission Tomography (¹⁸F-FDG PET)

Positron emission tomography (PET) is a molecular imaging technique used to detect abnormal behaviour of tissues. Fluorine-18-deoxyglucose (FDG) is a radionuclide labeled glucose analogue commonly used in clinical practice for molecular probing. This is based on the fact that abnormal tissue such as tumour exhibits markedly increased rates of glucose metabolism.

After administration, FDG is actively transported into cells, mediated by a group of structurally related glucose transport proteins. Once intracellular, FDG (like glucose) is phosphorylated by hexokinase as the first step in the glycolytic metabolism pathway. While normal glucose continues along the glycolytic pathway for energy production, FDG cannot enter the glycolytic pathway and becomes effectively trapped intracellularly as FDG-6-phosphate. Most tumour cells are highly metabolically active with high mitotic rates and display increased numbers of glucose transporters as well as higher levels of

hexokinase. These mechanisms allow tumour cells to take up and retain higher levels of FDG when compared to normal tissues.

PET/CT fuses functional information from PET and anatomic information from CT scan simultaneously and allows for these to be viewed and interpreted together. The multidetector CT apparatus and the PET detectors are mounted in the same gantry, one immediately behind the other, and scans are performed with the patient lying in the same position on the imaging table. For interpretation, the PET data is actually superimposed upon the CT data resulting in improved anatomic localisation of normal and abnormal FDG activity.

^{18}F -FDG PET CT has been widely used in clinical setting to stage, monitor response to chemotherapy and detect metastasis and recurrence in wide variety of cancer (Facey et al, 2007). It has also been used as a prognostic biomarker. High uptake of ^{18}F -FDG has also been associated with tumour angiogenesis and found to be predictive of aggressive features of cancer cells in early breast cancer patients (Ueda et al, 2008; Groves et al, 2011), and other cancers including lung and oesophagus (Reske et al, 2001).

5.3 DCE-MRI and FDG PET in desmoid tumour

The use of DCE-MRI in the characterisation and assessment of behaviour of desmoid tumour has not been reported before. There are limited reports in the literature of use of PET CT to assess desmoid tumour. In a small series of patients, Basu et al have suggested a change in FDG uptake by desmoids as a

marker of response to chemotherapy and discussed its potential role in predicting their aggressive behaviour (Basu et al, 2007). Previous clinicopathological studies in desmoid tumour have reported a correlation of increased vascularity with aggressive behaviour of desmoid and tendency to recurrence (Kouho et al, 1997; Yokohama et al, 1989). From these studies one can hypothesize that aggressive desmoids are potentially more vascular and metabolically more active. Therefore combination of DCE-MRI, which provides functional (vascularity) information, and FDG PET/CT, which provided metabolic information, may be useful in providing complementary information on tumour behaviour.

5.4 Aim of the study

The aim of this study was to investigate whether vascular (DCE-MRI) and metabolic imaging (FDG PET CT) can predict the aggressive desmoid tumours in FAP patients, allowing these to be identified and treated early.

5.5 Materials and method

5.5.1 Patient selection

FAP patients with confirmed desmoid tumours based on previous radiological and clinical evidence were identified from the St Mark's Hospital Polyposis Registry and prospectively recruited. Inclusion criteria included FAP patients with known desmoids and exclusion criteria included claustrophobia and presence of metal implant for MRI and known diabetes

for PET. All patients underwent MRI, including DCE-MRI sequences, and FDG PET CT imaging of the abdomen and pelvis with scans performed on the same day. A conventional follow up MRI scan was performed after one year to determine tumour growth.

5.5.2 Regulatory approvals

Regional ethics committee approval (Harrow Research and Ethics Committee reference number: 07/H0719/100) and informed consent from enrolled patients was obtained for this study.

5.5.3 MRI and DCE-MRI examinations

MRI was performed using a 1.5-Tesla MR scanner (Siemens Avanto, Siemens Medical Solutions, Erlangen, Germany) and a multichannel surface coil. T1, T2-weighted, and short tau inversion recovery (STIR) sequences of the abdomen and pelvis in the axial, coronal and sagittal planes were supplemented by dynamic contrast enhanced MRI. The acquisition parameters are listed in Table 5.1.

Sequence	TR (ms)	TE (ms)	TI (ms)	FA (°)	NEX	Slice thickness (mm)	FOV (mm)	Matrix
T1 axial bh Abdo and pelvis	138	4.76	-	70	1	8	300*263	256*179
T2 axial bh Abdo and Pelvis	97	2180	-	150	1	5	300*300	512*512
STIR axial	3800	93	150	160	1	5	300*300	256*256
Proton Density axial for DCE	7.38	4.76	-	2	6	5	300*300	512*512
T1W DCE	7.38	4.76	-	18	1 *40 repeats	5	300*300	512*512

Table 5.1 - MRI diagnostic acquisition parameters

Respiratory gating was used to reduce movement artifacts caused by breathing, and 10mg of intravenous hyoscine-N-butylbromide (Buscopan; Boehringer Ingelheim, Bracknell, Berkshire, England) was administered to reduce peristalsis.

The DCE-MRI studies were performed following 0.1mlkg^{-1} gadolinium-based intravenous contrast injection (Magnevist™, Bayer, Burgess Hill, West Sussex, UK), using a 3D VIBE sequence and the following parameters: TR 7.38ms, TE 4.76ms, FOV 300mm*300mm, Matrix 512*512, Flip angle 20° (proton density) and 180° (dynamic scan), Slice thickness 5mm; dynamic scan interval 11.9s: total acquisitions 40.

5.5.4 Whole body ^{18}F -FDG PET/CT examinations

Patients were injected with 4.5MBq (^{18}F) FDG per kilogram body weight (maximum of 370 MBq) after a fasting period of at least six hours. Patients stayed in a quiet and darkened room for 60-90 minutes. (^{18}F) FDG imaging was performed on an integrated PET-CT system (GE Discovery VCT, GE Healthcare, Amersham, UK). Following CT for attenuation correction (140kV, 80mA, 0.8s/rotation, Pitch 1.5, Slice thickness 3.75mm), the PET emission scans covering the same anatomical area were carried out in 2D mode, consisting of an emission scan of 8 minutes/bed position. Transaxial emission images of 5.47×5.47×3.27mm (in plane matrix size 128×128) were reconstructed using OSEM with two iterations and 28 subsets. The axial field of view was 148.75 mm.

5.5.5 Image analysis

Images were transferred and viewed on a standard workstation (Leonardo, Siemens Healthcare, Erlangen, Germany) by two radiologists (with a specialist interest in gastrointestinal imaging) in consensus. The morphological sequences were viewed as a single contiguous volume of the abdomen and pelvis using the 3D taskcard to facilitate identification of all desmoid tumours in the abdomen and pelvis. The site, size and signal characteristics of the tumour were recorded.

The DCE-MRI images were transferred to a workstation, and assessed using proprietary software (Magnetic Resonance Imaging Workbench, Institute of

Cancer Research). Following motion correction and image registration, the DCE-MRI data were quantified to give T_1 values, and hence Gadolinium concentration. An extended Kety model (Tofts) (Tofts PS, 1997) with a modified Fritz-Hansen assumed AIF (Arterial Input Function) (Walker-Samuel S et al, 2006) was used to describe the uptake of contrast within the region of interest:

$$C_t(t) = K^{trans} \int_0^t C_p(t') \exp\left(\frac{-K^{trans}(t-t')}{v_e}\right) dt'$$

where C_t = tissue concentration of contrast agent, K^{trans} = transfer constant, v_e = extravascular extracellular volume and the derived rate constant k_{ep} = K^{trans}/v_e .

K^{trans} (transfer constant), v_e (extravascular extracellular space, EES), k_{ep} (rate constant) and $IAUGC_{60}$ (integrated area under the gadolinium-time curve at 60s) were derived by region of interest analysis. K^{trans} is a parameter related to vessel permeability and tissue blood flow, V_e is an estimate of the extravascular extracellular space, K_{ep} represents transfer constant from EES to plasma. The DCE-MRI parameters of each slice were recorded and averaged to obtain whole tumour DCE-MRI estimates.

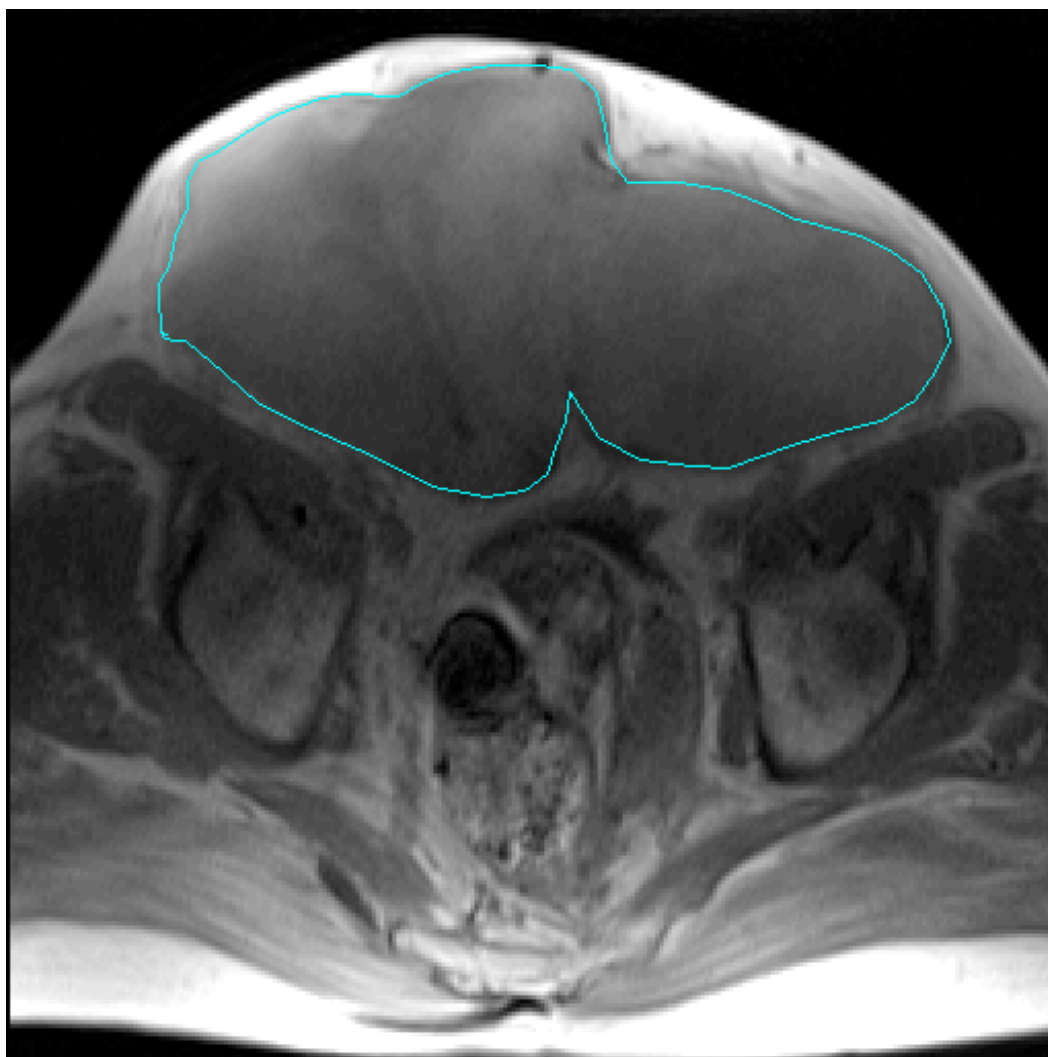


Fig 5.1 - DCE-MRI image of an abdominal wall desmoid with region of interest (ROI) drawn around the tumour

FDG-PET/CT images were reviewed by a radiologist experienced in desmoid imaging and a nuclear medicine specialist in consensus. For each desmoid tumour identified on PET CT, the maximum Standardised Uptake Value (SUV_{max}) was derived for the volume-of-interest (VOI) defined for the tumour.

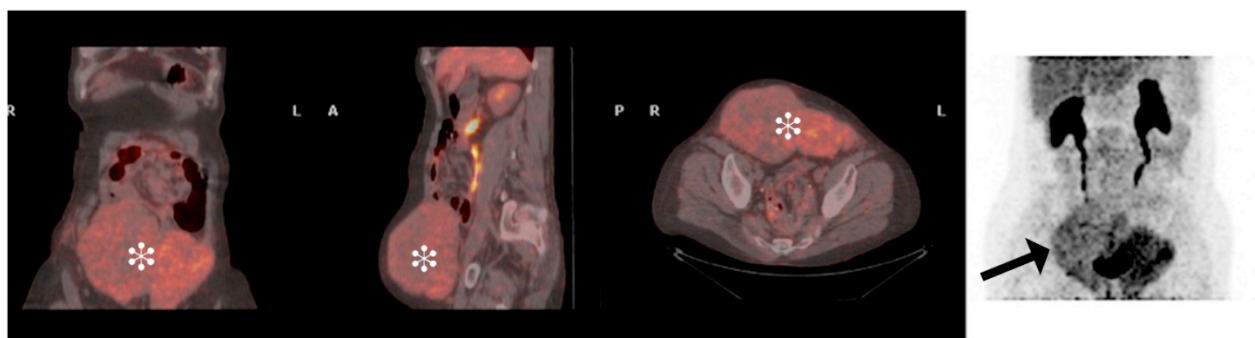


Fig 5.2 - ^{18}F FDG PET CT images of desmoid tumour. The abdominal wall/ pelvic desmoid tumour in this image had SUV_{max} value of 3.9 (asterisk and arrow)

5.5.6 Outcomes of interest

For each modality the number of desmoids was documented. The size of desmoid was obtained in MRI by automated region of interest analysis and the area was recorded in cm^2 . All desmoid lesions were classified by site into IAD, AWD or EAD. IAD were all mesenteric desmoids; AWD included desmoids located in anterior and posterior abdominal wall muscles; EAD incorporated desmoids at other sites. Desmoids were also classified according to change in size between first and follow up MRI scan. Increase in size by 10% or more was recorded as growth, decrease in size by 10% or more was recorded as regression and rest were classified as stable desmoids.

5.5.7 Statistical analysis

Statistical analysis was conducted using STATATM SE 10.1 for Macintosh (STATA Corp, Texas, USA). DCE-MRI parameters (K^{trans} , V_e , K_{ep} , IAUGC_{60}) were described as mean and standard deviation. Vascular Parameters (K^{trans} , V_e , K_{ep} , IUC) and SUV_{max} and the ratio of $K^{\text{trans}} / \text{SUV}_{\text{max}}$ were compared for each site (IAD, AWD and EAD) and for size change (increased, decreased or

stable) using one-way analysis of variance (ANOVA). SUV_{max} was correlated with vascular parameters using Spearman Rank correlation. A p-value less than 0.05 was considered statistically significant.

5.6 Results

Nine patients with FAP and desmoid disease underwent MRI and PET CT on the same day. Please refer to Table 4.1 for the demographic details of the included patients.

Twenty-three desmoids (9 IAD, 10 AWD and four EAD) were identified on MRI. PET-CT was quantifiable in 21 desmoids. Due to failure to cannulate one patient (patient 9, Table 4.1), DCE MRI was possible in only 8 patients. DCE MRI was quantifiable in 13/19 tumours (7 AW, four IA and two EA) in the remaining 8 patients. Quantification was not possible in 6 tumours (four IA and two AW) due to extensive fibrosis and poor vascularity. Two patients died between the studies leaving only 11 tumours on follow up MRI one year later for analysis of combined DCE-MRI and PET-CT parameters and subsequent growth. Table 5.2 summarises signal characteristics, DCE-MRI parameters, size change and SUV_{max} of these tumours.

Patient	Site	T2-Signal	K^{trans}	V_e	K_{ep}	IAUGC ₆₀	Initial MR Size (cm ²)	Follow up MR size (cm ²)	SUV _{max}	SUV _{max} / K^{trans}
1	IA	low	0.231	55.3	0.448	13.40	27.6	16.3	4.2	18.18
2	AW	mixed	0.080	59.4	0.137	9.85	191.2	-	8.3	103.75
3	EA	low	0.038	29.3	0.171	0.95	145.7	181.5	8.1	213.15
3	AW	mixed	0.143	64.3	0.234	16.88	32.2	24.2	4.1	28.67
3	EA	low	0.053	15.0	0.362	2.97	30.4	26.8	3.0	56.60
4	AW	mixed	0.195	67.9	0.295	17.17	47.3	24.5	4.3	22.05
4	AW	low	0.055	17.7	0.298	3.38	3.17	2.5	3.3	60
5	IA	low	0.174	60.0	0.312	9.82	70.7	-	4.2	24.13
6	AW	mixed	0.095	47.4	0.214	6.44	89.5	25.3	5.1	53.68
6	AW	mixed	0.378	55.8	0.671	21.34	2.5	5.5	3.9	10.31
7	IA	low	0.238	38.2	0.649	12.61	18.1	18.3	2.8	11.76
8	IA	low	0.234	39.6	0.612	11.97	25.3	34.8	5.3	22.64
8	AW	mixed	0.280	60.2	0.488	18.34	2.1	1.5	2.9	10.35

Table 5.2 - Description of the tumours

The mean (sd) value of DCE Kinetic parameters, K^{trans} , V_e , K_{ep} , IAUGC₆₀ was 0.175 (0.097), 0.507 (0.152), 0.366 (0.186), 12.23 (6.05). The mean (sd) SUV_{max} was 4.54 (1.78). SUV_{max} was significantly negatively correlated with K_{ep} but not with other vascular parameters (K^{trans} , V_e , IAUGC₆₀) (Table 4.4). Desmoid of size >30 cm² on the initial MRI had significantly higher SUV_{max} / K^{trans} ratio compared to size <30 cm² (p=0.03) (Table 5.3).

Variable 1	Variable 2	Spearman ρ	P value
SUV _{max}	K ^{trans}	-0.47	0.09
SUV _{max}	v _e	-0.11	0.72
SUV _{max}	k _{ep}	-0.56	0.04
SUV _{max}	IAUGC ₆₀	-0.47	0.10

Table 5.3 - Correlation analysis of SUV_{max} and DCE-MRI parameters

Five tumours decreased in size, three increased in size and three remained stable after a year. There was no significant difference in the SUV_{max} or DCE parameters (K_{trans}, V_e, K_{ep}, IAUGC₆₀) between the tumours that grew or shrank or between the tumour sites. However ratio of SUV_{max}/K^{trans} was significantly different for size change and between the sites of tumour (Table 5.4).

Parameters	Tumour Size				Tumour Site			
	Increased	Decreased	Stable	P value	IA	AW	EA	P value
K ^{trans}	0.21	0.17	0.17	0.32	0.21	0.17	0.09	0.13
v _e	0.41	0.49	0.56	0.83	0.48	0.53	0.46	0.61
k _{ep}	0.49	0.34	0.36	0.36	0.50	0.33	0.20	0.46
IAUGC ₆₀	11.63	11.74	15.24	0.31	11.95	13.34	8.91	0.06
SUV _{max}	4.93	3.96	4.13	0.16	4.12	4.55	5.55	0.30
SUV _{max} /K ^{trans}	78.78	32.02	24.08	0.001	19.18	41.45	117.62	0.001

Table 5.4 - Comparison of DCE parameters and SUV_{max} by tumour growth and site

All 21 tumours were quantifiable on PET CT (Mean SUV_{max} 3.91 (SD 1.71)); no correlation between SUV_{max} and change in size was noted in the whole cohort.

5.7 Discussion

This study found that desmoid tumours are generally hypovascular (low K^{trans}), with a large volume of distribution (high V_e) and poorly glucose avid (low SUV_{max}). FDG uptake in desmoid tumour was heterogeneous, consistent with the findings by Basu et al. However unlike their suggestion, FDG uptake did not predict aggressive tumours: desmoid tumour glucose uptake did not differ significantly between the tumours that grew or shrank. Furthermore the study by Basu et al included only one FAP associated desmoid. This is clinically relevant as FAP desmoids are genetically and biologically different from sporadic desmoids.

In this study the larger tumours had a higher metabolic-vascular ratio than smaller tumours – indicating a greater mismatch between glucose metabolism and vascularisation. This is consistent with data from cancer e.g. lung (Miles et al, 2006). SUV_{max} correlated negatively with K_{ep} indicating metabolic activity was higher in tumours with greater contrast agent retention, and slower washout. There was also a significant difference in the metabolic vascular ratio between the tumour sites; the clinical significance of this remains uncertain and needs exploring with further studies.

5.8 Limitations of this study

There are limitations to this study. Firstly the number of patients studied was small, however desmoid is an uncommon manifestation of a rare disease. Two patients died before follow-up imaging, one due to desmoid (Patient 2 Table 4.1). This tumour was the most avid for glucose uptake (SUV_{max} 8.3) but had low vascularity (Low k^{trans}) and high volume of distribution (high V_e). While only 11 of 23 tumours were imaged successfully by both DCE-MRI and PET-CT, and were assessed for growth after one year, this is the first study and largest such series evaluating the potential of non-invasive imaging in characterising the behaviour of desmoid tumours in FAP patients.

Secondly, eight out of these eleven tumours either remained stable or decreased in size. Only three showed interval growth. As only 10% of FAP desmoids show aggressive behaviour, it is not surprising that this small cohort of patients did not include many of aggressive type.

Thirdly, patients were on different treatments during the study and there is a possibility that this might have reduced growth in some of the tumours. However some tumours remained stable and others decreased or increased in size during the study period in the same patient implying different biology and response of individual tumour. Moreover it is difficult to comment on the effect of the treatment as there is no good trial evidence of efficacy.

As an exploratory study the primary aim was to study role of these imaging techniques as a predictor for aggressive behaviour in desmoids. Larger

studies in future involving patients on medication or without may help clarify this issue further.

5.9 Summary

Desmoids are a major cause of morbidity and mortality in post-colectomy FAP patients and a non-invasive test, which could potentially identify rapidly growing tumours early, may benefit these patients by allowing a more aggressive approach to treatment early.

This study has not conclusively shown ^{18}F -FDG-PET or DCE-MRI to be of use in this context, probably because there were insufficient actively growing desmoids in this small cohort. However, taken together with previous studies showing high ^{18}F -FDG avidity in some desmoids, this study has shown that a spectrum exists on this imaging modality, which warrants further exploration.

This study also highlights the difficulty of studying a rare and unpredictable tumour. In future international collaboration between centres specialising in these rare tumours and a trial involving large number of patients may help to further explore the use of modern imaging techniques in characterising desmoid tumour behaviour.

PART 3 – CLINICAL STUDY

Chapter 6

A clinical review of familial adenomatous polyposis associated desmoid tumours presenting with air fluid level

6.1 Background

Seventy percent of FAP-related desmoids occur in the abdomen, primarily arising in the small bowel mesentery and abdominal wall (Clark et al, 1999). IAD are associated with significantly poorer survival in comparison to AWD (Phillips et al, 2007). A relatively common clinical situation in aggressive IAD is the development of a liquefied centre containing gas, accompanied by abdominal pain and sepsis. Some authors have described this as desmoid fistula; however, the term 'fistula' refers to an abnormal communication between two epithelial surfaces, so this terminology is not used in our unit. The term 'air-fluid level (AFL)' is preferred, and it encompasses a spectrum of pathology ranging from necrosis, abscess formation and direct communication with the bowel. There is no clear guidance in the literature on the management of this difficult problem.

6.2 Aims of this study

The aims of the study were to understand the underlying mechanism of air-fluid level development in IAD and review our experience in the management of these complications of IAD grouped together as 'IAD with air-fluid level', and to formulate a treatment algorithm.

6.3 Methods

6.3.1 Patient selection

The St Mark's Hospital Polyposis Registry database was searched for patients with a diagnosis of FAP, defined by either an identified *APC* mutation or the presence of over 100 colorectal adenomas with no evidence of *MYH* mutation. Only those patients diagnosed with IAD containing air-fluid level on abdominal contrast enhanced CT or MRI were included in this study. These investigations were reported by experienced gastrointestinal radiologists familiar with imaging for patients with FAP, and were reviewed in a multidisciplinary setting. Patients developing only extra-abdominal desmoids or IAD without air-fluid levels were excluded.

6.3.2 Data collection

For included patients data were collected on patient demographics, type of surgery performed for FAP (IRA or RPC), age at primary surgery, site of IAD and age at diagnosis of IAD with AFL. Patients were grouped by treatment

received into non-operative, percutaneous drainage and open surgery categories.

6.3.2 Outcome of interest

The prospectively maintained dataset was analysed, the outcome of interest being change of treatment strategy and their outcome in the management of this complicated condition.

6.4 Results

Two hundred and forty six patients with DT were identified from the St Mark's Polyposis Registry database. Of these, a total of nine patients had an IAD with air-fluid level; seven were female. Table 6.1 outlines the demographics of the nine patients along with the type of primary surgery performed and the age of diagnosis of the IAD with air-fluid level and site of IAD.

Patient	Gender	Surgery for FAP	Age at surgery (Yrs)	Age at diagnosis of IAD with AFL (Yrs)	Site of IAD
1	F	RPC	33	37	SBM
2	F	IRA	19	22	SBM
3	F	IRA	20	24	SBM and pelvis
4	F	TPC	30	31	SBM
5	F	RPC	18	24	SBM and pelvis
6	M	RPC	34	41	SBM
7	F	IRA	21	24	SBM
8	F	IRA	18	20	SBM and retroperitoneum
9	M	Nil	Nil	36	SBM

Table 6.1 - Patient & DT demographic data

Patient 9 who presented with IAD with air-fluid level had attenuated phenotype of FAP and still had his colon in situ. In the remaining patients, the median time taken from primary surgery to DT development was 24 months (range 0 – 48 months) and the median time for progression to air-fluid level from desmoid diagnosis was 24 months (range 0 – 226 months). Figure 6.1 displays a typical IAD containing an air-fluid level on CT.



Fig 6.1 – Typical example of IAD with AFL on CT

In the nine patients, IAD diameter ranged from 10cm to over 20cm (Table 6.2). Possible communication from the IAD to the small bowel was reported on cross-sectional imaging in 8 patients; unusually there was communication to both the small bowel and the reproductive tract in an isolated case. This patient presented with discharge of enteric contents from the vagina.

Patients	DT max diameter (cm)	Communication	Microbiology culture from DT	Primary Procedure	Secondary Procedure
1	>20	Small bowel & Uterus	Coliform	Percutaneous drain	Surgery
2	17	Small bowel	Coliform	Percutaneous drain	-
3	15	Small bowel	Coliform	Surgery	-
4	>20	Small bowel	E. Coli & VRE	Percutaneous drain	Surgery
5	11	Small bowel	Coliform	Percutaneous drain	-
6	12	Small bowel	Nil	Non operative	-
7	>20	Small bowel	Coliform	Surgery	-
8	10	Nil	Coliform	Surgery	-
9	>20	Small bowel	Nil	Non operative	-

Table 6.2 - DT characteristics

Two patients presented with fever and abdominal pain and were successfully treated with antibiotics alone. Intravenous antibiotics were administered in accordance with our local guidelines for intra-abdominal sepsis (Co-amoxiclav 1.2g tds (or cefuroxime 1.5g tds if penicillin allergic) and metronidazole 500mg tds).

Four patients, who presented with abdominal pain and sepsis, were initially treated with intravenous antibiotics but failed to improve and underwent percutaneous drainage of AFL. However, this treatment was successful in

only two. Of these, ultrasound guided percutaneous drainage sufficed in one but the other required a small surgical incision to insert a drain. This patient had a hard rim of desmoid visible on ultrasound scan, which could not be traversed with a standard percutaneous drain.

The two patients who failed to settle after percutaneous drainage had multiloculated AFL on CT scan and developed progressive sepsis ultimately requiring surgery, which involved excision of desmoid with enterectomy in one and loop jejunostomy in the other. The latter patient has since successfully undergone multivisceral small bowel transplant.

The remaining three patients presented with generalised peritonitis and underwent urgent laparotomy. One had necrotic IAD with AFL and required drainage only. One required drainage of desmoid with loop jejunostomy that was later reversed. One patient underwent excision of desmoid with loop jejunostomy but developed progressive disease and died while awaiting multivisceral small bowel transplant. The treatment of the nine patients and outcomes are summarised in table 6.3.

Most invasive intervention	No of patients	Morbidity	30 day Mortality
Antibiotics only	2	Nil	Nil
Percutaneous drainage	2	Nil	Nil
Operative	5		
<i>Excision ± Proximal defunctioning</i>	3	1 (EC Fistula)	1
<i>Drainage ± Proximal defunctioning</i>	2	Nil	Nil

Table 6.3 - Management & outcome of DT with air-fluid level

Microbiology culture data from the IAD was available for seven of the nine patients and mainly grew coliform organisms. Five of these seven patients underwent laparotomy, desmoid enteric fistula was found in four and one patient only had necrotic desmoid, without connection to bowel.

Patient 1 developed an enterocutaneous fistula (ECF) through mesh placed to the reconstruct abdominal wall, following excision of desmoid of diameter greater than 20cm. This patient is currently awaiting further surgery for the ECF. The other patients had no significant morbidity.

6.5 Discussion

The complication of an air-fluid level in FAP related IAD is a rare phenomenon – only nine patients in this series (4%), but this represents approximately half of the significant morbidity associated with these desmoids. However this prevalence may be an overestimation as St Mark's Hospital is the tertiary centre specialising in these tumours and receives referral from across the country. Three out of nine patients in this series were referred from other units specifically for management of DT.

Apart from an isolated case report (Yu et al, 2009), the only publication focusing on this complication has been a case series of three patients. In that paper, Maldjian et al considered only percutaneous drainage of DT with abscess formation (Maldjian et al, 1995). To date, no studies have specifically addressed the management of DT communicating with the small bowel lumen, abscess formation or necrosis of DT, which has been termed DT with an air-fluid level in this study.

The mechanism by which an AFL develops within a DT is unknown. The development of an AFL involves a combination of central necrosis and liquefaction, coupled with either infection or communication with gas in the gut lumen. Microbiological data from this series, showing mainly coliform and E.coli, supports the source of infection being the translocation or communication between the DT and the bowel lumen, probably due to localised bowel ischemia/perforation. In four of the five patients who underwent surgery, a direct communication with small bowel (desmoid

enteric fistula) was found. The fifth patient had only necrotic desmoid with no obvious communication with bowel. However the fluid culture from this patient also detected coliform organism, suggesting the translocation of gut organism as explanation for the culture finding.

This series demonstrates a spectrum of management, ranging from conservative to highly interventional treatment ultimately leading to multivisceral transplantation. Church et al have described a classification system for IAD that may help with prospective management decisions. This system is based on DT size and resulting symptoms and described in table 6.4 (Church et al, 2005; Church et al, 2008).

Stage	Description
I	Asymptomatic, <10cm maximum diameter, and not growing
II	Mildly symptomatic, <10cm maximum diameter, and not growing
III	Moderately symptomatic or bowel/ureteric obstruction, or 10 to 20cm, or slowly growing
IV	Severely symptomatic, or >20cm, or rapidly growing

Table 6.4 - Desmoid tumour staging system (adapted from Church et al paper published in Diseases of colon and rectum, 2008 Jun; 51(6): 897-901)

All of the IAD in patients in this series with air-fluid levels had stage III or IV disease. Of the three patients with stage III disease, two were successfully treated with percutaneous drainage and antibiotics and one was successfully treated with antibiotics alone. Out of six remaining patients with stage IV

IAD, one was successfully treated with antibiotics alone and the rest required surgery. The two patients with the largest IAD, both larger than 20cm in diameter, were considered for small bowel multi-visceral transplantation. One patient underwent a transplant, and the other died while awaiting it. From our series, size of DT alone could not predict the need for surgery as one of the patients managed by percutaneous drainage had an IAD measuring 17cm and other managed with antibiotics alone had IAD of diameter more than 20cm.

Desmoid tumour with AFL presents a management challenge in clinical practice. Cytotoxic chemotherapy cannot be used in this situation due to underlying sepsis. Surgery is often complex requiring resection of large segment of bowel, defunctioning or bypass.

6.6 Summary

The main limitation of this study is the small patient number, however, desmoid tumours are rare and only a small number of desmoid patients present with this condition.

Based on the outcome of this study, a management algorithm for IAD patients with an air-fluid level is proposed (Figure 6.2). A small number of patients who are symptomatic but not severely septic (two in this cohort) can be managed with antibiotics alone. Patient presenting with sepsis and IAD with AFL, but not peritonitic on clinical assessment, can be managed with radiologically or surgically placed drains. Patients with suspicion of a hard

DT, where the DT has more than a two-centimeters solid rim on ultrasound or CT scanning, or anatomical access is hazardous, are difficult to drain percutaneously. In addition multi-locular collections and viscosity of these fluid may also hinder drainage. Open drain placement can overcome some of these obstacles. Failure of drainage or generalized peritonitis necessitates an open surgical approach usually requiring excision or drainage with or without defunctioning/exclusion.

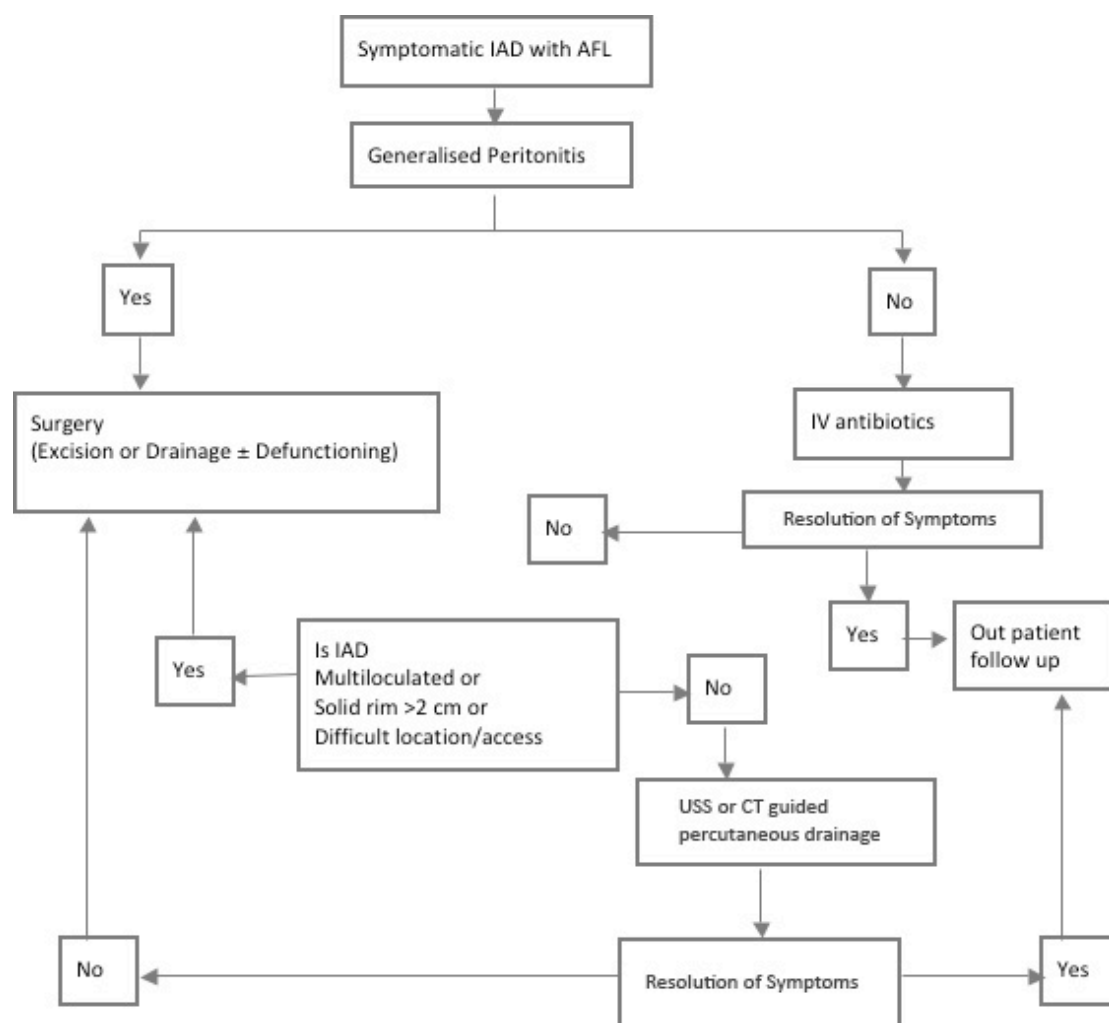


Fig 6.2 – Management algorithm for intra-abdominal desmoids with air-fluid levels

6.7 Conclusion

Management of IAD with air fluid level is complex often requiring surgery. However some patients can be managed with a more conservative approach of antibiotics alone or antibiotics combined with percutaneous drainage avoiding complex and potentially morbid surgery. We suggest managing these conditions in specialist centre on individual assessment basis and with involvement of multidisciplinary team.

PART 4 – LABORATORY STUDIES

Chapter 7

Laboratory materials and methods

7.1 Samples

Three desmoid cell lines (D1, D2 and D3) and one fibroblast cell line (C1) derived from patient with FAP but without desmoid have previously been established. These cell lines were established from tumour and tissue biopsy from FAP patients with prior ethical approval (Harrow Research and Ethics Committee, REC ref No: 08/H0719/75; 09/H0709/13) and patient consent (Sinha et al, 2010). These were first subjected to 'second hit' *APC* mutation analysis (Chapter 8).

The confirmed FAP desmoid cell line (D3) along with FAP fibroblast control (C1) and a commercially available established human fibroblast cell line from human foreskin (C2) were then used for choline metabolism and Wnt signal pathway studies. Formalin fixed paraffin embedded sections of the same desmoid tumour were used for immunohistochemistry. The desmoid tumour sections were obtained from the histopathology department at St Mark's Hospital.

7.2 Reagents

7.2.1 Tissue culture reagents

All reagents were sterile and of tissue culture grade.

Dulbecco's Modified Eagle's Medium (DMEM) containing, low glucose, pyruvate, L-glutamine (Cat no: 31885-023, Gibco Invitrogen, Paisley, UK)

10% Foetal Bovine Serum (FBS, First Link, UK)

Antibiotics (100Unitsml⁻¹ penicillin, 100µgml⁻¹streptomycin, Invitrogen, UK)

0.25% Trypsin-EDTA, Gibco (Invitrogen, UK)

Phosphate buffered saline (PBS), Gibco (Invitrogen, UK)

Bambanker Cell Freezing Media (Anachem,UK)

All plastic-ware for TC (Invitrogen, UK)

7.2.2 DNA purification reagents

10mM NaCl/10mM EDTA

75mM NaCl/25mM EDTA

10% SDS

20mg/ml Proteinase K

25:24:1 phenol:chloroform:isoamyl alcohol

24:1 chloroform:isoamyl alcohol extraction mix

3M sodium acetate (NaOAc)

100% ethanol

TE buffer

7.2.3 RNA purification reagents

Purification of total RNA from cells were performed using RNeasy Mini Kit (Qiagen, UK)

7.2.4 cDNA synthesis reagents

First strand cDNA was synthesised by using Superscript™ First-Strand Synthesis System for RT-PCR (Invitrogen, UK).

7.2.5 Real time polymerase chain reaction (RT-PCR) reagents

5X Green Go Taq® Flexi buffer (Promega UK)

25mM MgCl₂

PCR Nucleotide mix (Promega, UK)

Forward Primer

Reverse Primer

GoTaq® DNA Polymerase (Promega, UK)

Nuclease-Free Water

Agarose (Invitrogen, UK)

Ethidium Bromide

Loading sample buffer

500bp and 1Kb DNA ladders

7.2.6 Quantitative RT-PCR (qPCR) reagents

qPCR for choline kinase alpha (CHKA) expression was performed by using Platinum[®] SYBR[®] Green qPCR Supermix-UDH with ROX (Invitrogen).

qPCR for *WNT11* and *AXIN2* expressions were performed by using PerfeCTa[®] SYBR[®] Green SuperMix, Low ROX[™] (Quanta Biosciences).

7.2.7 SDS-PAGE Reagents/ Western Blot Reagents

Tetramethylethylenediamine (TEMED)

30% (w/v) Acrylamide/bis-acrylamide

10% Ammonium persulphate (APS)

10% Sodium Dodecyl Sulphate (SDS)

1.5M Tris, pH 8.8 (resolving gel)

1.0M Tris pH 6.8 (30.285g TRIS, 200ml ddH₂O) (stacking gel)

Double distilled water (ddH₂O)

Prestained Precision Plus protein standard

Protran Nitrocellulose membrane (GE Healthcare, UK)

Stripping Buffer

Blocking solution (3% Albumin, 1% Albumin from chicken egg white in TBST)

10x Running Buffer (30.3g TRIS, 188g Glycine in 1l ddH₂O). For 1x solution 10ml 10% SDS was added.

Transfer Buffer (5.82g TRIS, 2.93g Glycine, 3.75ml 10% SDS, 200ml MeOH, ddH₂O was added to make 1l solution)

ECL Western Blotting Substrate (Thermo Fisher Scientific, UK)

Amersham Hyperfilm ECL (GE Healthcare, UK)

7.2.8 β -catenin indirect immunofluorescence staining reagents

4% Paraformaldehyde

Purified mouse anti- β -catenin (BD Transduction Laboratories, UK, 1:400 dilution)

Alexa fluor-488 goat anti-mouse IgG (Invitrogen, UK, 1:500 dilution)

DAPI staining for cell nuclei (Sigma- Aldrich, UK)

7.2.9 Immunohistochemistry reagents

Histoclear

100% Ethanol, 70% Ethanol, ddH₂O, 1.5% H₂O₂ in methanol

10mM Sodium Citrate Buffer, pH 6

1x phosphate buffered saline (PBS)

1.5% Horse Serum

VectaStain Elite ABC reagent

DAB (3,3' Diaminobenzidine) substrate (DAKO, UK)

Haematoxylin

Pertex Mounting Media (Leica Biosystems, UK)

7.3 Antibodies

Summary of antibodies used for the studies is listed below.

Antigen	Specificity	Antibody type	Use	Dilution	Raised in	Company
Wnt-11	Human/Mouse	PAb	IC IH WB	1:1000 1:1000	Goat	R&D System
β -catenin	Human, Dog, Rat, Mouse, Chick	MAb	WB IC IH	1:500	Mouse	BD Transduction
HSP60	Human, Mouse, Rat, bovine, canine, chicken, drosophila, guinea pig, hamster, monkey, pig, rabbit, sheep, xenopus	MAb	WB	1:1000	Mouse	Stressgen
Peroxidase conjugated anti-mouse	Mouse	PAb	WB	1:10000	Donkey	Jackson Immunoresearch
HRP conjugated anti-goat	Goat	PAb	WB	1:10000	Donkey	Santa Cruz Biochem

Table 7.1 - Summary of antibodies

7.4 Primers for RT-PCR and qPCR

AXIN2 (for RT-PCR)

Forward: TTATGCTTTGCACTACGTCCCTCCA

Reverse: CGCAACATGGTCAACCCCTCAGAC

AXIN2 (for qPCR)

Forward: AAGTGCAAACCTTTCGCCAAC

Reverse: ACAGGATCGCTCCTCTTGAA

***WNT11* (for RT-PCR)**

Forward: CGATGCTCCTATGAAGGTGAAA

Reverse: CTTCCGTTGGATGTCTTGTG

***WNT11* (for qPCR)**

Forward: AGACCGGCGTGTGCTATG

Reverse: CACCTGTGCAGACACCAGAC

***CHKA* (for qPCR)**

Forward: CGGAAGTATCCCACCAAGAA

Reverse: TCCCCAGAGGAAATGAGATG

***GAPDH* (for RT-PCR control)**

Forward: TGTTGCCATCAATGACCCCTT

Reverse: CTCCACGACGTACTCAGCG

***PPIA* (house keeping gene for *CHKA* qPCR)**

Forward: CTGCACTGCCAAGACTGA

Reverse: CATTCCTGGACCCAAA

***36B4* (house keeping gene for *AXIN2* and *WNT11* qPCR)**

Forward: GTGTTGACAAATGGCAGCAT

Reverse: AGACACTGGCAACATTGCGGA

7.5 General methods

7.5.1 Cell culture

The frozen cells were thawed in a water bath at 37°C and immediately transferred to a 10cm petri dish containing cell culture medium (DMEM with 15% FCS with penicillin V and streptomycin). Cells were re-fed every 48 hours. After confluence, cells were detached from the culture plate by adding 3ml trypsin solution and four times the volume of culture medium (12ml) was then added to neutralise trypsin. The cell suspension was then centrifuged at 1000rpm for 5 minutes, the supernatant was discarded and the cell pellet was re-suspended in culture medium. Cells were then divided and passaged at 2×10^5 cells per plate.

7.5.2 Cell freezing

After centrifuging the cells as described in section 7.5.1, the cell pellet was re-suspended in 1ml cell freezing solution and transferred in a cryovial. The cryovial was then gradually frozen at -80°C and subsequently stored in liquid nitrogen after 48 hours.

7.5.3 DNA extraction

Pelleted cells were first re-suspended in 10mM NaCl/10mM EDTA (500µl) and centrifuged at 13000rpm for one minute. The supernatant was discarded and 75mM NaCl/25mM EDTA (500µl), 10%SDS (50µl) and Proteinase K (25µl)

was then added to cells and left for overnight incubation at 37°C. Next day phenol/chloroform/isoamyl alcohol (25:24:1, 500µl) was added to the solution, vortexed briefly and centrifuged at 13000rpm for 1 minute. The top layer of the solution was then carefully transferred to a new tube and chloroform/isoamyl alcohol (24:1, 500µl) was then added to the solution, mixed well and centrifuged at 13000rpm for 1 minute. The top layer of supernatant was then carefully transferred to a new tube and 100% EtOH (1ml) and 3M NaOAc (50µl) was then added to the tube and gently mixed until precipitated DNA could be seen floating. The solution was spun down at 13000rpm for 2 minutes; the supernatant was carefully discarded without disturbing the pellet. 70% ethanol (1ml) was then added to the pellet, vortexed very gently and spun down at 13000rpm for 2 minutes. The supernatant was discarded and the pellet was resuspended in 50µl of TE and incubated at 56°C for 1-2 hours to fully dissolve the DNA. The concentration of DNA was measured using a Nanodrop spectrophotometer and further diluted by adding TE to obtain a final concentration of 50ngµl⁻¹.

7.5.4 Purification of total RNA

Cells were grown to confluence and pelleted as described in section 7.5.1. Pelleted cells were lysed by adding buffer RLT (Qigen) (350µl) and homogenised by centrifugation at 30000rpm for 2 minutes in a QIA shredder spin column placed in a collection tube. 70% alcohol (350µl) was added to the homogenised lysate and mixed by pipetting. The sample (700µl) was then transferred to an RNeasy spin column placed in a collection tube and centrifuged for 15s at 15000rpm and the flow-through was discarded. On-

column DNA digestion was performed by washing the RNeasy spin column membrane first with buffer RW1 (Qigen) (350µl) and centrifuging at 15000rpm for 15s and then adding DNase I incubation mix to the spin column membrane at room temperature for 15 minutes. The membrane was further washed by adding buffer RW1 (350µl) and buffer RPE (Qigen) (500µl) and centrifuging each time at 15000rpm. RNA was then eluted on a collection tube by adding 30µl of RNase-free water to the spin column membrane and centrifuging at 15000rpm for one minute. Total RNA thus eluted was then quantified by measuring in a NanodropTM spectrophotometer (Thermo Scientific). The quality of the isolated RNA was determined by measuring the optical density (OD) in the Nanodrop. By determining the 260/280 ratio > 2, pure RNA isolation and low protein contamination was ensured.

7.5.5 cDNA preparation

The RNA/primer mixture was first prepared by adding 3µg RNA, 1µl 10mM dNTP mix, 1µl Random Hexamer (50ng/µl) and DEPC-treated water to make 10µl total volume and incubated at 65°C for five minutes and immediately cooled on ice for one minute. The reaction mix prepared by adding 2µl 10x RT buffer, 4µl 25mM MgCl₂, 2µl 0.1M DTT, 1µl RNaseOUTTM (40U/µl) was then added to RNA/primer mixture and incubated at 25°C for 10 minutes. The mixture was further incubated at 42°C for 50 minutes after adding 1µl SuperScriptTM II RT and then at 70°C for 15 minutes and chilled on ice. cDNA synthesis was completed by adding 1µl RNase H to the mixture and incubating at 37°C for further 20 minutes. The synthesised cDNA was either used immediately for PCR or stored at - 20°C for future use. All the thermal

cycles described were performed using Gene Amp® PCR System 2700 (Applied Biosystems).

7.5.6 RT-PCR

2µl cDNA was added to 23µl master mix to make 25µl PCR solution. The master mix was prepared by adding the following reagents.

5x Green Go Taq Buffer 5µl

25µm MgCl₂ 1.5µl

PCR nucleotide mix (dNTPs) 0.5µl

Forward primer 0.5µl

Reverse primer 0.5µl

Go Taq polymerase 1.25µl

RNase free water 13.75µl (to total volume 25µl)

RT-PCR was carried out using Gene Amp® PCR System 2700 (Applied Biosystems) by using the following thermal cycles:

94°C for 5 minutes

(94°C for 30s, 60 °C for 30s, 72°C for 30s) for 30 cycles

72°C for 10 minutes

5µl aliquots of the RT-PCR product were then resolved on a 1% agarose gel containing ethidium bromide. 500bp and 1kb DNA ladders were included on each gel.

7.5.7 Quantitative Real Time PCR (qPCR)

qPCR for *CHKA* expression was performed in a 7900HT Fast Real Time PCR System (Applied Biosystems, UK) and analysed using Applied Biosystems SDS software V2.4. Platinum@SYBR@Green qPCR Supermix-UDH with ROX (Invitrogen, UK) and invitrogen probes specific for *CHKA* were used as per manufacturer instructions. *PPIA* was used as house keeping gene. Reactions were carried out in triplicate, each in 10µl volumes. Thermal cycling condition for qPCR was as follows: 2 minutes each at 50°C and 95°C; then 3s at 95°C and 30s at 60°C for 40 cycles followed by dissociation for 15s each at 95°C and 60°C.

qPCR for *AXIN2* and *WNT11* expressions were performed in a ViiA™ 7 System (Applied Biosystems) using using PerfeCTa® SYBR® Green SuperMix, Low ROX™ (Quanta Biosciences). 36B4 gene was used as housekeeping gene. Reactions were carried out in triplicate, each in 25µl volumes. Thermal cycling condition for qPCR was as follows: 2 minutes at 95°C; then 15s at 95°C and 30s at 60°C for 40 cycles followed by dissociation for 15s each at 95°C and 60°C.

7.5.8 Protein extraction

Lysis buffer was first prepared by adding 1ml 1x RIPA buffer, 10µl Protein Inhibitor Complex (PIC) and 4µl EDTA reagents. 500µl of the lysis buffer was then added to the confluent cells in a 10cm culture plate and the lysate was collected by scraping. The solution was then centrifuged at 13000rpm for 30

minutes at 4°C. The supernatant was collected in a tube and either used immediately for western blot analysis or stored at -20°C for future use.

RIPA Buffer (Radio Immuno Precipitation Assay buffer)

150mM sodium chloride

1.0% NP-40 or Triton x-100

0.5% sodium deoxycholate

0.1% SDS (sodium dodecyl sulphate)

50mM Tris, pH 8.0

7.5.9 Western blotting

7.5.9.1 *Sample preparation*

Protein samples were added to 2x Laemmli buffer (120mM Tris pH 6.8, 4% SDS, 10% β-mercaptoethanol, 10% glycerol, 0.004% bromophenol blue), boiled for 5 minutes at 95°C.

7.5.9.2 *Sodium dodecyl sulphate polyacrylamide gel electrophoresis*
(SDS-PAGE)

For gel recipes, please see section 18.53 of Molecular Cloning (Sambrook et al, 1989). Proteins were separated on 8% Tris-Glycine SDS polyacrylamide gels. 5µl pre-stained precision plus protein standards were loaded alongside the samples to allow determination of protein band sizes. Gels were run in

protein gel tanks (Bio-Rad, UK) in 1x Tris-glycine running buffer for approximately 2 hours at 80mV.

1xTris-glycine running buffer

25mM Tris base

190mM glycine

0.1% SDS

7.5.9.3 Transfer of proteins to nitrocellulose membrane

After electrophoresis, the separated proteins were transferred onto nitrocellulose transfer membrane (ProtranTM, Whatman, GE Healthcare) in transfer buffer using a Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad, UK) at 10V for 30 minutes. After transfer the membrane was washed with 1x TBST and blocked at room temperature for one hour in blocking solution.

Primary antibody diluted in blocking solution was applied directly to the membrane and the membrane was incubated at 4°C overnight on a slow rocker. The membrane was then washed for 3x 10 minutes in 1x TBST and appropriate horseradish peroxidase (HRP) conjugated secondary antibody diluted in blocking solution was added to it. The membrane was then incubated at room temperature for at least one hour and washed in 1x TBST for 3x 10 minutes. Enhanced chemiluminescence (ECL) substrate (Amersham) was applied to the membrane and the protein bands were detected by X-ray film.

Transfer Buffer

5.82g Tris base

2.93g Glycine

3.75ml SDS (10%)

200ml methanol

Water up to 1L

1x TBST (Tris-Buffered Saline with Tween)

25mM Tris pH 7.4

150mM NaCl

2mM KCl

0.1% Tween-20

7.5.10 β -catenin immunocytochemistry

Cells grown on coverslips were first washed with PBS 2 times and fixed with 4% formaldehyde for 15 minutes at room temperature. The cells were then washed 3x 5 minutes with PBS and permeabilised with 0.1% Triton-x100 in PBS for ten minutes at room temperature. The cells were further washed with PBS 3x 5 minutes and blocked with 1% BSA/0.1% Triton/PBS (PBST-BSA) for one hour at room temperature. The appropriate dilution of primary antibody in PBST-BSA was then added to the cells and incubated overnight at 4°C. Next day the cells were washed with PBS 3x10 minutes and incubated with appropriate dilution of secondary antibody in PBST-BSA for one hour at room

temperature in the dark (foil covered). The coverslips were then washed with PBS 3x10 minutes and mounted onto slides using a wet mount technique (with DAPI to stain nuclei) and the slides were analysed using a Zeiss Fluorescent microscope.

7.5.11 Immunohistochemistry

7.5.11.1 *Dewaxing*

All the slides were placed in a metal slide holder and placed in each of the following solutions for five minutes:

Histoclear

Histoclear

100% ethanol

100% ethanol

70% ethanol

The slides were then placed in a pot of distilled water for three minutes.

7.5.11.2 *Antigen retrieval*

The slides were transferred to a plastic holder containing in pre-warmed Trisodium citrate solution (pH 6) and microwaved (750W) for 6 minutes. The box containing the slides were then cooled down by placing in a box of distilled water placed on a shaker for five minutes at room temperature.

Tri-sodium citrate solution

0.5g Tri-sodium citrate crystals

500ml distilled water

HCl added to pH 6

7.5.11.3 Blocking endogenous peroxidase activity

The slides were placed in 1.5% H_2O_2 in methanol (50ml Methanol, 750 μl H_2O_2) for 30 minutes and washed three times in PBS.

7.5.11.4 Primary antibody staining

The slides were first blocked with 1.5% horse serum for one hour. The appropriate dilution of primary antibody prepared in horse serum was then added to the slides and the slides were incubated overnight at 4°C.

7.5.11.5 Secondary antibody staining

The slides from step 7.5.10.4 were first washed 3x5 minutes in PBS solution. The appropriate dilution of secondary antibody (Vector laboratory pan specific biotinylated anti-mouse, anti-rabbit, anti-goat antibody) in horse serum was then added to the slides. The slides were left at room temperature for 30 minutes and washed 3x5 minutes in PBS solution. The slides were then stained with VECTASTAIN® Elite ABC reagent (Vector laboratories, UK) for

30 minutes at room temperature. After 30 minutes the slides were washed 3x5 minutes in PBS solution.

7.5.11.6 *Diaminobenzidine (DAB) reaction*

The slides were stained for 2-5 minutes using high-sensitivity substrate chromogen system (liquid DAB substrate, Dako) while constantly checking under microscope for development of appropriate level of chromogen signal intensity (brown stain). The slides were then washed twice with distilled water and counterstained in Haematoxylin for 1-2 minutes. The slides were washed again two times in distilled water.

7.5.11.7 *Dehydration*

The dehydration step was completed by dipping the slides in the following solutions for 5 minutes each

70% Ethanol

100% Ethanol

100% Ethanol

Histoclear

Histoclear

The slides were then immediately mounted using the pertex mounting medium (Leica Biosystems) and studied under the microscope.

Chapter 8

***APC* mutation analysis in FAP desmoids and fibroblast cell lines**

8.1 Background and aims

Cell culture is a commonly used laboratory technique to characterise animal cells and investigate tumorigenesis. Culture of desmoid cell lines provides a unique opportunity to investigate these rare tumours in vitro. However, there is no commercially available established desmoid tumour cell line. Previous attempts at primary cell culture of desmoid have proven difficult due to overgrowth of fibroblasts (Sturt et al, 2004). Recently Sinha et al described a model of FAP desmoids and reported successful establishment of primary desmoid cell lines from surgical biopsy of tumours (Sinha A, 2010). Establishment of desmoid cell lines as opposed to fibroblast overgrowth was confirmed by the presence of nuclear β -catenin in the desmoid cell lines (Sinha A, 2010). β -catenin is an important regulator of the Wnt signaling pathway and is upregulated in FAP desmoid cells owing to mutations in both alleles of the *APC* gene.

The studies described in this chapter and subsequent chapters uses primary desmoid tumour cell lines created and stored by Sinha et al. A major problem in primary cell lines established from desmoid tumour biopsy is the

overgrowth of background fibroblasts with subsequent passage of cells in the culture. To clarify this possibility, this study aimed to confirm the true selection of desmoid cells in culture by performing whole sequence *APC* gene mutation analysis.

According to Knudson's 'second hit' hypothesis, both alleles of *APC* should be mutated in a desmoid tumour. Previous studies have demonstrated that desmoid in FAP are monoclonal proliferations, and the 'second hit' somatic mutations have been identified and characterised in these tumours (Middleton et al, 2000; Lamlum et al, 1999). Hence, the identification of two mutations in the cells in culture would confirm that the growing cells are true desmoid cells and not the overgrowth of contaminating fibroblasts.

8.2 Materials and methods

8.2.1 Samples

Three primary desmoid tumour cell lines (D1, D2 and D3) and one primary FAP fibroblast cell line (C1) established and stored by Sinha et al (2010) were subjected to *APC* mutation analysis. The primary desmoid cell lines were established from surgical excision of desmoid tumours and the primary fibroblast cell line was established from mesenteric biopsy of a patient with FAP but free of desmoid tumour. The cell lines were established using an explant technique (Sinha A, 2010). Ethical approval from the Harrow Research and Ethics Committee along with research and development approval from the North West London Hospitals NHS Trust and informed

consent from the patients were obtained for the study. Table 6.1 outlines the patient characteristics and known germline *APC* mutations in the patients.

Samples	Patient age (Yrs)	Tumour Site	Mutation
D1	22	AWD	c.4666dupA p. Thr1556Asn fsX3
D2	27	IAD	c.4226delC
D3	34	AWD	c.3393G>T p.Glu1132X
C1	27	Biopsy from mesentery	c.4226delC

Table 8.1 - Patient characteristics and known germline mutations

8.3 Cell culture

Frozen vials of the desmoid cell lines (D1, D2 and D3) and fibroblast cell line (C1) were thawed and cell culture was performed as described in section 7.5.1. Two million cells per cell line were pelleted and subjected to DNA extraction for *APC* mutation analysis.

8.4 DNA extraction

DNA was extracted from the samples using the protocol described in section 7.5.3.

8.5 High-throughput method for whole gene sequencing of *APC*

A high-throughput PCR method was used for sequencing all fifteen coding exons of the *APC* gene for full mutation screening. The primers used for PCR and sequencing was optimised to amplify at one temperature. Primer sequence used for this experiment is listed below:

<i>APC</i> exon1	Forward:	GAGTTTGTTCCTTTACCCCTTTC
	Reverse:	AAAGTGTCTTACCTCAAGTTTACAAGA
<i>APC</i> exon2	Forward:	TATATAAGGTGCGTGCTTTGAGAGT
	Reverse:	AAATCGAGAGAAGCTGTACTTGGA
<i>APC</i> exon3	Forward:	TGTTTTCAGTCATGTATATTTGTGG
	Reverse:	AAATGCAAAATGCTCTAAGTGTTA
<i>APC</i> exon4	Forward:	TGCTCTTCTGCAGTCTTTATTAGC
	Reverse:	AGGCCTAAAGTTGGGTAAACATA
<i>APC</i> exon5	Forward:	CACCATGACTGACGTATTTGCTTA
	Reverse:	AATAGAGCCAAAATAAACACAGCC
<i>APC</i> exon6	Forward:	CATAACCCTGAGCTTTTAAGTGGT
	Reverse:	TGTAAACTGACAGCTAAAGTAAGGTATCTA
<i>APC</i> exon7	Forward:	CTACACCATTTTTGCATGTACTGA
	Reverse:	CCATCTTGCTTCATACTTTTCTGA
<i>APC</i> exon8	Forward:	CAGACACTTCATTTGGAGTACCTTA
	Reverse:	TGTCTTAGCAAAGTAGTCATGGCA

APC exon9	Forward:	CACTGATTACTTCATCCTGGAAAG
	Reverse:	ACATTTGCTTTGAAACATGCAC
APC exon10	Forward:	TCCACTAAAAATCCGTGAATTAGG
	Reverse:	GATAACAGAAGTTGGTGGCCTTA
APC exon11	Forward:	CTGGCATAAAATGGAATAATTGTC
	Reverse:	GATAAGCGAATGTGAAGCACAGG
APC exon12	Forward:	AATAAAGCTTGGCTTCAAGTTGTC
	Reverse:	AGATTGCACAACCTGCCCTCTAAG
APC exon13	Forward:	GCTAGCCAGAATTTCTTTCTTAATAG
	Reverse:	GGTAAGAAATTAGGAAATCTCATGG
APC exon14	Forward:	TTCTTAATTTACCAGTGAGGGAC
	Reverse:	GGCTACACCTCTCAACTATAATTTGC
APC exon15_1	Forward:	GGAGATGTGGAATACTTGAATTTAT
	Reverse:	ATTATCATCATGTCGATTGGTGTC
APC exon15_2	Forward:	CAATATTATGTCTCCTGGCTCAAG
	Reverse:	AACATGTCCTATTTGAATTTTCCG
APC exon15_3	Forward:	AAGAAGTGTGAGCCATTCATACCT
	Reverse:	ACCTGTATGGAGAAACACATTCCT
APC exon15_4	Forward:	CCGACCTAGCCCATAAAATACATA
	Reverse:	CATATGTTTCGGTTTTACTGCTTTG

APC exon15_5 Forward:	AATGTAAGCCAGTCTTTGTGTC
Reverse:	AGTCTGCTGGATTTGGTTCTA
APC exon15_6 Forward:	ATCAGCTGAAGATGAAATAGGATG
Reverse:	CATTTACTGCAGCTTGCTTAGGT
APC exon15_7 Forward:	CTCAAACCAAGCGAGAAGTACC
Reverse:	ATTTCAATATCATCATCATCTGAATCATCT
APC exon15_8 Forward:	TAAGAATAATGCCTCCAGTTCAGG
Reverse:	CTGGTCCATTATCTTTTTTCACACG
APC exon15_9 Forward:	TTCTTACAGAAGGCAGAAGTACAG
Reverse:	CAGTAAGGAGTTCCTTCAATAGGC
APC exon15_10 Forward:	CCACAAAATACTGAATATAGGACACG
Reverse:	GAGGTTTACTTGGTTCTCCCTGT
APC exon15_11 Forward:	ACCCATACTTCAGAAACAATCCAC
Reverse:	CAGCAGCAGCTTGATGTAAACTAC
APC exon15_12 Forward:	GTGAAGATCTGACACTTGATTTG
Reverse:	AACAGGGCTTAATTCTGATTTAC
APC exon15_13 Forward:	TATTCCAGGAGTTCGAAATAGCTC
Reverse:	TGTTTGTTTGGTAAGGTTCTGTTG
APC exon15_14 Forward:	TTATCTCAACTTCCAAGGACATCA
Reverse:	TCAGAATGAGACCGTGCAATATC
APC exon15_15 Forward:	TCATTTGAATCTCTTTCTCCATCA
Reverse:	GTTAATGGGACAGTCCTCAATTCT

APC exon15_16 Forward:	CATGTGAACTCTATTTTCAGGAACC
Reverse:	GCTTGGGTTGTAATTAAAAGGAGT
APC exon15_17 Forward:	CAAAATAATCCTGTCCCTGTATCA
Reverse:	AGCAATAATTAAAGGAAGTTGGGA

To perform the PCR, all the primers were first spotted on 96-well plates using a robotic setup and a predefined protocol. PCR mix was made by adding 250µl Megamix royal, 25µl DNA (50ngµl⁻¹) and 375µl water for injection. The Tecan Evo 150 robot and endoware software package was used to aliquot predetermined amount of PCR mix to the primer spotted 96-well PCR plates. The PCR was performed with following set up in MJ thermo cycler (MJ Research, USA).

95⁰C for 5 minutes

94⁰C for 1 minute, 60⁰C for 1 minute, 72⁰C for 1 minute (30 cycles)

72⁰C for 10 minutes

The PCR product was then run on a 2% agarose gel to confirm that the reaction had worked. Individual PCR reactions were repeated for any failed reactions.

For DNA sequencing, the unconsumed primers and dNTPs were first removed by using ExoSAP-IT reagent. For this 5µl of PCR product was added to 2µl ExoSAP-IT and incubated at 37⁰C for 15 minutes and then 80⁰C for 15 minutes. The solution was then cooled to 4⁰C.

For individual sequencing plates, the sequencing mix was first prepared by adding Big Dye v1.1 (51µl), Big Dye buffer (178.5µl) and water for injection (735µl). Separate forward and reverse sequencing primer spotted plates were thawed and 9.5µl of sequencing mix was added to each well of the primer spotted plates. 0.5µl of post ExoSAP PCR product was then added into the sequencing plate and the thermocycle reaction was performed using the following set up:

96°C for 1 minute,

96°C for 10s, 50°C for 5s, 60°C for 4 minutes (25 cycles)

Hold at 4°C

Unincorporated Dye (ddNTPs) was removed from the sequencing product by using DyeEx 96 plate. The sequence was then run on an ABI 3730 sequencer using the standard sequencing protocol provided by Applied Biosystems.

DNA extraction, PCR and APC Sequencing described above was performed at the Kennedy Galton Centre, Northwick Park and St Mark's Hospital.

8.6 Results

8.6.1 APC mutation analysis in desmoid tumour cell lines (D1, D2 and D3)

The whole *APC* gene sequencing of the three primary desmoid tumour cell lines identified the germline mutations already known in the respective patients. However, a 'second hit' somatic mutation was identified only in the

cell line D3. Fig 8.1 demonstrates the result of APC sequencing of the D1 cell line and Fig 8.2 (a, b) demonstrates the result of APC sequencing of the D3 cell line.

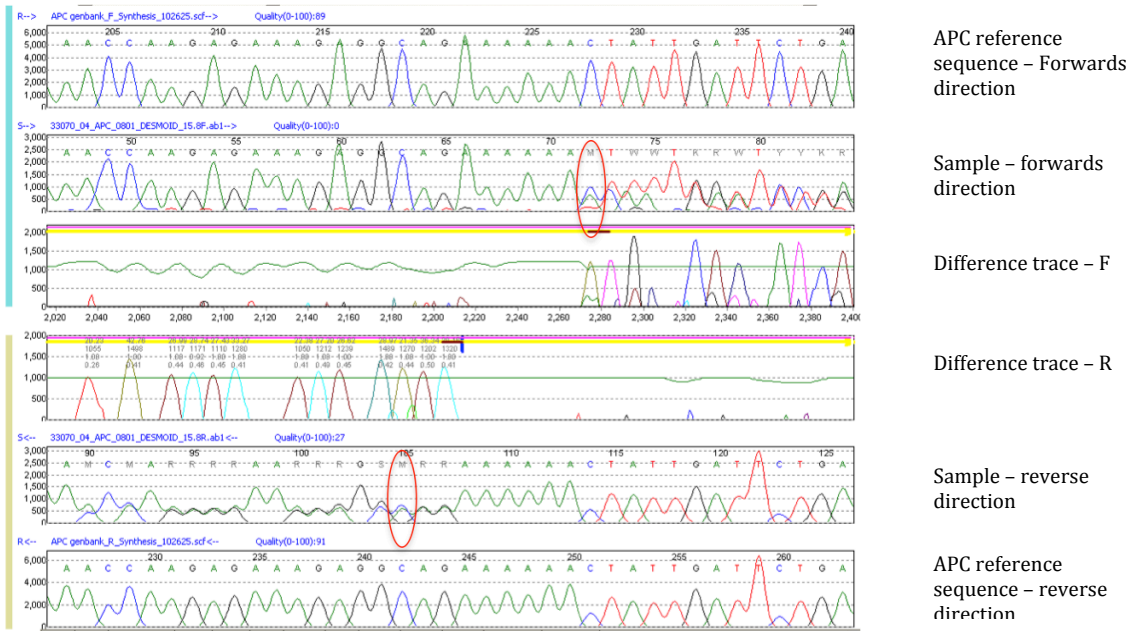
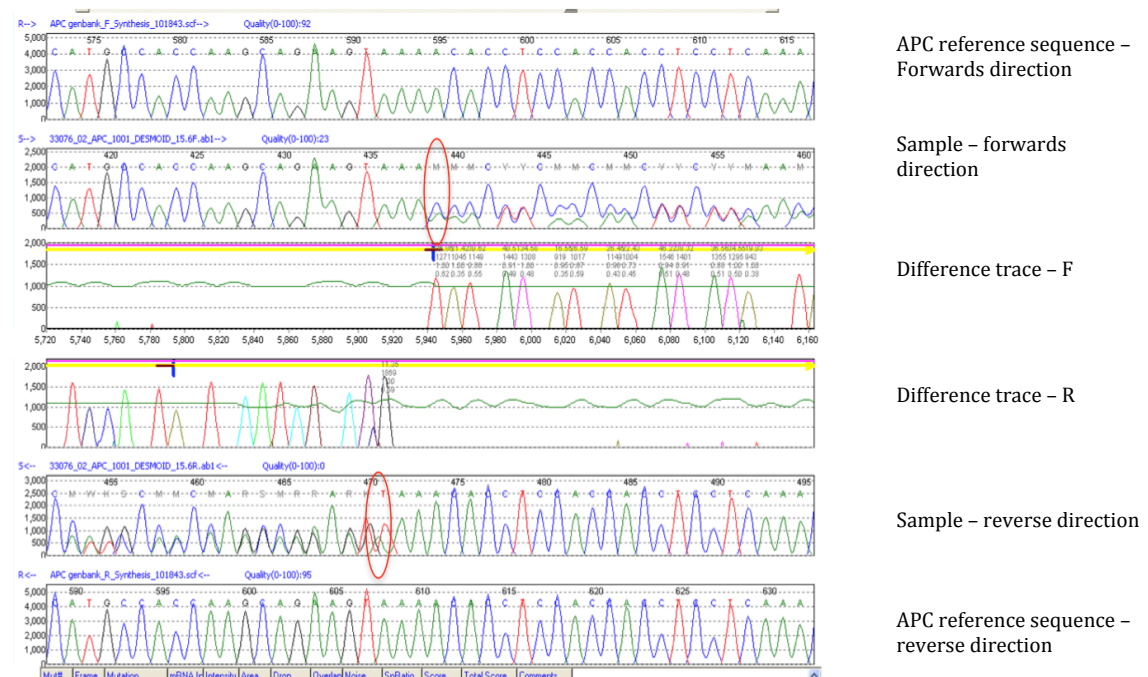


Fig 8.1 - DNA sequencing showing duplication of adenine (red circle) at nucleotide sequence no 4666 (c 4666dupA). The result of mutation analysis identified the known germline mutation in this sample; however, no ‘second hit’ somatic mutation was detected



A



B

Fig 8.2 - DNA sequencing showing glutamine replaced by thymine at nucleotide sequence 3393 (germline mutation, codon 1132) (A, red circle) and deletion of adenine at nucleotide sequence 4312 (somatic mutation, codon 1438) (B, red circle). The whole exon APC sequencing detected the known germline mutation and also a second hit somatic mutation in this sample

8.6.2 APC mutation analysis in FAP fibroblast cell line (C1)

APC sequencing only detected the known germline mutation in the patient. No other mutation in APC was detected. Fig 6.3 shows the result of mutation analysis in this sample.

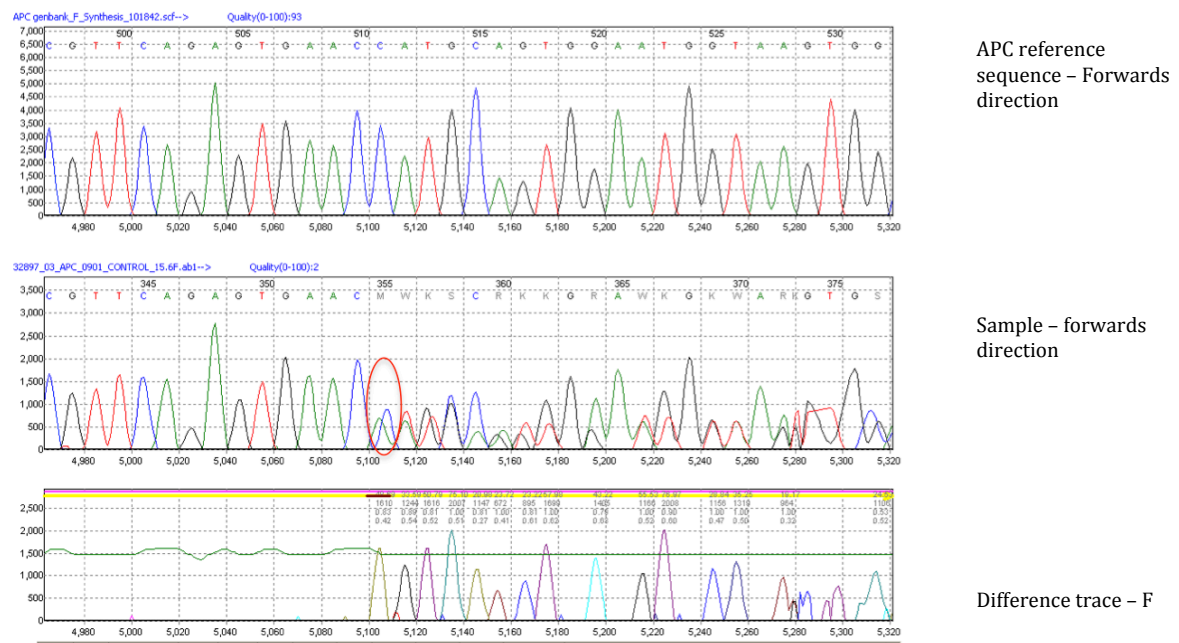


Fig 8.3 - DNA sequencing showing deletion of cytosine (red circle) at nucleotide sequence 4226 (c.4226delC)

8.7 Discussion

APC is a tumour suppressor gene, and consistent with Knudson's two-hit hypothesis, both alleles are mutated in FAP associated desmoid tumours; one in the germline and the other in the soma (Knudson, 1985). A relationship between the first (germline) and second (somatic) hit in desmoids has been previously described: desmoids from patients with germline mutations 5' to

codon 1400 are more prone to have a truncating somatic mutation 3' to codon 1400 and desmoids carrying a germline mutation 3' to 1400 are more likely to have loss of heterozygosity (LOH) as their second hit. Consistent with this hypothesis, desmoid cells in culture should also harbour two mutations in *APC*, one germline and one somatic mutation. In 2006 Martinico et al published the result of endostatin gene therapy in desmoid tumours by using primary desmoid tumour cell lines established from FAP patients. In this study, the selection of desmoid tumour cells in culture was confirmed by detection of two *APC* mutations (germ line and somatic) in the cells in culture (Martinico et al, 2006).

The results of *APC* mutation in the current study show that two of the cell lines established from desmoid tumour (D1 and D2) did not harbour an identifiable second hit somatic mutation. The likely explanation of this finding is that the desmoid cells have been overgrown and replaced by fibroblasts in these cell lines. As will be the case with every cell in the FAP patient, such fibroblast cells in culture harboured the known germline mutation. As expected, the fibroblast cell line C1 only harboured the known germline mutation.

On the other hand, desmoid tumour cell line D3 was found to harbour two mutations. One of these mutations (germline) was on codon 1132, which is not in the 'desmoid-prone mutation' region. Consistent with the known desmoid genetic and somatic mutation relationship, as explained above, however, the second somatic mutation in this cell line was detected in codon 1438, within the 'desmoid-prone mutation' region.

8.8 Summary

In summary the result of the *APC* mutation study in D3 cell line confirms that true desmoid cells are present in the culture and therefore D3 is a true desmoid tumour cell line.

Chapter 9

Study of choline metabolism in desmoid tumour in FAP

9.1 The choline metabolism pathway (Kennedy Cycle)

Choline is an essential nutrient in humans, primarily supplied by diet and absorbed in the intestine. It is a precursor for the biosynthesis of choline phospholipids such as phosphatidylcholine (PC), which is required for the formation and maintenance of cell membrane. In the Kennedy cycle, choline first enters the cell through the plasma membrane by passive transport diffusion or by choline-specific transport. It is then phosphorylated by the enzymatic effect of choline kinase (ChoK), resulting in phosphocholine (PCho). PCho is converted to CDP-Choline through the action of CDP-phosphorylcholine cytidyltransferase and subsequently to phosphatidylcholine (PC) through the action of DAG-choline phosphoryltransferase. Phosphatidylcholine-specific phospholipase D (PC-PLD) hydrolyzes PC to generate choline in addition to phosphatidic acid (PA), thus completing the cycle. The Kennedy cycle is outlined in Fig 9.1.

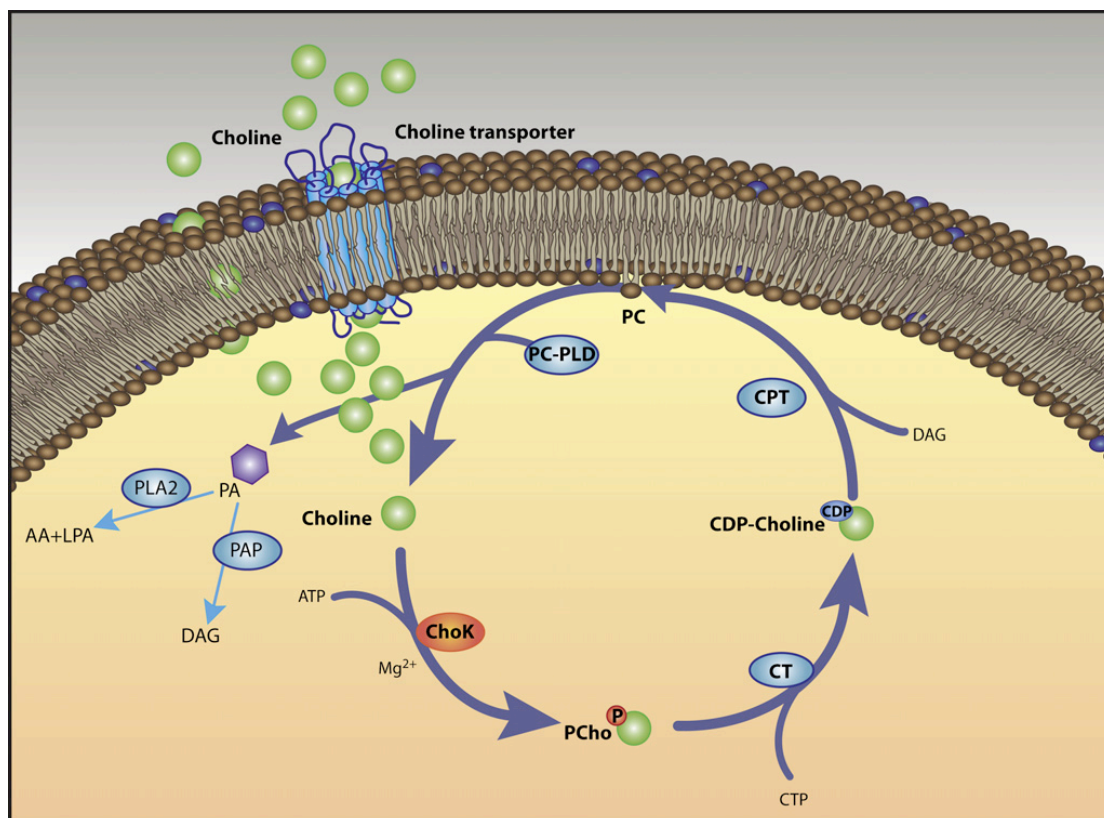


Fig 9.1 - Outline of the Kennedy pathway (adapted from Gallego-Ortega et al. *Advances in Enzyme Regulation*; 51 (2011), 183-194)

9.2 Choline metabolism in tumours

A high concentration of phosphocholine (PCho) is commonly detected in many cell lines derived from human tumours (Bhakoo et al, 1996). It has been suggested that an alteration of the phospholipid metabolism resulting in increased intracellular PCho levels leads to cell transformation to tumour (Cuadrado et al, 1993; Lacal et al, 1987). Studies using nuclear magnetic resonance (NMR), which are capable of detecting abnormal phospholipid metabolism, have demonstrated that the levels of PCho are much higher in many tumours in comparison to corresponding normal tissues (Aboagye and Bhujwalla, 1999; Eliyahu et al., 2007). All these studies have pointed at the

PCho as a malignancy marker useful for the diagnosis and monitoring of the cancer progress (Beloueche-Babari et al, 2010). Furthermore using PET technology, choline metabolism has been used for cancer diagnosis and more importantly for monitoring response to drug therapy in patients with breast cancer (Kenny et al, 2010).

Enhanced choline transport and ChoK mediated phosphorylation are the major mechanism of PCho accumulation in tumour cells (Glunde et al, 2004). Previous studies have also found that the ChoK, especially its isoform Choline kinase alpha (*CHKA*), is constitutively activated in many human tumour cells (Aoyama et al, 2004). In other studies, *CHKA* is found to act as prognostic factor in cancers such as lung and bladder (Ramirez de Molina et al, 2007; Hernando et al, 2009).

Though non-metastasizing, desmoid tumours are locally invasive. Choline metabolism in desmoid tumour has not been investigated before.

9.3 Hypothesis and aim

The aim of this study was to investigate *CHKA* as a marker of choline metabolism in desmoid tumour using the in-vitro model. Detection of over expressed *CHKA* would then allow for further studies of the choline metabolism pathway in these tumours. This may ultimately offer the opportunity to explore the potential role of choline based PET scan in the early identification of aggressive desmoids.

9.4 Materials and methods

9.4.1 Samples

One confirmed desmoid cell line (D3), one FAP fibroblast cell line (C1) and one non -FAP fibroblast cell line (C2) were used for this study.

9.4.2 Methods

Cell culture (section 7.5.1), RNA extraction (section 7.5.4) and cDNA synthesis (section 7.5.5) was performed as described in the chapter 7. *CHKA* expression was studied by qPCR using Platinum@SYBR@Green qPCR Supermix (Invitrogen, UK) in ABI prism 7900 HT Sequence Detector System (Applied Biosystems). Primer sequences (section 7.4) and thermal cycles used for qPCR (section 7.5.7) are detailed in chapter 7. qPCR reactions were performed in triplicates and analysed using SDS software V 2.4 (Applied Biosystems). To determine the mRNA fold change of *CHKA* the $\Delta\Delta C_t$ quantitation method was used taking *PPIA* as house keeping gene.

9.5 Results

The mRNA expression analysis by qPCR did not detect significant up regulation of *CHKA* in the desmoid tumour cell line. The result of this experiment is summarised in fig 9.2.

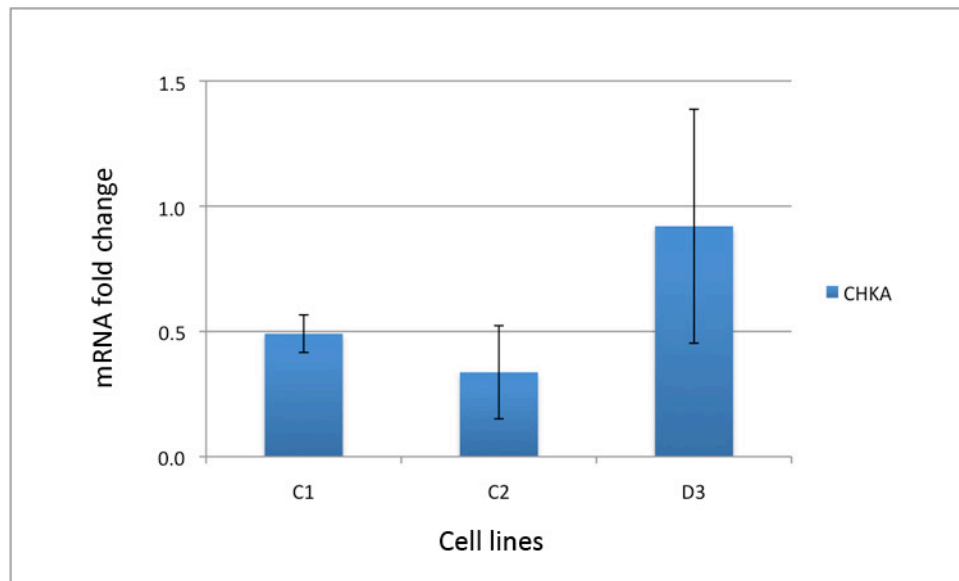


Fig 9.2 - qPCR for *CHKA* in desmoid tumour cell line (D3) and FAP fibroblast cell line (C1) in relation to non-FAP fibroblast cell line (C2) normalised to endogenous control *PPIA*; means and SD are shown

9.6 Conclusion

While increasing evidence in the literature has pointed towards the role of *CHKA* in cell proliferation, transformation and carcinogenesis and supported the use of this enzyme as a novel target for treatment of various tumours, this did not seem to be the case in the desmoid tumour cell line in the current study.

However only one desmoid cell line was used and there were no cell lines to compare between the aggressive and relatively benign desmoid tumours. The cell line used in this study was established from an AWD with relatively benign course. It may be possible that the *CHKA* is selectively more active in the aggressive form of the tumour. Further studies are needed using the cell

lines established from quiescent and aggressive desmoid tumours to compare and confirm whether or not choline metabolism is activated in these tumours.

Chapter 10

In vitro studies of Wnt signalling pathways in desmoid tumour in FAP

10.1 Background

Wnt proteins are secreted morphogens that are required for basic developmental processes such as cell fate specification, cell proliferation and the control of asymmetric cell division. Based on their biological activity, the Wnt proteins are primarily divided into two classes. The canonical Wnts (Wnt1/Wg class) which include Wnts 1, 3, 3a and 8 and the non-canonical Wnts (Wnt5a class) which include Wnts 4, 5a, 5b and 11. Based on these proteins, two Wnt signalling pathways are described, canonical Wnt signalling and non-canonical Wnt signalling (Uysal-Onganer and Kypta, 2011).

10.2 Canonical Wnt signalling pathway

In the canonical Wnt pathway, the major effect of Wnt ligand binding to the frizzled/LRP5/6 receptor complex is the stabilisation of cytoplasmic β -catenin through inhibition of the β -catenin degradation complex formed by APC, GSK3 and Axin proteins. β -catenin is then free to enter the nucleus and activate Wnt-regulated genes through its interaction with TCF family

transcription factors. In the normal pathway, APC and Axin prevent β -catenin from travelling to the nucleus by engaging it in the destruction complex. However, an APC deficiency (as in FAP) prevents β -catenin degradation and leads to excessive cell proliferation and formation of tumours.

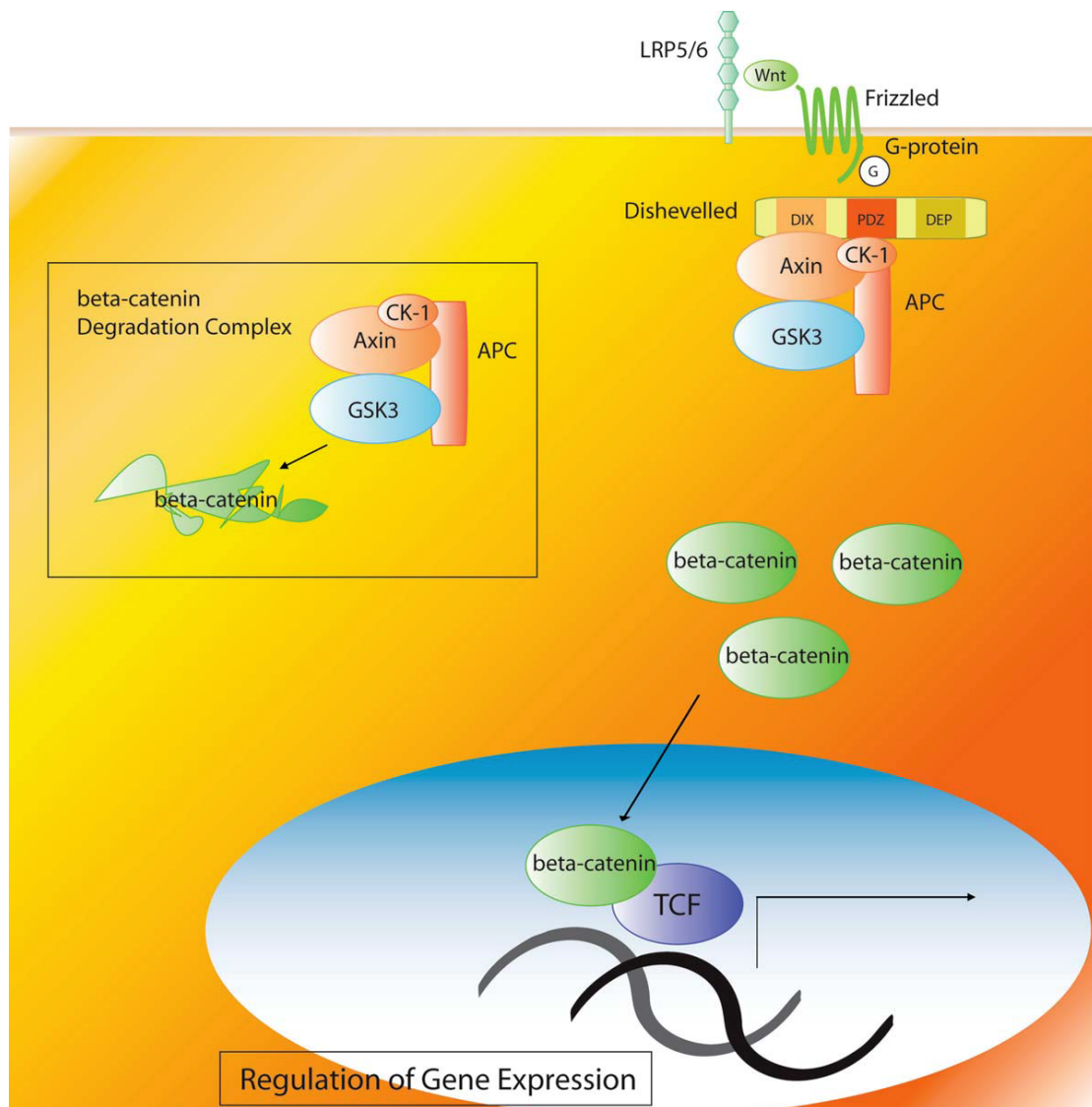


Fig 10.1 - Canonical Wnt signalling pathway (adapted from Sugimura et al, Birth Defect Research (Part C) 90: 243-256 (2010))

10.3 Non-canonical Wnt signalling pathway

Binding of Wnt ligands to the frizzled and other receptors (such as ROR2) initiates non-canonical Wnt signalling pathways. Signalling is mediated by dishevelled proteins, which can activate the JNK pathway to regulate cell motility. Intracellular calcium is another important mediator of non-canonical Wnt signalling. An increase of calcium ions activates the CaMKII-TAK1-NLK, PKC-Cdc42, and calcineurin-NFAT pathways that regulate polarity of cells. Non-canonical Wnt signalling regulates early embryo development, neurogenesis, skin morphogenesis and bone formation; the mechanism is through regulation of cellular motility and polarity. Also, non-canonical Wnt signalling antagonises canonical Wnt signalling (Sugimara et al, 2010).

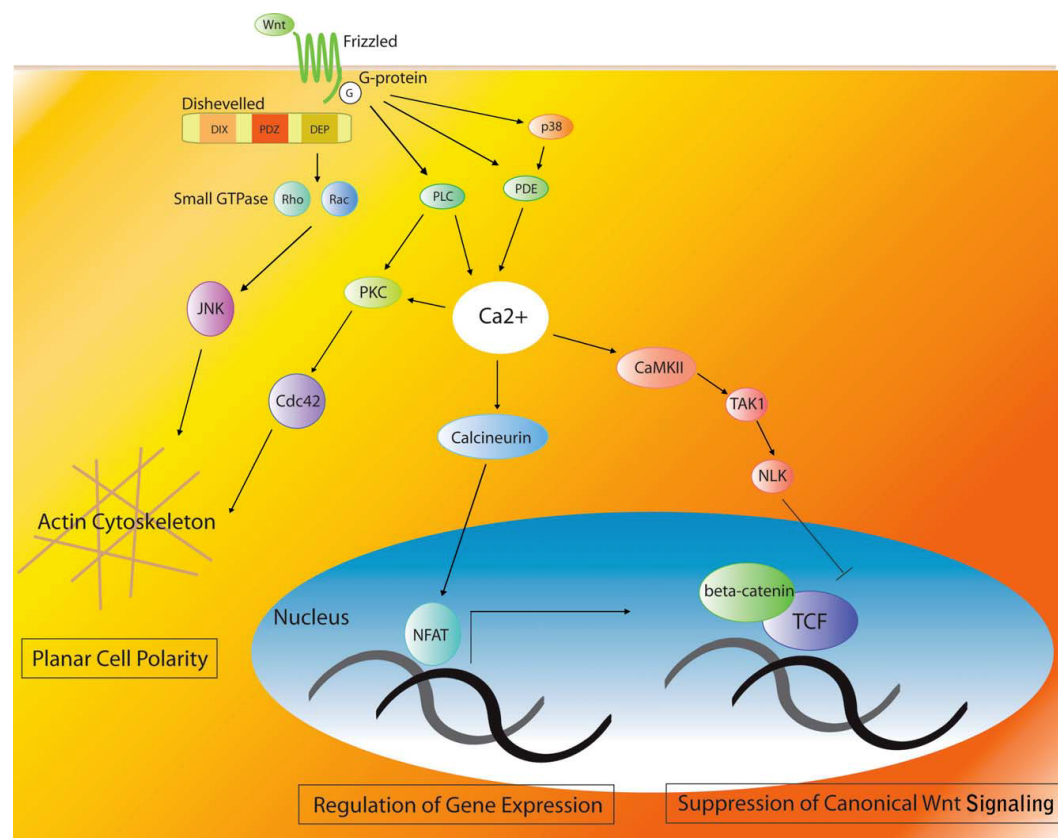


Figure 10.2 - Non-canonical Wnt signalling pathway (adapted from Sugimura et al, Birth Defect Research (Part C) 90: 243-256 (2010))

10.4 Evidence of Wnt signal activation in desmoid tumours

The majority of data regarding Wnt signal activation in desmoid tumours come from studies of sporadic desmoid tumours and primarily relate to the canonical Wnt signalling pathway. Tejpar et al studied forty-two surgically resected sporadic desmoid tumours and reported increased β -catenin protein expression in all the lesions. They found that 22 of 42 tumours contained a β -catenin mutation and 9 contained an *APC* mutation, both resulting in the stabilisation of β -catenin protein in the cells (Tejpar et al, 1999). A further study using 10 sporadic desmoid tumours identified universal expression of TCF-3 in these tumours, in contrast to colon cancer, which expresses TCF-4 and TCF-1. This study has suggested that the interaction unique to TCF-3 could be responsible for the expression of different β -catenin mediated target genes in desmoid tumours. This could partially explain the difference in cell behaviour observed between desmoid tumours and other neoplastic process such as colon cancer that exhibits β -catenin stabilisation (Tejpar et al, 2001). Using six cases of sporadic desmoid from the extremities, Alman et al have reported a higher level of β -catenin protein expression in desmoid tumour compared to surrounding normal tissue (Alman et al, 1997).

Due to the 'double hit' mutation in the *APC* gene resulting in abnormal APC, the β -catenin destruction complex formed by APC, Axin and GSK3 is inefficient in FAP-associated desmoid tumours. This results in the stabilisation of β -catenin, which would be predicted to lead to constitutive activation of canonical Wnt signal in FAP-associated desmoid tumours.

The non-canonical Wnt signalling pathway has not been studied in desmoid tumours. The available data, mainly from animal studies, suggest that the non-canonical Wnt signalling plays an important role during embryo development and the regulation of tissue development and homeostasis (Sigmura et al, 2010). Wnt-11, a secreted non-canonical Wnt protein is primarily responsible for modulating cell growth, differentiation and morphogenesis during development. However, high expression of Wnt-11 detected in gastric, prostate and renal cell carcinoma cell lines and some primary colorectal adenocarcinomas, has suggested a role in human cancer development (Kirikoshi et al, 2001). Moreover in prostate cancer, Wnt-11 was found to induce neuroendocrine-like differentiation of cancer cells (a more aggressive form of cancer), enhance cancer cell survival and promote cancer cell migration (Uysal-Onganer et al, 2010).

10.5 Aims of the study

The main aim of this study was to investigate the activation of canonical and non-canonical Wnt signalling pathways in FAP associated desmoid tumour.

10.6 Materials and methods

10.6.1 Sample

A FAP associated desmoid tumour cell line (D3); FAP fibroblast cell line (C1) and a non-FAP fibroblast cell line (C2) were used. Formalin fixed paraffin

embedded sections of desmoid tumour were used for immunohistochemistry analysis.

10.6.2 Methods

The cells were grown using standard cell culture technique. RNA extracted from each cell line using an established protocol was used to prepare cDNA. Using RT-PCR and qPCR Wnt target genes *AXIN2* (canonical) and *WNT11* (non-canonical) were studied in the desmoid and control cell lines. Protein was extracted from the cells using standard technique (see section 7.5.8) and Western blot analysis was performed for β -catenin and Wnt-11 proteins. Cells were grown on cover slips and stained with β -catenin antibody for immunocytochemistry analysis.

Paraffin embedded sections of the desmoid tumour from which the cell line was established were stained with β -catenin and Wnt-11 antibodies for immunohistochemistry analysis. The histology slides were reviewed with the pathologist experienced in desmoid tumour reporting.

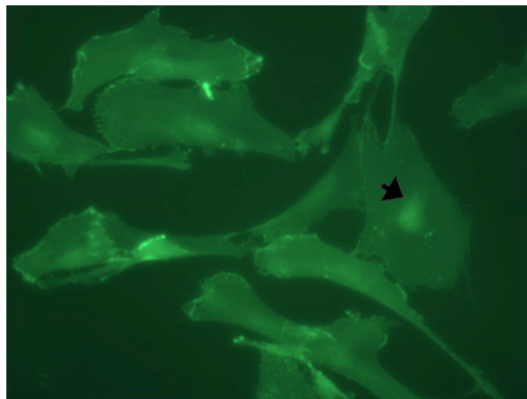
The details of materials, methods, primers and antibodies used in these experiments are listed in the chapter 7.

10.7 Results

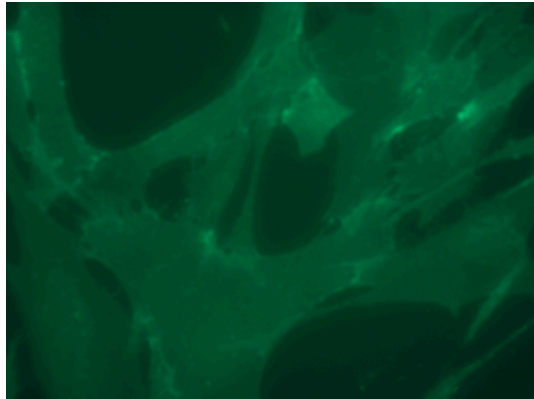
10.7.1 Studies of the canonical Wnt signalling pathway

10.7.1.1 *β-catenin immunocytochemistry*

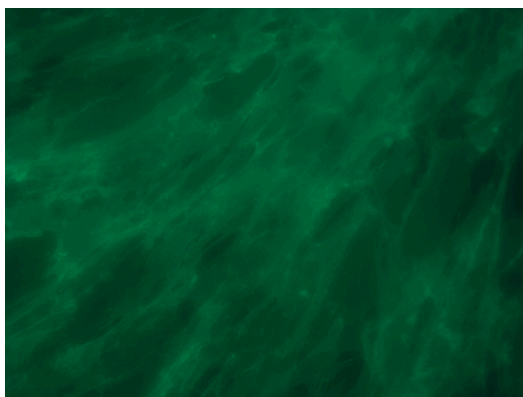
Immunocytochemistry detected cytoplasmic β -catenin expression in all the cell lines (D3, C1 and C2). However nuclear localisation of β -catenin was only observed in the desmoid tumour (D3). Fig 10.3 demonstrates the result of immunocytochemistry analysis on these cell lines.



A



B



C

Fig 10.3 - β -catenin immunocytochemistry in desmoid (A), FAP fibroblast (B) and non-FAP fibroblast (C) cell lines. Nuclear localisation of β -catenin is demonstrated in the desmoid tumour cell line (arrow, A)

10.7.1.2 *β-catenin western blot*

Western blot analysis of β-catenin protein in the desmoid and control cells did not show any significant difference in the total protein level. Fig 9.4 depicts the result of western blot analysis of β-catenin protein; heat shock protein 60 (HSP60) was used as loading control in this experiment.

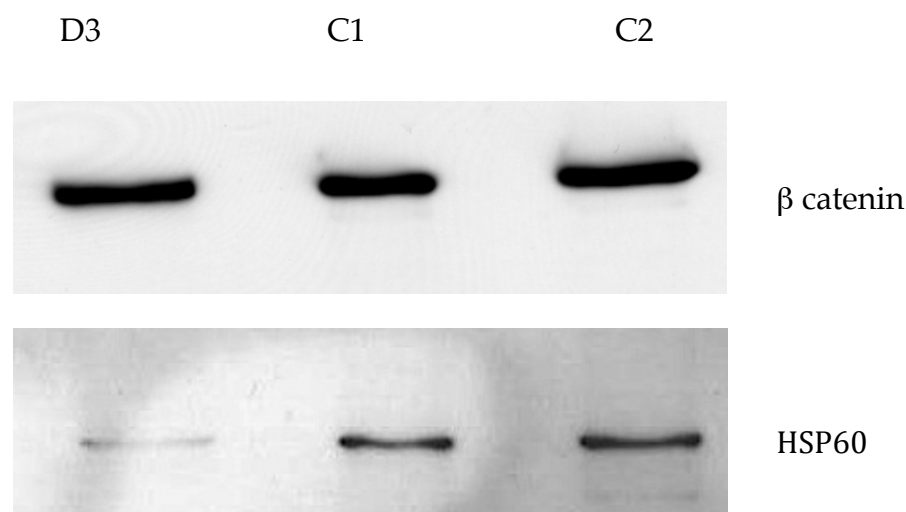


Fig 10.4 - Representative result of western blot analysis of β-catenin protein in desmoid tumour (D3), FAP fibroblast (C1) and Non-FAP fibroblast (C2) cell lines

10.7.1.3 *β-catenin immunohistochemistry*

Widespread nuclear β-catenin expression was evident in the section from the desmoid tumour from which the cell line was created. Fig 9.5 demonstrates the histology findings.

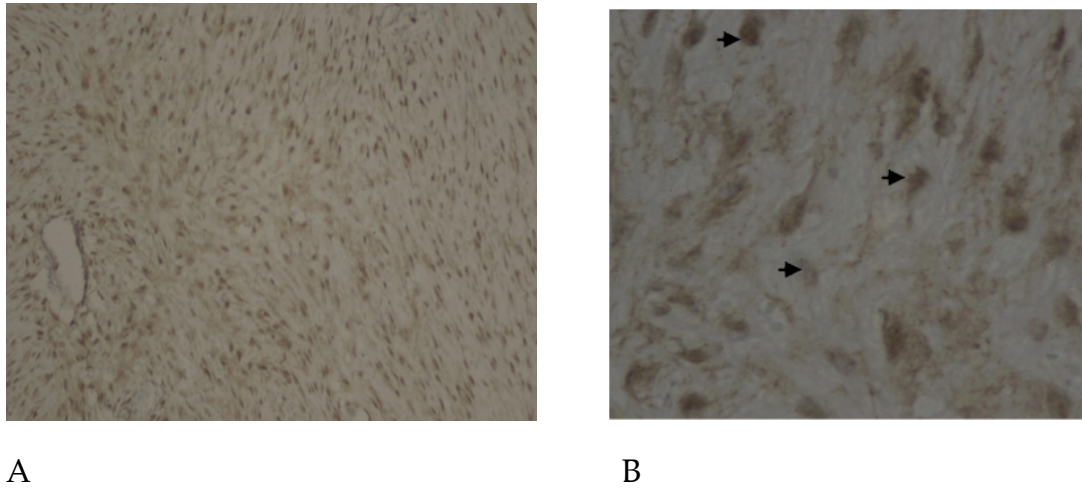


Fig 10.5 - Low (A, 10x) and high (B, 40x) resolution pictures of histology slides of desmoid tumour (D3) demonstrating widespread nuclear β -catenin expression

10.7.1.4 *AXIN2 PCR and qPCR*

AXIN2 as a downstream target of canonical Wnt activation was studied using RT-PCR and qPCR techniques. The desmoid tumour cell line demonstrated overexpression of *AXIN2*, compared to control cell lines by RT-PCR. This overexpression was found to be 22-fold in the desmoid in comparison to control cells using qPCR. Fig 10.6 and 10.7 depict the results of PCR and qPCR studies in these cell lines respectively.

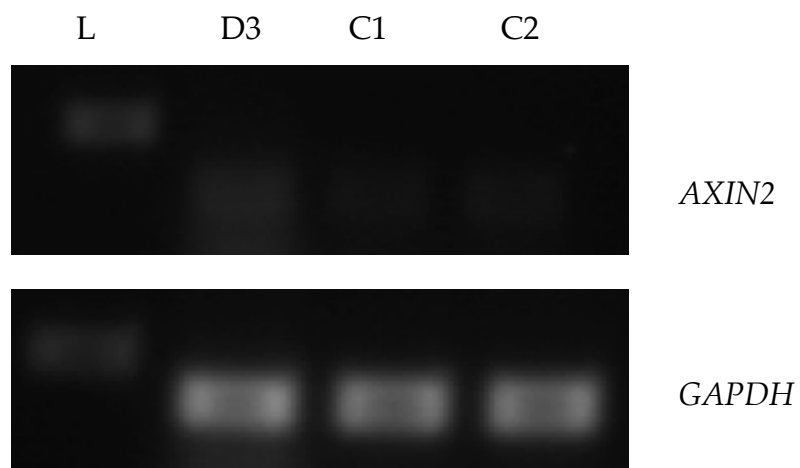


Fig 10.6 – RT-PCR depicting overexpression of *AXIN2* in desmoid cell line (D3). *GAPDH* used as loading control. L: DNA ladder

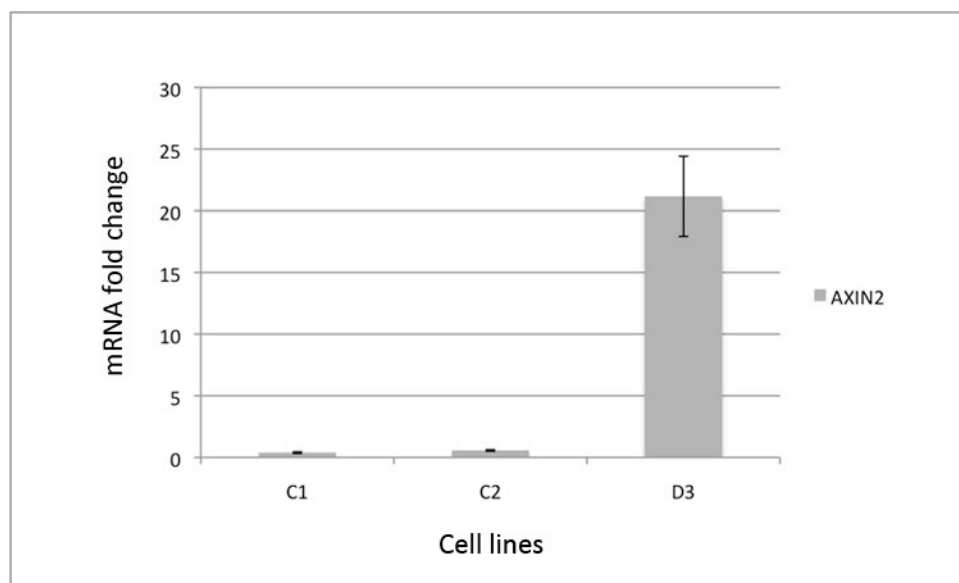


Fig 10.7 - qPCR analysis of *AXIN2*. A 22-fold over expression of *AXIN2* was detected in desmoid tumour (D3) cells; means and SD are shown

10.7.2 Studies of non-canonical Wnt signalling pathway

10.7.2.1 WNT11 PCR and qPCR

WNT11 was studied using RT-PCR and qPCR techniques to investigate the non-canonical Wnt signalling pathway. Both RT-PCR and qPCR methods detected overexpression (55-fold by qPCR) of WNT11 in desmoid tumour cells, compared to controls. Fig 10.8 and 10.9 demonstrate the results of these studies.

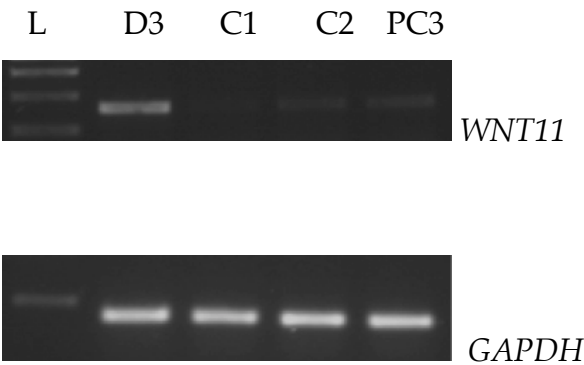


Fig 10.8 - WNT11 RT-PCR. An over expression of WNT11 was detected in desmoid tumour (D3) cell line. L: DNA ladder, PC3: prostate cancer cell line, positive control for WNT11

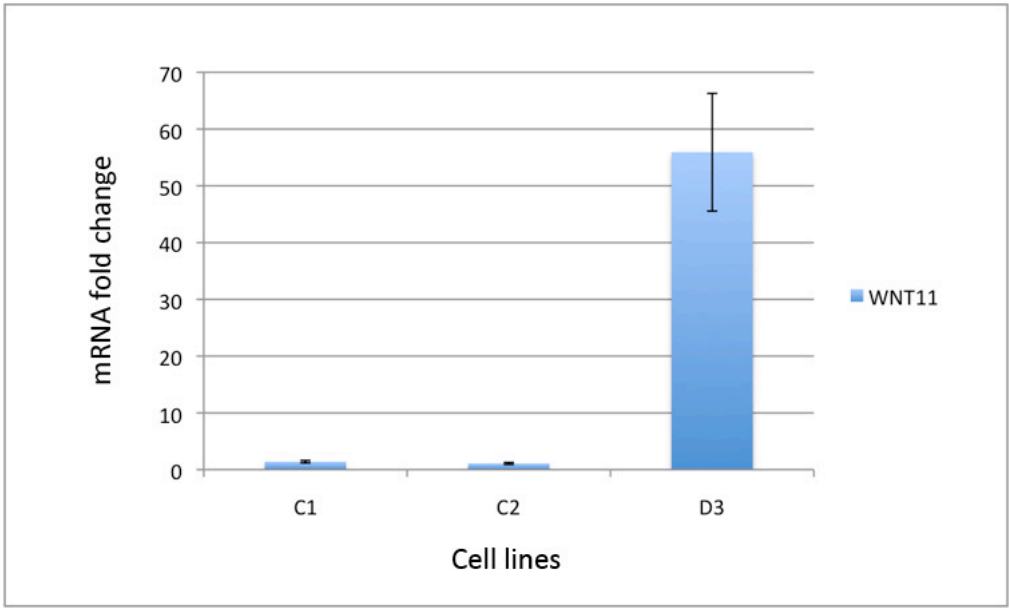


Fig 10.9 - WNT11 qPCR. WNT11 expression was 55-fold higher in desmoid tumour (D3), means and SD are shown

10.7.2.2 *Wnt-11 western blot*

No significant difference in the expression of Wnt-11 protein was demonstrated by western blot analysis among the cell lines. Fig 9.10 demonstrates the result of western blot analysis.

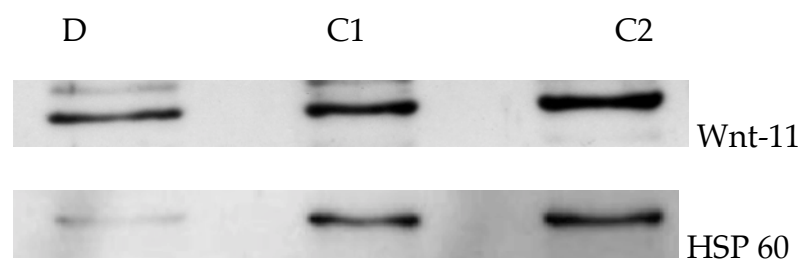
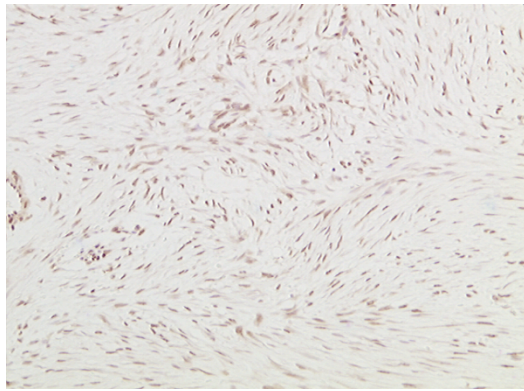


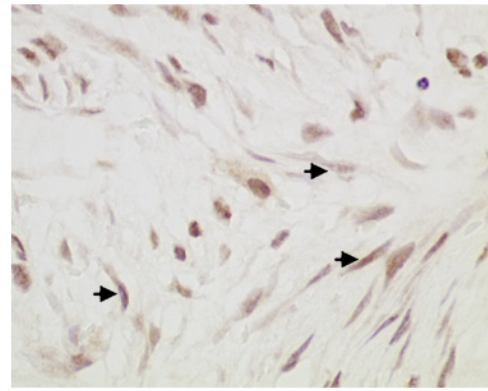
Fig 10.10 - Representative result of Wnt-11 western blot analysis. No significant difference in expression of Wnt-11 protein was detected among the cell lines

10.7.2.3 *Wnt-11 immunohistochemistry*

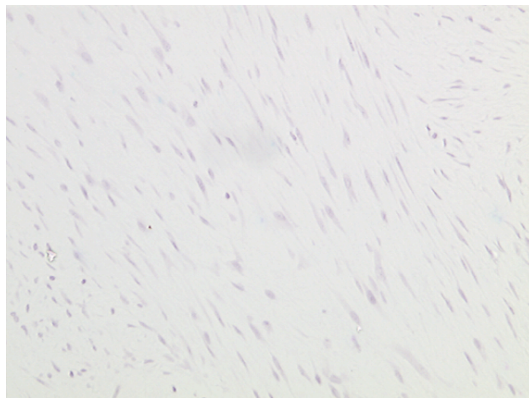
Immunohistochemistry analysis of Wnt-11 on the desmoid tumour sections detected wide expression of Wnt-11. For control these slides were only stained using secondary antibody. Fig 9.11 depicts Wnt-11 histology results.



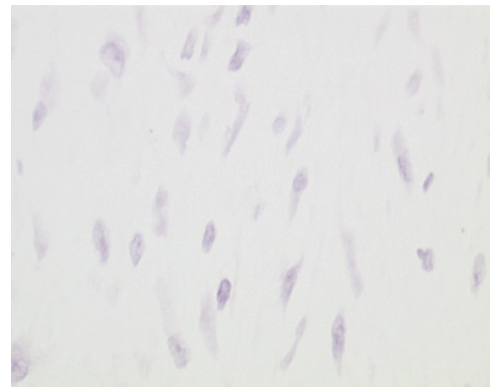
A



B



C



D

Fig 10.11 - Immunohistochemistry of Wnt-11 in desmoid tumour. A and B are 10x and 40x magnification of the desmoid tumour sections; widespread cytoplasmic and nuclear expression of Wnt-11 (brown stain) is demonstrated. C and D are the 10x and 40x magnification of control slides stained with secondary antibody only

10.8 Discussion

In vitro studies using a desmoid tumour cell line detected nuclear localisation of β -catenin and overexpression of the canonical Wnt target gene *AXIN2*. In this study, the β -catenin protein level was not found to be significantly different among the desmoid and control fibroblast cell lines. However the nuclear localisation of β -catenin in desmoid tumour was evident by

immunohistochemistry, which detected widespread nuclear β -catenin localisation in the desmoid tumour section from which the primary cell line was established.

The finding of nuclear localisation but not overtly increased β -catenin in the desmoid tumour cells in this study suggests that while the level of β -catenin protein is same in all the cell lines, the inefficient degradation complex in desmoid due to *APC* mutation results in β -catenin stabilisation resulting in its translocation to nucleus to interact with TCF factor. In addition, any increase in cytoplasmic and nuclear β -catenin in the desmoids may be masked by the pool of cadherin-associated β -catenin in the membrane.

AXIN2 is a negative regulator of the Wnt/ β -catenin pathway. J Eek-Hoon et al reported that activation of the Wnt/ β -catenin/TCF signalling pathway induces the transcription of *AXIN2*, using a mouse model and mouse cell lines (Eek-hoon et al, 2002). In a further study using 30 human cell lines derived from different origins, Yan et al detected *AXIN2* overexpression only in human colon cancer cell lines that were known to have activating mutations in the Wnt/ β -catenin pathway (Yan et al, 2001). The findings from these studies suggest that the overexpression of *AXIN2* provides firm evidence of the Wnt/ β -catenin pathway activation. Hence the findings of *AXIN2* overexpression combined with the nuclear localisation of β -catenin in the desmoid tumour cell line in this study confirms that the Wnt/ β -catenin pathway is activated in the desmoid tumour.

WNT11, the non-canonical Wnt, was overexpressed in desmoid tumour cells in these experiments. The immunohistochemistry also detected expression of the Wnt-11 in the desmoid sections from which the cell line was established. However at the protein level no significant difference in the expression was detected amongst desmoid and control cell lines. Wnt-11 is a secreted protein so its level in cell extracts may not reflect total expression levels. The nuclear staining for Wnt-11 is unexpected, but not unprecedented. Nuclear Wnt-11 has been observed in chronic myeloid leukemia.

Wnt-11 is reported to promote cellular motility and enhance cellular survival. The overexpression of Wnt-11 in desmoid tumour cells may be the contributing factor for selection of desmoid tumour cells and their survival in the culture. Further studies are required to confirm the interesting finding of the current study.

Antibodies to Wnt-11 are still being purified and available antibodies are not 100% specific. This could be one of the reasons for Wnt-11 protein result in this experiment. The unstable nature of Wnt-11 protein could be another contributing factor. Further studies in future with more purified antibody to Wnt-11 will help clarify this further.

10.9 Summary

In summary, the in vitro studies of Wnt signalling pathways confirmed that Wnt/ β -catenin (canonical Wnt signal pathway) pathway is activated in the desmoid tumour. Wnt-11, the non-canonical Wnt is also overexpressed in

desmoid tumour. Further work using more desmoid cell lines is needed to validate and explore these findings in greater detail. Validation of these findings will provide a unique opportunity to explore the role of Wnt inhibitors in desmoid tumour. This may ultimately lead to identification and development of novel therapy to treat this intriguing condition.

PART FIVE – CONCLUSION

Chapter Eleven

Conclusion

Desmoids are a major cause of morbidity and mortality in patients with FAP. The variable nature of the desmoid tumours, and the lack of reliable molecular, pathological or clinical markers of aggressive disease, makes early identification of aggressive desmoid difficult. Surgical excision incurs high recurrence, and high morbidity in case of IAD. Furthermore, there is lack of high quality evidence on the efficacy of available non-surgical/medical treatment. Desmoids therefore remain as an outstanding challenge in the management of FAP patients.

The available literature usually combines sporadic and FAP associated desmoids, which are biologically distinct entities. The studies described in this thesis focus exclusively on FAP-associated desmoids and attempt to further characterise these tumours.

A potential role of modern functional and metabolic imaging in identifying the aggressive minorities of desmoid tumours has been explored and in-vitro studies have investigated the molecular pathways in this enigmatic tumour.

11.1 Imaging studies

The radiology studies described in Part Two of this thesis investigated the role of modern imaging modalities in the evaluation of desmoid tumours. The first study in Chapter Three of this thesis demonstrated that modern day MRI is at least equivalent and probably superior to cross-sectional CT for the radiological evaluation of desmoids in patients with FAP. MRI also has the advantage of lack of radiation exposure to young patients with FAP, who are frequently affected by desmoids.

Chapter Four explored the use of diffusion weighted imaging (DTI) in desmoid tumours. This is the first reported study in FAP desmoids and confirms that DTI is feasible in these tumours. Desmoid tumours show anisotropic diffusion, which is less directional than in muscle. This forms a foundation to explore the role of this imaging modality in desmoid tumour in future using a bigger study population.

Chapter Five of this thesis has investigated the potential role of functional (^{18}F FDG PET CT) and vascular imaging (DCE-MRI) in identifying growing desmoid tumours. This study found that desmoid tumours were generally hypovascular and, contrary to the other few studies showing high glucose avidity, were poorly glucose avid. While the study was not conclusive for finding any definite parameter predictive of aggressive tumour behaviour, it demonstrated a spectrum of findings in FAP-associated desmoid tumours that will be an invaluable asset to refer to for the future studies using larger sample size.

11.2 Clinical study

The clinical study described in Part Three of the thesis has reviewed management of IAD presenting with air-fluid level in cross sectional imaging. The study showed that some patients with IAD with air-fluid level could be treated with a conservative approach of antibiotics with or without a percutaneous drain; the others ultimately required complex surgery. Based on this study a management algorithm has been proposed to treat such patients.

11.3 Laboratory studies

Using the primary cell lines established from desmoid tumours, the first laboratory based study (Chapter 8) was performed to identify *APC* mutations in the cells. This study successfully identified a 'second hit' somatic mutation in the *APC* gene in one of the three cell lines investigated and confirmed the establishment of a true desmoid tumour cell line.

The confirmed desmoid tumour cell line was then used along with controls to study the choline metabolism pathway (Chapter 9) and the Wnt signalling pathways (Chapter 10). While the study described in this thesis failed to demonstrate up-regulation of choline metabolism in desmoid tumour cells, the activation of canonical Wnt signalling pathway was established. Interestingly Wnt-11, a non-canonical Wnt, was found up regulated in desmoid tumour cells. Further studies are needed to confirm whether expression of Wnt-11 may be responsible for the selection and survival of true desmoid tumour cells in culture.

Chapter Twelve

Future work

12.1 Prognostication of desmoid with modern functional imaging

The DTI, DCE-MRI and ^{18}F FDG PET CT imaging studies described in this thesis have confirmed the feasibility of such studies and shown a spectrum of findings in FAP associated desmoid tumours. However only a small number of patients were studied and the included subjects were already on available treatment before the commencement of the study, which might have influenced some of the study findings. Moreover only one patient had a really aggressive desmoid tumour who unfortunately died due to desmoid before the follow up scan could be performed.

A study involving larger number of patients, with a practice of imaging the desmoid with functional and metabolic imaging first and then starting the medical treatment, may help to clarify the role and the value of these imaging modalities in the early assessment of aggressive phenotype of desmoid tumours, and also the assessment of success or failure of a particular modality of treatment.

12.2 Validation of Wnt signal activation in desmoid tumours

The laboratory-based studies described within this dissertation have established the activation of the Wnt signalling pathway in desmoid tumour. The demonstration that the β -catenin protein is stabilised in these tumours suggests blockade of the Wnt-APC- β catenin pathway as potential treatment. However this interesting finding has been described in this thesis using the in-vitro model of single cell line. There is need for establishment of more desmoid tumour cell lines and reproducing the result of these experiments to validate the findings of this initial work. As more is learned about the role of mediators in the Wnt signaling cascade and the downstream genes that regulate proliferation, a novel therapeutic approach may be devised based on blockade of this signalling pathway. Adjuvant treatments based on manipulation of cellular growth control may improve the outcome for individuals with this disorder and perhaps a treatment based on modulation of the function of Wnts or manipulation of downstream mediator could be devised. Establishment of more desmoid tumour cell lines and further work on Wnt signalling pathway is currently underway in our institution.

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APPENDICES

A1 Collaborations and assistance

A1.1 Imaging studies

The 64-MDCT was performed at the Intestinal Imaging Centre, St Mark's Hospital, under supervision of Dr Arun Gupta and Dr David Burling. The 1.5T MRI, DTI, DCE-MRI and ¹⁸F FDG PET CT were performed at Paul Strickland Scanner Centre, Mount Vernon Hospital, Northwood under supervision of Professor Vicky Goh and Dr Luke Sonada. Ethical approval for imaging studies was obtained by Mr Salman Rana, Clinical Research Fellow, Polyposis Registry, St Mark's Hospital, Harrow. Patients were recruited for initial study by Mr Ashish Sinha, Clinical Research Fellow, St Mark's Hospital. I was involved in organising follow up scans, data gathering and analysis as explained in individual studies below.

Chapter 3 - A prospective comparison of 64-slice multidetector computer tomography and 1.5- Telsa magnetic resonance imaging in the assessment of desmoid tumours in familial adenomatous polyposis

The 64-MDCT and MRI images were interpreted by Professor Vicky Goh, Dr Arun Gupta and Dr David Burling. I collected the data and performed statistical analysis for this study.

Chapter 4 - Diffusion tensor imaging (DTI) in the assessment of desmoid tumours in familial adenomatous polyposis

I gathered the quantitative data from the DTI images using commercial tensor software (Neuro3D, Siemens Healthcare) under direct supervision of Professor Vicky Goh. I also performed the data analysis and the statistical tests for this study.

Chapter 5 - Role of Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) and Fluorine-18 Flurodeoxyglucose Positron Emission Tomography (18F-FDG PET) in predicting behaviour of desmoid tumours in familial adenomatous polyposis

Under direct supervision of Prof Vicky Goh, I generated the quantitative DCE-MRI data after drawing ROI around individual tumour on a workstation using proprietary software (MRIW, Royal Marsden Hospital). Dr Luke Sonada helped to generate the FDG PET data for the study. I also analysed the data and performed all the statistical tests for the study.

A1.2 Clinical study

Chapter 6 - A clinical review of familial adenomatous polyposis associated desmoid tumours presenting with air fluid level

I identified the study patients from the Polyposis Registry database and reviewed all the notes. I collected the data for this study, analysed it and developed the treatment algorithm described in the study.

A1.3 Laboratory studies

Chapter 8 - *APC* mutation analysis in FAP desmoids and fibroblast cell lines

I performed cell culture and submitted the pellets of desmoid and control cell lines for the *APC* mutation analysis. The full sequence *APC* mutation analysis was performed at the Kennedy-Galton Centre, Northwick Park and St Mark's Hospital, under supervision of Dr Stewart Payne using automated DNA sequencer.

Chapter 9 - Study of choline metabolism in desmoid tumour in FAP

This work was done, in collaboration with Professor Eric Aboyage and Dr Rohini Sharma, at Comprehensive Cancer Imaging Centre, Cyclotron Building, Hammersmith Hospital. I designed and performed all the laboratory experiments (TC, RNA extraction, cDNA synthesis, qPCR for CHKA and data analysis) under supervision of Dr Rohini Sharma.

Chapter 10 - In vitro studies of Wnt signalling pathways in desmoid tumour in FAP

This work was performed at the Institute of Reproductive and Developmental Biology (IRDB, Hammersmith Hospital, Imperial College London) under supervision of Dr Robert Kypta. I designed and performed all the laboratory experiments for this study, except the qPCR for AXIN2 and WNT11. Dr Nora

Bengoa (Cell Biology and Stem Cells Unit, CIC bioGUNE, Bizkaia, Spain) performed the qPCR for AXIN2 and WNT11, under supervision of Dr Robert Kypta (I supplied the cDNA required for the experiment). Dr Morgen Moorghen, St Mark's Hospital, reviewed the immunohistology slides of this study.

A2 Regulatory approvals

Ethical approval from the Harrow Research and Ethics Committee along with research and development approval from the North West London Hospitals NHS Trust was obtained for the studies described in this thesis. Listed below are the details of these approvals.

Title	Harrow REC Number	NWLH NHS R&D Number	Date of Approval
Carcinogenesis of desmoid disease in familial adenomatous polyposis	08/H0719/75	RD8/080	25th February 2009
Immunohistochemical analysis and treatment of familial adenomatous polyposis related desmoids	09/H0709/13	RD9/011	21st April 2009
The role of imaging in the assessment of desmoid tumour growth: a comparison of CT, MRI and PET scanning	07/H0719/100	RD7/090	5th March 2008

A3 Publications in peer reviewed journals

The following paper publications in peer-reviewed journals have resulted from the studies described in this thesis.

1. Chapter three: Imaging assessment of desmoid tumours in familial adenomatous polyposis: is state-of-the-art 1.5 T MRI better than 64-MDCT?

Sinha A, Hansmann A, Bhandari S, Gupta A, Burling D, Rana S, Phillips RK, Clark SK, Goh V. Br J Radiol. 2012 Jul;85(1015):e254-61.

2. Chapter four: Diffusion tensor imaging (DTI) of desmoid tumours in familial adenomatous polyposis: initial experience. Bhandari S, Sinha A, Tam E, Stirling JJ, Simcock I, Clark S, Goh V. Eur J Radiol. 2012 Nov;81(11):3646-51.

3. Chapter five: Can combined 18F-FDG-PET and dynamic contrast-enhanced MRI predict behavior of desmoid tumors in patients with familial adenomatous polyposis? Bhandari S, Taylor NJ, Sinha A, Sonoda L, Sanghera B, Wong WL, Goh V, Clark SK. Dis Colon Rectum. 2012 Oct;55(10):1032-7.

4. Chapter six: Familial adenomatous polyposis-related desmoids presenting with air-fluid level: a clinical review and management algorithm. Bhandari S, Ranchod P, Sinha A, Gupta A, Clark SK, Phillips RK. Dis Colon Rectum. 2012 Jul;55(7):810-4.

A4 Presentations of the research work

The following presentations resulted from the work carried out in this dissertation.

A4.1 Oral presentations

1. In-vitro studies of the Wnt signalling pathways in FAP desmoid. Society of Academic and Research Surgery (SARS), 9-10 Jan 2013.
2. Is modern day MRI better than CT for assessment of desmoid tumours in familial adenomatous polyposis? The Association of Surgeons in Training (ASiT), 23-25 March 2012; 2011 Colorectal Tripartite Meeting, Cairns, Australia, 3-7 July, 2011

A4.2 Poster presentations

- 1) In-vitro studies of the Wnt signalling pathways in desmoid tumour in familial Adenomatous polyposis. European Society of Coloproctology(ESCP), Vienna, 26-28 September 2012.
- 2) Can combined Fluorine- 18 Fluorodeoxyglucose Positron Emission Tomography (18 F – FDG PET) and Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI) predict behaviour of desmoids tumours in familial adenomatous polyposis (FAP) patients? Society of Academic and Research Surgery (SARS), Nottingham, 4-5 Jan 2012.

3) Diffusion Tensor Imaging (DTI) of desmoids tumours in familial adenomatous polyposis : initial experience. Society of Academic and Research Surgery (SARS), Nottingham, 4-5 Jan 2012.

4) Dynamic contrast enhanced magnetic resonance imaging of desmoid tumours in familial adenomatous polyposis. 4th Biennial Meeting: International Society for Gastrointestinal Hereditary Tumours (InSiGHT), San Antonio, Texas, March 31- April 2, 2011.