Predicting Serious Drug Side Effects in Gastroenterology (PRED4)

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PROTOCOL FULL TITLE:

PREDICTING SERIOUS DRUG SIDE EFFECTS IN GASTROENTEROLOGY

Protocol Short Title/Acronym

PRED*4*

Trial Identifiers

REC Number 11/SW/0222

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1. Study Synopsis

Title of Research Study	PREDICTING SERIOUS ADVERSE DRUG REACTIONS IN GASTROENTEROLOGY
Protocol Short Title/Acronym	**PRED4**
Study Phase if not mentioned in title	Not applicable
Sponsor name	Royal Devon and Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW
Chief Investigator	Dr Tariq Ahmad
REC number	11/SW/0222
Medical condition or disease under investigation	Patients who have suffered serious, but rare, side effects due to commonly used drugs.
Purpose of clinical trial	To investigate the genetic contribution to serious adverse drug reactions.
Primary objective	To identify clinically useful genetic markers that predict serious drug side effects, so that these drugs can be avoided, or monitoring intensified, in genetically high risk patients. A simple, cheap, diagnostic test will be developed using these data which can be rapidly adopted into medium and large sized hospitals
Secondary objective(s)	(a) to understand the mechanisms underlying drug side effects (b) through a knowledge of the mechanisms, to learn about particular functional chemical groups which predispose to toxicity, and thereby facilitate more rational drug design. (c) to develop a network of interested UK clinicians for further pharmacogenetic research projects.
Study Design	Case-control association study
Sample Size	2/300 patients in each subgroup
Summary of eligibility criteria	All major criteria listed must be met
Demyelination complicating anti-TNF therapy in Inflammatory bowel disease and other inflammatory disorders.	• History of exposure to anti TNF-α antibody at any time in the past. • No history of demyelinating neurological symptoms prior to exposure to anti TNF-α antibody. • Neurological symptoms lasting at least 24 hours.

	• MRI brain and/or spinal cord shows changes consistent with CNS demyelination or electrophysiological studies(nerve conduction or evoked potentials) are consistent with PNS or CNS demyelination and confirmed by a neurologist. • Neurological opinion implicates anti TNF-α medication as possible cause of demyelination, and if the patient is still receiving the drug, it is withdrawn.
Proton Pump inhibitor induced interstitial nephritis.	• ≥ 30% rise in serum creatinine *or* ≥ 25% fall in eGFR any time after introduction of PPI • No other risk factors for renal disease • Medical opinion implicating PPI justifies drug withdrawal, even if temporary
Thiopurine induced pancreatitis in Inflammatory bowel disease.	• History of ulcerative colitis or Crohn's disease. • Episode of acute severe abdominal pain • History of thiopurine exposure **within** the previous 7 days of **pancreatitis occurring** • Rise in serum pancreatic enzymes (amylase/lipase) ≥2 times upper limit of normal. • Episode of acute pancreatitis within 3 months of starting thiopurine. • Medical opinion implicates thiopurine as the mostly likely cause of pancreatitis, and drug withdrawn.
Thiopurine induced myelosuppression in Inflammatory bowel disease.	• History of ulcerative colitis or Crohn's disease. • History of thiopurine exposure **within** the previous 7 days of **Leucopaenia occurring** • Normal total white cell count and/or neutrophil count at baseline. • Fall in total white cell count **to** ≤ $2.5x10^9$/L, or reduction in neutrophil count **to** ≤ $1.0x10^9$/L or less • Medical opinion implicating thiopurine leads to dose reduction or drug withdrawal even if temporary
Sulfasalazine Induced Neutropaenia in inflammatory bowel disease or Rheumatoid arthritis	• History of inflammatory bowel disease or Rheumatoid arthritis • History of sulfasalazine exposure in the previous 30 days • Normal total white cell count and neutrophil count at baseline • Fall in neutrophil count to ≤$0.5 x10^9$/L • Medical opinion implicating sulfasalazine leads to dose reduction or drug withdrawal (even if temporary) •
Thiopurine induced Liver Injury in inflammatory bowel disease	• History of inflammatory bowel disease • Normal ALT and bilirubin at baseline • No pre-existing liver disease • Elevation of ALT and/or bilirubin to >= 5 times upper limit of normal (defined by local lab)

	• History of thiopurine exposure in the previous 30 days prior to this abnormal blood test • Medical opinion implicating thiopurine in development of hepatotoxicity leads to dose reduction or drug withdrawal (even if temporary)
Thiopurine Hypersensitivity Reaction	**Inclusion criteria** • Aged 6 years or over • History of Inflammatory Bowel Disease • History of thiopurine exposure in the previous 7 days before the onset of adverse event. • Flu like symptoms severe enough to lead to drug withdrawal even if temporary (symptoms should include fever, muscle ache, joint pain) • Onset of symptoms within 4 weeks of starting thiopurine. • Symptoms resolved within 14 days of drug withdrawal. **Exclusion criteria** • Patient tolerated re-challenge with the same thiopurine regardless of dose. • Drug withdrawn due to nausea and/or diarrhoea without any other symptoms, • Objective evidence of Infection.
Version and date of final protocol	**Version 5final dated 11th November 2014**
Version and date of protocol amendments	Amendment No 1 dated 20th September 2011 (Children info sheets) Amendment No 2 dated 24th November 2011(PPI) **Amendment No 3 dated 9th January 2012 (Scottish Sites)** **Amendment No 4 dated 4th November 2013**

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2. Background & Rationale

In the European Union adverse drug reactions are increasing at twice the rate of prescriptions. The European Commission estimated in 2008 that adverse reactions kill 197 000 EU citizens annually, at a cost of €79 billion. In the UK 6.5% of all hospital admissions are caused by adverse drug reactions.

Adverse drug reactions are often classified into two groups. Type A reactions are predictable from the known mode of action of the drug and can be alleviated by either dose reduction or drug withdrawal. Examples of adverse drug reactions in this group include hypoglycemia induced by diabetic drugs, leucopaenia induced by thiopurines, and bleeding induced by warfarin. Type B reactions cannot be explained by the mode of action of the drug and usually require drug withdrawal. Examples in this group include Thiopurine induced pancreatitis, Flucloxacillin induced jaundice, and Abacavir hypersensitivity.

The factors predisposing individuals to adverse reactions are in most cases unknown. Recent studies have demonstrated that modern genetic technology can be successfully employed to identify genetic factors that determine adverse drug reactions, promising a safe individualized therapeutic strategy for patients. Importantly these studies have confirmed that some rare side effects are associated with large effect variants which can be identified using a relatively small number of rigorously characterized cases. Thus the HLA class II allele HLA-B*5701 genotype has been shown to be a major determinant of flucloxacillin-induced cholestatic hepatitis with an odds ratio of 80 using a cohort of only 51 patients. This same HLA allele was earlier found to associate with Abacavir hypersensitivity, a finding that has translated into clinical practice to reduce the burden of this serious adverse reaction in a cost effective manner - in Europe HLA-B*5701 testing is now mandatory before prescribing Abacavir. For other adverse drug reactions the genetic effect size will be low or moderate suggesting that phenotype expression requires the interaction of multiple genetic and environmental factors. These genetic factors may influence pharmacokinetic (for example, the drug metabolism and transporter genes responsible for drug disposition) or pharmacodynamic (genes coding for drug targets, immune response genes, cytokines etc) pathways. The advent of unbiased genetic technologies, for example genome-wide screening and whole genome sequencing, permits the identification of genetic variants in all pathways, provided sufficient numbers of well-characterized patients with such adverse reactions can be recruited. This requires a collaborative, comprehensive nationwide approach, which our UK and international network is capable of delivering.

These studies will investigate the genetics of 7 rare adverse reactions from drugs commonly used in gastroenterology (and other diseases). These case control association studies will involve patients with the following documented serious adverse side effects

- Demyelination complicating anti-TNF therapy in inflammatory bowel disease (IBD) and other inflammatory disorders.
- Proton Pump inhibitor (PPI) induced interstitial nephritis.
- Thiopurine induced pancreatitis in inflammatory bowel disease.
- Thiopurine induced myelosuppression in inflammatory bowel disease.
- **Sulfasalazine Induced Neutropaenia in inflammatory bowel disease or Rheumatoid arthritis**
- **Thiopurine induced Liver Injury in inflammatory bowel disease**
- **Thiopurine Hypersensitivity reaction in inflammatory bowel disease**

Participation requires a single patient visit to obtain consent, completion of a questionnaire and venepuncture. Control subjects have already been recruited and will comprise patients who have been

exposed to the drug without adverse effect. The investigation of other adverse drug reactions will require submission of a protocol amendment.

SUMMARY OF CURRENT KNOWLEDGE OF SERIOUS ADVERSE EVENTS

Demyelination complicating anti-TNF therapy in inflammatory bowel disease and other inflammatory disorders.

New demyelination events, as well as clinical and radiological exacerbation of existing multiple sclerosis, have been reported in a number of patient groups following exposure to anti-TNF drugs. These include patients with IBD and patients with inflammatory arthropathies and skin diseases (1). In IBD, cases of demyelination were reported in the landmark index clinical trials with a growing number highlighted by subsequent case reports and series. Amongst CD patients treated worldwide with adalimumab in placebo-controlled trials, the incidence of demyelination/optic neuritis is reported to be 2 events per 1000 patient-years (2). Lees et al reported the Edinburgh experience and identified 3 patients out of 202 anti-TNF treated patients with suspected demyelinating disease and definite neurological abnormalities (3). In the UK, 36 cases of CNS demyelination and 1040 other neurological events complicating anti-TNF therapy had been reported to the Medicines and Healthcare products Regulatory Agency (MHRA) by November 2010.

These observations suggest a possible association between anti-TNF therapy and demyelination. Further evidence to suggest that TNF-alpha blockade may cause, or contribute to the development of, demyelination is provided by the observed temporal association with drug exposure, the observed improvement in symptoms reported by the majority on anti-TNF withdrawal, and the reappearance, or exacerbation, after re-exposure. Nevertheless it is recognised that there is a small increased incidence of MS, demyelination and optic neuritis in patients with IBD (for CD, incidence rate ratio (IRR) 2.12, 95% CI 0.94-4.50; for UC, IRR 2.63, 95% CI 1.29-5.15) (4) and this risk may be conferred by shared susceptibility genes. Convincing epidemiological data that this adverse events occurs more frequently in patients exposed to anti-TNF agent compared to naïve patients remains limited.

There is however considerable experimental and clinical data, in particular disease deterioration on exposure to anti-TNF drugs, to suggest that TNF and the TNF receptor systems play a pivotal role in the pathogenesis of MS (5).

The development of experimental autoimmune encephalitis, an established animal model for human MS, is inhibited by both polyclonal and monoclonal anti-TNF-α antibody preparations. Similarly, the severe, progressive demyelinating disease which develops in transgenic mice selectively over-expressing TNF-α in the CNS, can be reversed with the administration of a monoclonal anti-TNF antibody. Conversely, TNF-α homozygous knockout mice have been demonstrated to develop extensive CNS demyelination; treatment with recombinant TNF-α reduces disease severity. There are two TNF-α receptors; TNFR1 and TNFR2. Murine experiments have concluded that TNF-α signalling mediated via the TNFR2 pathway promotes CNS progenitor cell proliferation, which are later required for remyelination. Overall, these pre-clinical studies suggest that although TNF-α accelerates the process of acute demyelination, its presence in the CNS is required for remyelination and repair processes (5).

Proton Pump inhibitor induced interstitial nephritis.

Proton pump inhibitors (PPIs) are used to suppress gastric acid secretion for the treatment of acid-related gastrointestinal disorders including gastro-oesophageal reflux, dyspepsia and peptic ulcer disease. This class of drug is widely used in Europe and the US where it is the second most frequently prescribed medication (after statins) (6). PPIs are generally safe and well-tolerated. Common adverse effects include: headache, nausea, diarrhoea, abdominal pain, fatigue, and dizziness. Long-term use is associated with hypomagnesaemia and Vitamin B12 deficiency. Infrequent adverse effects include rash, itch, flatulence, constipation, anxiety, and depression. Rarely PPI cause ‘idiosyncratic’ reactions

such as erythema multiforme, pancreatitis, Stevens Johnson syndrome, and acute interstitial nephritis (AIN) (6–9)

The first case of omeprazole-induced AIN was reported by Ruffenach *et al* in 1992 (10). Subsequent reports have implicated pantoprazole, rabeprazole, lansoprazole and esomeprazole confirming that this phenomenon is a class effect (8,11,12). The incidence of PPI induced interstitial nephritis is uncertain with all data derived from case reports and case series. Ray et al found six cases of AIN associated with PPIs after examining 210 kidney biopsies (in a renal centre serving a population of 1.1 million) (7). Between 1992 and December 2009, there were 74 cases reported in the UK through the Medicines and Healthcare products Regulatory Agency (MHRA) reporting scheme. A systematic review by Sierra et al found 64 cases of PPI-associated interstitial nephritis in the worldwide literature, with 59 of these confirmed by renal biopsy (6). The majority of these were due to omeprazole. The mean age of the patients was 78 years, and the mean duration of PPI treatment before the onset of nephritis was 13 weeks (range between 2 weeks and 52 weeks).

The mechanism of drug-induced AIN is unknown, but an immunological basis is suspected, with supporting evidence from in vitro lymphocyte tests (7). There are no known risk factors and no relation between dosage, latency, time to recovery, age or gender reported (6,13).

PPI-induced interstitial nephritis generally has a good prognosis. The mainstay of treatment is conservative management with drug withdrawal. Most patients will have a complete recovery after withdrawal of the medication, though some patients may need corticosteroids (up to one third of patients) (6,13). Data by Gonzales *et al* suggested that early initiation of steroid therapy within two weeks of cessation of the causative agent may lead to improved renal function (14). There have been three reported cases that have needed dialysis, with one requiring permanent dialysis (6).

A recent study comparing the experience of PPI-induced interstitial nephritis by Consultant Nephrologists in the UK to the cases reported to the Medicines and Healthcare products Regulatory Agency (MHRA) in 2009 showed that it is a widely recognised problem in clinical practice, with significant under reporting of the problem to the MHRA (15).

Thiopurine induced pancreatitis in inflammatory bowel disease.

Thiopurines (Azathioprine [AZA] and Mercaptopurine [MP]) are widely-used in inflammatory bowel disease with proven efficacy (16–18). Azathioprine is a pro-drug which is metabolized to 6-mercaptopurine following oral ingestion. Mercaptopurine impedes DNA synthesis and thus inhibits the proliferation of cells in particular T-cells and B-cells. Current indications for treatment with thiopurines include maintenance of remission, corticosteroid-sparing in chronic active disease, prevention of postoperative recurrence, and treatment of perianal and enteric fistulae (19). In clinical practice, in 68% of IBD patients the initial therapeutic goal (including end-points such as mucosal healing, elimination of steroids, healing of internal fistulae, pain relief) is achieved (20). A long-term follow-up study found that after initial response, efficacy is reasonably well-sustained with remission rates of 95%, 69% and 55% after 1, 3 and 5 years respectively (21).

Despite their widespread use, there are still concerns regarding their safety, both short- and long-term. They are generally well-tolerated but in about 15% of patients, the drug has to be discontinued due to adverse effects (22,23). Adverse drug reactions can be divided into dose-dependent (Type A) and dose-independent (Type B). Acute pancreatitis is an example of dose-independent whilst myelotoxicity (which includes pancytopaenia, leucopaenia, thrombocytopaenia and anaemia) falls into the "dose-dependent" category.

There is agreement that acute pancreatitis occurs more frequently in patients with IBD than in the general population, with the incidence varying from 4.8 to 38/100,000 patients with IBD (19,23,24). It is more common in patients with Crohn's disease (4-fold increase compared to the general population) than in ulcerative colitis (2-fold increase). Drugs, in particular thiopurines and 5-aminosalicylates, are the commonest cause of acute pancreatitis in patients with IBD.

The incidence of pancreatitis in patients treated with a thiopurine is 1.2-5% (19,23,24). It is thought to be an idiosyncratic reaction and not related to TPMT genotype (19). The pathogenesis of thiopurine-induced pancreatitis is unknown, though it has been postulated that an immune-mediated mechanism or hypersensitivity mechanism may be likely. There have been reports of autoantibodies against the pancreas being found in patients with thiopurine-induced pancreatitis with Crohn's disease (approximately 30%)(23) .

Thiopurine-induced pancreatitis most commonly occurs within the first month after commencement of therapy, and re-challenge with either AZA or MP leads to recurrence of symptoms (21,23–25). Most patients have symptoms of acute severe abdominal pain accompanied by nausea and vomiting, though clinically symptoms of acute pancreatitis can be difficult to differentiate from those caused by the activity of IBD, or by its complications. Most patients will have a rise in serum pancreatic enzymes (amylase or lipase), with an acceptable cut-off of 2-3 times the upper limit of normal. There may be changes seen on abdominal imaging such as CT (24). Most episodes of acute pancreatitis are mild and resolve after the discontinuation of the drug, although more severe cases can occur (with local and systemic complications of pancreatitis, including death) (26).

Thiopurine induced myelosuppression in inflammatory bowel disease.

A review by Gisbert and Gomollon (22), which included 66 studies, evaluated the incidence of thiopurine-induced (Azathioprine, Mercaptopurine) myelotoxicity in IBD patients. Using the cut-off for leucopaenia of 3 x 10^9/L, and the number of neutrophils 1.5 x 10^9/L, the cumulative incidence of AZA/MP-induced myelotoxicity was 7% (95% CI 6-8%), and the incidence rate of myelotoxicity was 3% (95% CI 3-4%) (22). There appears to be similar risk for both drugs, with cumulative incidence of myelotoxicity of 7% (95% CI 5-8%) for azathioprine and 9% (95% CI 5-12%) for mercaptopurine.

Thiopurine-induced myelotoxicity occurs most frequently within the first few weeks or months of treatment, with the duration of treatment ranging from 12 days to 27 years. When only studies with ≤12 months of follow-up were included, the incidence rate of thiopurine-induced myelotoxicity was higher, at 11%, compared to the incidence rate of 3% when all studies were included (22). Myelosuppression can occur over a period of several months or occur quite suddenly. In a study by Connell et al, 9 cases of severe leucopaenia was reported, of which 6 occurred abruptly (having had normal blood counts 1 month previously) (27).

Most patients with AZA/MP-induced leucopaenia (neutropaenia in particular) are asymptomatic. Those that present with symptoms typically have severe sore throat and fever (28). Infections are the only significant consequence of neutropaenia. The cumulative incidence of infections among patients suffering from AZA/MP-induced myelotoxicity was 6.5% (95% CI 3.2-9.8%) (22). The common sites of infection include the oral cavity and mucous membranes and the skin. There have been three deaths reported due to bone marrow suppression in IBD patients treated with AZA/MP, with the risk of mortality in patients with AZA-induced myelotoxicity being approximately 1% (95% CI 0.32-2.70%) (22).

In cases of mild leucopaenia, dose reduction (for example, to 50%) may be sufficient to restore counts (although there have been reports that show that leucopaenia can resolve spontaneously without a change in dose). Re-introduction of the drug is recommended at a lower dose to avoid the reappearance of bone marrow toxicity. In cases with absolute neutrophil counts of less than 1 x 10^9/L, drug cessation is recommended. If the leucocyte counts normalise, there have been suggestions that the drug can be re-started at a lower dose, and ceased permanently if leucopaenia recurs after the re-challenge (22).

Both azathioprine and mercaptopurine are pro-drugs that need to be bio-activated and metabolised to 6-TGN (6-thioguanine nucleotides) through a complex enzymatic pathway to exert their therapeutic effect (19,22). TPMT (thiopurine S-methyltransferase) plays an important role in this process. TPMT metabolises MP into inactive 6-methylmercaptopurine (6-MMP), and hypoxanthine-guanine phosphoribosyltransferase (HPRT) anabolises MP into 6-TGN, the molecule responsible for both the

therapeutic activity and drug-related leucopaenia. A reduction in TPMT activity predisposes to bone marrow suppression because of preferential metabolism of MP into 6-TGN (19,22,29).A trimodal distribution of TPMT activity is observed in the general population and this activity correlates with TPMT genotype (29). Most centres routinely measure TPMT phenotype/genotype to aid dosing patients with these medications. However TPMT genotype / activity explains only 20% of the observed episodes of bone marrow suppression supporting the existence of other genetic or environmental factors. A recent study examining HPRT activity in relation to 6-TGN concentrations and thiopurine-induced leucopaenia found that high levels of HPRT activity predicted unsafe 6-TGN concentrations and leucopaenia (30). The authors postulated that this could partly explain the therapeutic response or toxicity that is not adequately explained by the polymorphisms of TPMT.

Sulfasalazine induced neutropenia in IBD and RA

Sulfasalazine is used for the treatment of Inflammatory Bowel Disease (IBD) as well as Rheumatoid Arthritis (RA). Taken orally, Sulfasalazine is reduced by colonic bacterial azoreductases into Sulfapyridine and 5-aminosalicyclic acid (5-ASA). 5-ASA released in the colon has topical anti-inflammatory and immunosuppressive effects and is responsible for the efficacy of sulfasalazine in IBD while many of the toxic effects, experienced by 10-45% of IBD patients have been attributed to the sulfapyridine carrier moiety. Second generation 5-aminosalicylates (mesalazines) were subsequently developed using alternative mechanisms to deliver 5-ASA to the colon. These drugs are generally better tolerated by patients and have largely replaced Sulfasalazine in clinical practice. However mesalazines are 7 fold more expensive, may be less efficacious (a recent Cochrane review concluded that Mesalazine is statistically inferior to Sulfasalazine in maintenance of remission in UC (31)), and are associated with other serious side effects not seen with sulfasalazine (e.g. pancreatitis, nephrotoxicity).

In contrast to IBD, the active moiety of sulfasalazine in RA is thought to be sulfapyridine. Indeed mesalazines are not effective in the treatment of RA. Sulfapyridine decreases secretion of inflammatory cytokines like IL8 and also inhibits nuclear factor-kappa B (NFkB) which induces the transcription of central mediators of the immune response. Sulfasalazine is used as a Disease Modifying Anti Rheumatic Drug (DMARD) either as monotherapy or in combination with other DMARDs. Haematological disturbances, most commonly mild neutropaenia, are experienced by 2-3% of patients using sulfasalazine (32). In most cases, this is benign and self-limiting. However, idiosyncratic agranulocytosis (defined as neutrophil count less than 500/uL), which predisposes to serious infections, is also seen. Indeed sulfasalazine is currently one of the commonest causes of drug induced agranulocytosis. Data collected from GP records in Britain estimated the frequency of this side effect to be 0.6 per 1000 IBD patients treated with sulfasalazine but approximately 10 times higher (6.1/1000) in patients with RA (33). A large Swedish study showed a fatality rate of 6.5% in patients with agranulocytosis (34). Seven deaths due to blood dyscrasias associated with the use of sulfasalazine were reported to the UK Committee on Safety of Medicines between 1991 and 1998.

Neutropaenia and agranulocytosis typically occurs within 3 months of starting sulfasalazine. The median time to develop agranulocytosis with Sulfasalazine treatment was 43 days amongst cases reported to Swedish Adverse Drug Reactions Advisory Committee (34). In another study of RA cases, 76% of adverse reactions necessitating drug withdrawal were in the first 3 months (35). In order to detect neutropaenia early the British society of Rheumatologists recommends a monthly full blood count for the first 3 months and 3 monthly thereafter. If, following the first year, dose and blood results have been stable, frequency of blood tests can be reduced to every 6 months for the second year of treatment. Thereafter, monitoring of blood for toxicity may be stopped. Less stringent monitoring is recommended by the British society of Gastroenterologists.

Mechanisms

As myelotoxicity is not usually seen with Mesalazine and other sulfonamides are also implicated in agranulocytosis, it is likely that the sulfapyrine component is responsible for neutropaenia. Mild neutropaenia might be dose dependant. Agranulocytosis is more likely to be an idiosyncratic hypersensitivity reaction. The mechanisms are not understood but might include immune mediated destruction of neutrophils, or direct toxic effects upon marrow granulocytic precursors. Both

mechanisms might be mediated by reactive metabolites following the oxidation of sulfasalazine by the neutrophil NADPH oxidase / myeloperoxidase system.

Candidate gene studies reported no association between polymorphisms in the NAT2 and ELA2 genes and sulfasalzine neutropaenia (36). The sulfapyridine (SP) component of Sulfasalazine is inactivated to N-acetyl SP via NAT2 and the neutophil elastase gene (ELA2), is implicated in congenital neutrpaenia. To date no genome wide association studies have been carried out in sulfasalazine induced neutropaenia (37).

Thiopurine induced Liver injury in inflammatory bowel disease.

Thiopurine drugs (6-mercaptopurine and azathioprine) are effective at maintaining remission in patients with Crohn's disease and ulcerative colitis (38,39). Despite this their use is limited by side effects and the requirement for regular blood monitoring. Thiopurine drugs are one of the few drugs in the world where pharmacogenetic testing is routine. The TPMT enzyme assay is accurately able to predict the metabolic products of thiopurine metabolism, which are primarily responsible for bone marrow suppression in a dose dependant fashion thus preventing administration to at risk patients (40).

Non-dose dependant, rare idiosyncratic reactions, that are not predicted with TPMT genotyping are seen in up to 4% of patients (41). The most common non-dose related serious adverse events include pancreatitis and hepatitis. Following an idiosyncratic adverse drug event, as many as 17% of patients have to subsequently discontinue their treatment (42). Interestingly of those patients who restarted a thiopurine after cessation, 40% developed the same idiosyncratic adverse event again suggesting a genetic aetiology (42).

Hepatotoxicity associated with thiopurine administration is well documented and is seen in approximately 4% of all patients (42). Indeed thiopurines have been estimated to be responsible for 4% of all cases of hepatotoxicity (43). Two isolated pictures of liver damage have been described for thiopurines. The most common idiosyncratic reaction is a hypersensitivity syndrome that typically presents with elevated transaminases within the first few weeks of treatment initiation. Cholestatic jaundice is much less common, but necessitates complete and often permanent cessation of the offending drug. These two mechanisms of liver injury are distinct from the dose-dependant liver injury, nodular regenerative hyperplasia, the incidence of which is related to the level of 6-thioguanine, a metabolite of 6-mercaptopurine most commonly observed in patients with low TPMT levels (40).

Thiopurines are metabolised via three competing pathways. The first is to 6-thioguanine, the metabolite responsible for its therapeutic effects, but also its most severe side effects such as bone marrow suppression and nodular regenerative hyperplasia. The second is via xanthene oxidase to 6-thiouric acid (this pathway is responsible for the drug interaction between allopurinol and thiopurines). The third pathway is via TPMT, which methylates the metabolic products (44). There is evidence that rather than 6-TG levels, the levels of methylated metabolic products correlate with the development of hepatotoxicity (45). This is likely, however, to be a TPMT independent phenomenon as TPMT genotype/activity does not appear to be related to the likelihood of developing liver injury (45).

At present very little is known about the predisposing risk factors for thiopurine induced liver damage, although there appears to be an increased risk in the male sex (46). The aim of this research project would be to recruit 300 patients who have suffered a hypersensitivity type hepatotoxicity reaction to thiopurines. In this context, hepatotoxicity is defined as an ALT or conjugated bilirubin that has risen to greater than five times the upper limit of normal for the individual laboratories range within a defined time period of commencing treatment (as defined by the International DILI Expert Working Group (47)).

A detailed clinical database would be built up to identify for the first time the clinical characteristics of patients who have experienced hepatotoxicity. A subsequent genome wide association study would then be undertaken to identify predisposing genetic variants. Although the genetic analysis will provide a useful insight, a real strength of the approach we have taken in Exeter is to aspire to build a combined clinical and genetic risk prediction model. The clinical data is invaluable and provides not

only a more accurate definition of likely cases, but we believe this approach when combined with genetic analysis is more likely to be translatable to the clinic with more immediate benefits to patients.

Thiopurine Hypersensitivity Reaction in Inflammatory Bowel Disease

Thiopurine hypersensitivity reactions are dose independent and occur in 5-10% of patients treated with Azathioprine and Mercaptopurine. Most hypersensitivity reactions are mild, presenting with a flu-like illness within the first 4 weeks of therapy, resolving rapidly on drug withdrawal. Symptoms and signs of mild hypersensitivity reactions are poorly defined in the literature but include fever, myalgia, arthralgia, headache and fatigue often leading to drug cessation. These symptoms can be associated with an acute inflammatory response, supported by a rise in serum markers eg CRP, mimicking active IBD. We believe the more common gastrointestinal side effects of nausea, vomiting and diarrhoea are not typical of this hypersensitivity reaction and such patients will not be included in this study.

A rare more serious thiopurine hypersensitivity syndrome has been described that can present with hypotension, cutaneous eruptions (typically a neutrophilic dermatosis), leukocytosis, and liver and/or renal dysfunction. The clinical picture can overlap with severe thiopurine pancreatitis and may mimic sepsis.

The mechanism of thiopurine hypersensitivity is unknown. It has been proposed that the imidazole component of AZA may be responsible by binding to endogenous proteins resulting in hapten formation and immune activation. This might explain why a small proportion of patients who develop flu-like illness in response to AZA therapy are subsequently able to tolerate 6MP. However, this theory must be challenged as there is no evidence to suggest that the syndrome is more common with azathioprine than mercaptopurine and a number of patients experience identical reactions to rechallenge with 6-MP.

This hypersensitivity syndrome does not appear to be associated with TMPT genotype and is not dose related suggesting an idiosyncratic mechanism. An association with flu-like hypersensitivity to thiopurines and an exonic variant in ITPA has been described in a case-control study, however, it is unlikely this will stand-up to replication.

The aim of this study is to prospectively and retrospectively evaluate patients started on a thiopurine and identify those who develop flu-like symptoms that cause sufficient morbidity that the drug is stopped. Once patients have been identified a detailed clinical history will be collected and a DNA sample taken. A genome-wide association study will be undertaken to identify genetic risk variants that predispose to the development of this hypersensitivity reaction.

3. Study Objectives and Design

3.1 Study Objectives/Aim

The main aim of this study is to identify clinically useful genetic and non-genetic factors that predict serious adverse drug reactions, so that these drugs can be avoided, or monitoring intensified, in high risk patients. A simple, cheap, diagnostic test will be developed using these data which can be rapidly adopted into medium and large sized hospitals. This will allow the development of strategies to individualise drug therapy to maximise benefits and minimise harm.

The secondary objectives are:

a. to understand the mechanisms underlying drug side effects
b. through a knowledge of the mechanisms, to learn about particular functional chemical groups which predispose to toxicity, and thereby facilitate more rational drug design.
c. to develop a network of interested UK clinicians for further pharmacogenetic research projects.

3.2 Study Design

These are a series of case control studies involving patients with documented drug toxicity.

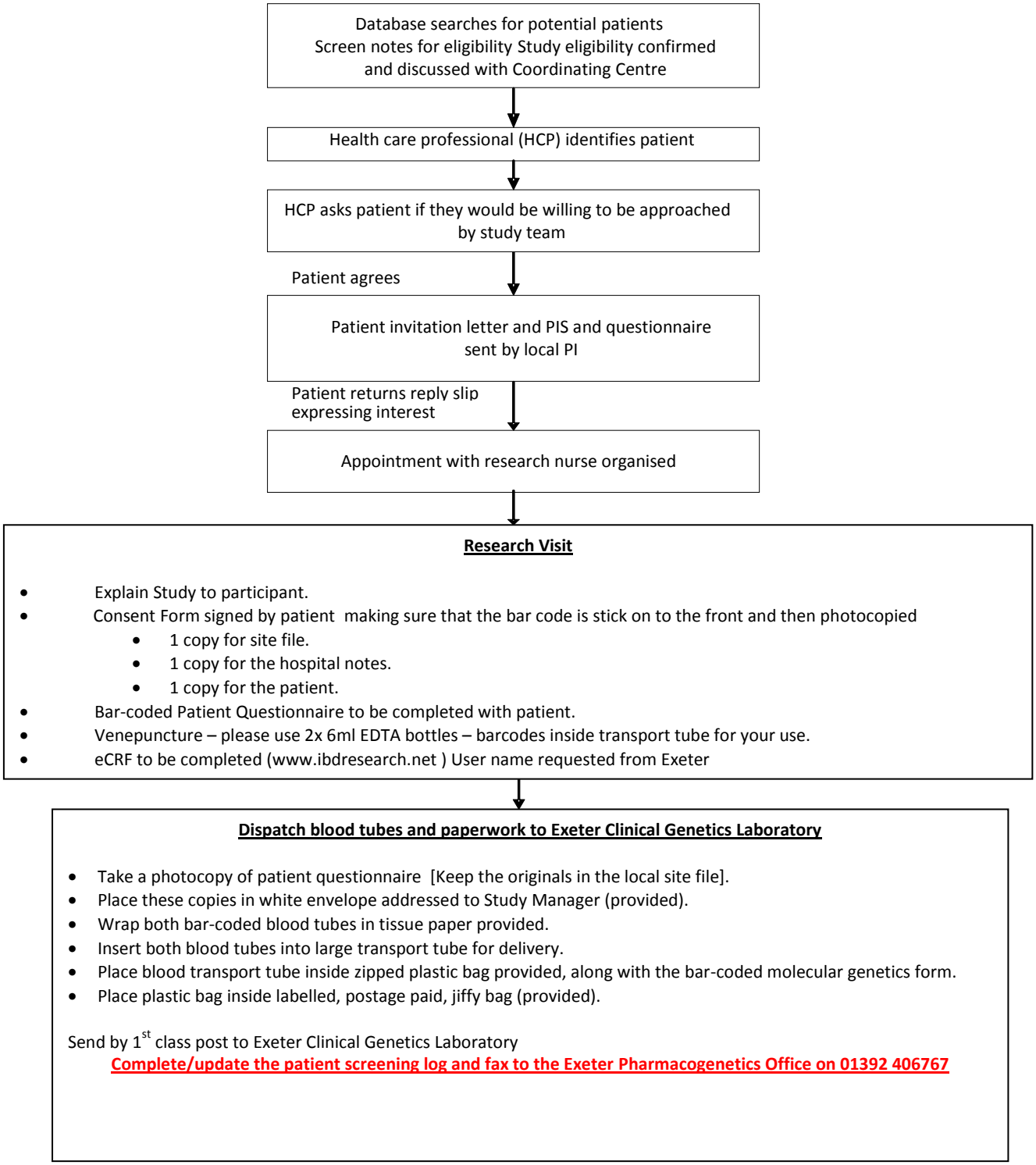
Definitions of drug toxicity

In the absence of a diagnostic test for serious adverse drug reactions, definitions are clinical. Factors implicating a drug as the cause of an adverse reaction include:

- The temporal relationship between the introduction of the drug and occurrence of the reaction
- Improvement following drug withdrawal or dose reduction
- Recurrence on rechallenge with the drug
- Exclusion of other drug or non-drug causes

Patients may be included in these studies if they meet all the major criteria specific to each adverse drug reaction. A further series of minor criteria, which typically includes the factors above, permits a semi-quantitative assessment of diagnostic certainty.

3.3 Study Flowchart



4. Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

- Patient willing to take part.
- Any patients including children.
- Patient meets all major criteria for specific adverse drug reaction
- Written informed consent obtained from patient or parent

Major criteria for cases:

Demyelination complicating anti-TNF therapy in Inflammatory bowel disease and other inflammatory disorders

- History of exposure to anti TNF-α antibody at any time in the past.
- No history of demyelinating neurological symptoms prior to exposure to anti TNF-α antibody.
- Neurological symptoms lasting at least 24 hours.
- MRI brain and/or spinal cord shows changes consistent with CNS demyelination or electrophysiological studies (nerve conduction or evoked potentials) are consistent with PNS or CNS demyelination and confirmed by a neurologist.
- Neurological opinion implicates anti TNF-α medication as possible cause of demyelination, and if the patient is still receiving the drug, it is withdrawn

Proton Pump inhibitor induced interstitial nephritis.

- ≥ 30% rise in serum creatinine *or* ≥ 25% fall in eGFR any time after introduction of PPI
- No other risk factors for renal disease
- Medical opinion implicating PPI justifies drug withdrawal, even if temporary

Thiopurine induced pancreatitis in Inflammatory bowel disease History of ulcerative colitis or Crohn's disease

- Episode of acute severe abdominal pain
- History of thiopurine exposure **within** the previous 7 days of **pancreatitis occurring**
- Rise in serum pancreatic enzymes (amylase/lipase) (≥2 times upper limit of normal)
- Episode of acute pancreatitis within 3 months of starting thiopurine
- Medial opinion implicates thiopurine as the mostly likely cause of pancreatitis, and drug withdrawn

Thiopurine induced myelosuppression in Inflammatory bowel disease.

- History of thiopurine exposure **within** the previous 7 days of **Leucopaenia occurring**
- Normal total white cell count and/or neutrophil count at baseline.
- Fall in total white cell count to ≤ 2.5×10^9/L, or reduction in neutrophil count to ≤ 1.0×10^9/L or less
- Medial opinion implicating thiopurine leads to dose reduction or drug withdrawal even if temporary

Sulfasalazine induced neutropenia in inflammatory bowel disease and rheumatoid arthritis

- History of inflammatory bowel disease or Rheumatoid arthritis
- History of sulfasalazine exposure in the previous 30days
- Normal total white cell count and neutrophil count at baseline
- Fall in neutrophil count to ≤0.5 x109/L
- Medical opinion implicating sulfasalazine leads to dose reduction or drug withdrawal (even if temporary)

Thiopurine induced liver injury in inflammatory bowel disease

- History of inflammatory bowel disease
- Normal ALT and bilirubin at baseline
- No history of chronic liver disease
- Elevation of ALT and/or bilirubin to >= 5 times upper limit of normal (normal range as per local laboratory)
- History of thiopurine exposure in the previous 30 days prior to abnormal blood test
- Medical opinion implicating thiopurine in development of hepatotoxicity

Thiopurine Hypersensitivity Reaction

Inclusion Criteria

- **Aged 6 years or over**
- **History of Inflammatory Bowel Disease**
- **History of thiopurine exposure in the previous 7 days before the onset of adverse event.**
- **Flu like symptoms severe enough to lead to drug withdrawal even if temporary (symptoms should include fever, muscle ache, joint pain)**
- **Onset of symptoms within 4 weeks of starting thiopurine.**
- **Symptoms resolved within 14 days of drug withdrawal.**

Exclusion Criteria

- **Patient tolerated re-challenge with the same thiopurine regardless of dose.**
- **Drug withdrawn due to nausea and/or diarrhoea without any other symptoms,**
- **Objective evidence of Infection.**

Controls:These have already been recruited as part of earlier work:

- Patient willing to take part.
- History of drug usage without occurrence of serious adverse event
- Has the same underlying disease as the index patient.

Written informed consent obtained.

4.2 Exclusion Criteria (please also refer to the Thiopurine Hypersentivity exclusion above)

- Patient unwilling to take part.
- Unable to obtain written informed consent.
- Patient is, in the opinion of the investigator, not suitable to participate in the study.

4.3 Identification of potential Participants

UK Patient Identification

(i) Patients will be identified by their healthcare professional (PI's) within any participating NHS hospital Trust (research site). PI's will also be recruited via the British Society of Gastroenterology, The Renal Association, the Association of British Neurologists, the British society for Rheumatology and the British Association of Dermatologists.

(ii) Engagement with the UK IBD patient group, (NACC - 40,000 members.) and other appropriate patient groups. We will advertise this study directly to patients using our own website (www.ibdresearch.co.uk), patient group websites and newsletters.

Patients contacting the research office will be sent a study information pack comprising patient invitation letter, participant information sheet and participant questionnaire. Patient's willing to take part will be asked to complete and return a reply slip and questionnaire. If the patient's routine clinical care is carried out at an institution designated as a research site then they will be directed to the local PI for recruitment. As a last resort and no research site can be opened close to the patient's home then arrangements will be made for a member of the Exeter research team to visit the patient at home to discuss the study, take face to face informed consent, assist with the patient questionnaire and carry out venepuncture, having confirmed eligibility with the patient's own clinical team. Research passports and letter of access will be obtained, if necessary, and staff will follow the RD&E's lone workers policy.

(iii) The Yellow Card Reporting System. Following approval of this method of recruitment by the MHRA, we will utilise the yellow card database to identify potential patients. We will ask the clinician who originally reported the adverse drug reaction to approach the patient

International Patient Identification

To ensure we recruit adequate numbers of participants and / or to replicate our findings in a second stage experiment we will engage with other genetics consortia, many of whom we are currently collaborating with, as part of the work of the international IBD genetics consortium. Patients will be identified from existing international IBD DNA banks. Eligible patients will have previously given broad informed consent for use of their DNA for IBD genetic studies.

4.4 Withdrawal of Subjects

Subjects will be informed that they are free to withdraw from the study at any time up until the samples and data are coded but not anonymised. When the sample is fully anonymised the participants will still be able to withdrawal however their samples and any data will be retained to be used in the analysis. The Investigator may remove a subject if, in his / her opinion, it is in the best interests of the subject. If a patient permanently withdraws from the study, or is lost to follow-up, the reason will be recorded.

4.5 Expected Duration of Trial

Case identification and recruitment will continue for another year.

5 Study Procedures

5.1 Screening – Medical records

Once the patient has been identified their medical records will be reviewed by a healthcare professional to ensure that met all of the major eligibility criteria. Once this is confirmed the patient will be contacted by their healthcare professional.

The patient will then be sent a patient information sheet and questionnaire to complete at home. An appointment will be made for the patient to be seen by the local research team either at the patient's usual hospital or at home.

Research visit

The study will be explained to the patient. Any questions regarding participation will be discussed.

Each patient will be identified by a numerical barcode which will be supplied to the site by the central coordinating team.

The patient will be asked to read the consent form, initial the boxes and sign and date it; this will be also signed by the delegated healthcare professional taking the consent. The original consent form will be photocopied and a copy given to the patient, a copy placed in the hospital notes and one held in the site file.

The questionnaire will be reviewed and any missing data completed. The healthcare professional will then take a blood sample from the patient (2x4.5 ml is required). Tubes containing EDTA must be used. The patient's unique barcode will be attached to the blood tubes and then placed in the larger transport tubes provided. The patient's initials must be inserted on the laboratory request form, and the transport tubes placed inside the plastic bag containing the request form. These will be put in the jiffy bag and posted to the molecular genetics laboratory at the Royal Devon and Exeter Hospital.

This completes the patient visit. The recruitment log can now be completed and sent to the central Exeter team to alert them of a pending blood sample. The healthcare professional can then complete the case report form (CRF) by using the medical records.

All CRFs are to be completed in a clear, legible manner. Black ink must be used to ensure accurate interpretation of data. Any changes should be made by drawing a line through the data to be changed, entering the corrected information, and signing (or initialling) and dating the change.

Every effort should be made to have the CRFs completed and as soon as possible following recruitment of a participant. Once completed the CRF's and the questionnaire will be copied and sent to the coordinating team in the pre-paid envelope.

Research visit – Children

We are recruiting children of all ages to the study. Children represent a significant minority of patients affected by IBD, and other inflammatory diseases and are as likely to develop these serious side effects as adult age patients. The genetic predictors of these side effects may be different in children. Children will only have a blood sample for these studies taken when they are having bloods taken for routine care.

5.2 Handling of clinical data

Once the eCRF is completed missing data and queries will be raised by the Exeter research team. Research nurses and data managers will liaise with the relevant clinicians regarding data corrections.

The Chief Investigator will be updated on a regular basis and will review the reports produced by the statistician in order to ensure consistency and accuracy of the data. Once CRF's and corresponding queries and reports are reviewed, the CRF will be signed off by the Chief Investigator or designated person.

The database especially designed for this project will be held at the Royal Devon and Exeter Hospital in the Department of Gastroenterology Research Office. It will be password protected and will undergo back up on a daily basis.

Patient confidentiality will be maintained at all times and will be protected in accordance with the Royal Devon and Exeter NHS Foundation trust data protection policy. Data will be pseudoanonymised. A unique study ID will be given to each participant by the local research site and no personal details will be sent to the main research site. Access to the secure file linking study ID with personal details will be held by the local research site only, on a protected computer. Access will be limited to the local principal investigator and research nurse.

5.3 Storage and testing of DNA

DNA will be extracted at the Peninsula Molecular Genetics Laboratory, Exeter by members of Professor Sian Ellard's team. All DNA samples will be stored at the Peninsula Clinical Research Facility in locked, alarmed -80C freezers. Designated members of the research team will have access to the samples. Dr Tariq Ahmad will act as custodian.

All DNA samples will be pseudo-anonymised as we may wish to recruit patients to future studies investigating serious drug side effects based upon their genotype. This is explained to the patients on the patient information sheet. The file linking the sample code to personal identifiable information will be held by the local principal investigators.

Coded DNA samples will be sent to other centres in the UK and USA for genetic analyses but all samples will be fully anonymised.

We will use the latest GWAS genotyping platform (e.g. Illumina 1M chip) and additionally carry out high resolution class I and II HLA typing, taking advantage of any facilities made available by the international serious adverse events consortium. We will draw on publicly available Wellcome Trust Case Control Consortium (WTCCC) data for country and sex-matched population controls.

Genotypic data will be kept by the Department of Gastroenterology at the Royal Devon and Exeter Hospital under secure conditions. Participants and/or their GP's will not be told of the genotypes identified from the studies at any stage during the study.

6 Assessment of Safety

6.1 Risks and benefits for the participant

There is a risk of bruising at the venepuncture site. To minimize this risk venepuncture will only be carried out by healthcare professionals competent at carrying out this procedure.

There are no direct patient benefits from participation.

6.2 Procedures for Recording and Reporting (Serious) Adverse Events

In the event that the patient should experience any untowards events during the visit then the patient will be reviewed by a clinicians and the event will be reported by the normal methods to the sponsor and CI.

7 Statistics

7.1 Sample Size

For each serious adverse event a sample size of 300 cases and 1200 controls provides greater than 95% power to identify an association at "beyond doubt" p values of $1x10^{-12}$ at odds ratios of at least 5.0 with an allele frequency of greater than 2%.

MAF	Sample size	Power
0.05	100	0.26
	200	0.98
	300	0.99
0.1	100	0.85
	200	0.99
	300	0.99
0.2	100	0.99
	200	0.99
	300	0.99

7.2 Analyses

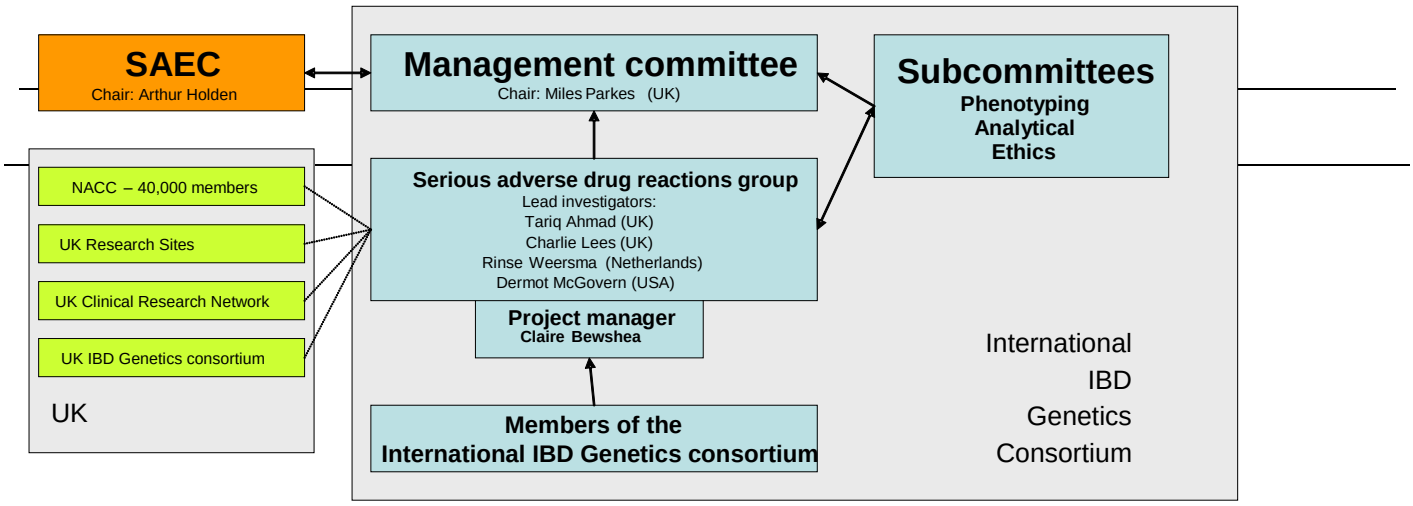
Statistical methodology for genetic association studies is a rapidly developing field, and the most up to date methods will be applied to bring the most powerful statistical methods to bear on the data analysis, and thus extract the maximum information possible from the genotype data. A detailed statistical analysis plan will be prepared prior to starting the analysis by Dr Jeff Barrett, Dr Carl Anderson and Professor Tim Frayling.

Prior to the association analyses, a test for Hardy Weinberg equilibrium will be undertaken at each SNP, using Fisher's exact test. Any marker found to deviate significantly ($p<0.001$) will be flagged and the reasons for deviation explored. Population substructure will also be tested for, and adjusted for in the analysis if any is detected. The extent of missing genotype data per SNP and per patient will be examined and the reasons for this explored. Tests to ensure that any missing genotype data is at random will also be conducted. Multiple imputation methods will be used should missing genotypes be extensive.

For assessing association between a SNP and the risk of an ADR, two tests for association will be undertaken to compare genotype frequencies between cases and controls. The first will be a Chi-squared test, which makes no assumption regarding the underlying mode of inheritance, and the second will be a Cochrane-Armitage test for trend, which assumes an additive mode of inheritance. In the event that it is necessary to adjust for the effect of potential confounding factors, two logistic regression models will be fitted – the first including covariates to represent the confounding factors only and the second including covariates to represent both the confounding factors and the SNP – and a likelihood ratio test used to assess for association. The regression analysis will be conducted twice under the two different assumptions regarding mode of inheritance. In addition to the p-value, the false discovery rate will be calculated to assess for statistical significance whilst accounting for the multitude of tests undertaken.

In the event that copy-number variants (CNVs) are investigated in addition to SNPs, the most up to date methods to assess for association with CNVs will be applied.

8 Study Management Structure



9 Direct Access to Source Data and Documents

The Investigator(s) will permit monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (eg patients' case report forms, consent forms etc. On going central monitoring will be carried out by the sponsor. CV's, delegations logs, CRF's and questionnaires will be deem source data.

10 Ethics & Regulatory Approvals

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to South West - Exeter Research Ethics Committee (REC). Annual progress and safety reports and a final report at conclusion of the trial will be submitted to the Sponsor and the REC. All protocol amendments will be submitted to the REC and Regulatory Authorities for approval.

Consent

All patients recruited to the study will be required to give written informed consent. This will be carried out in person. Patients who lack capacity to consent will not be recruited. All subjects will be given the opportunity to contact a member of the research team to discuss the project in more detail if desired. All subjects will be informed of the nature and purpose of the study, its requirements and possible hazards, and their rights to withdraw at any time from the study without prejudice and without jeopardy to any future medical care at the study site. For children under the age of 16 will obtained parental consent.

Confidentiality

This study adheres to the Caldicott principles for the use of identifiable data. Data will be pseudoanonymised. A unique study ID will be given to each participant by the local research site and

no personal details will be sent to the main research site. Access to the secure file linking study ID with personal details will be held by the local research site only on a protected computer. Access will be limited to the local principal investigator and research nurse.

Individual data will not be made available to participants or their doctors unless the results could potentially impact on the individual's clinical care. Results would then be shared with the participant and their GP/consultant. This decision would be made by the Chief and Principal Investigators.

Use of tissue samples in future research

Consent will be sought in line with the Human Tissue Act (2004) from the patients to use their DNA samples in future work which may involve recruitment based on their genotype. Pseudoanonymisation of the data will allow us to go back to individual patients via the local principal investigators. We will submit a new ethics application for any future studies using these samples.

Patients will be told that their DNA sample will be:

- Considered a gift to the Royal Devon and Exeter NHS Foundation Trust, which will act as custodian of the sample.
- Tested for multiple genes in the future using new genetic techniques
- Made available in an anonymised form only to other researchers working in the field after careful consideration of their study protocol and approval by the relevant REC
- Any commercial use of the findings is unlikely to occur in the short−term and that this is a long−term project.
- Furthermore, any commercial exploitation of the findings is unlikely to be due to single samples, but is more likely to be due to the findings in a large number of patient samples.

11 Quality Assurance

Monitoring of this study will ensure compliance with Good Clinical Practice. Scientific integrity will be managed and oversight retained, by the Research and Development Directorate/ Peninsula College of Medicine and Dentistry.

12 Data Handling

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

- Patient data will be anonymised once it leaves the local research site.
- All anonymised data will be stored on a password protected computer.
- All study data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act.

13 Data Management

The data will be obtained in a paper CRF which will be copied and sent to the central co-ordinating team in Exeter. The CRF will be deemed source data and any queries relating the data in the CRF will be resolved by Data Clarification Forms (DCF's). Once the DCF is completed then it will be signed and dated by the PI.

All CRF's and DCF's will be coded and not hold any identifiable information. The originals will be stored at the coordinating centre and the copies will be kept in the site files. The data will be entered in to an electronic database which will be password protected for analyses.

14 Publication Policy

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals.

15 Insurance / Indemnity

NHS Indemnity will apply.

16 Financial Aspects

Funding to conduct the study is provided by:

1. The international serious adverse events consortium
2. CORE Grant Award – Award letter dated 22nd July 2011

17 Signatures

Date: 11 November 2014

Dr Tariq Ahmad D.Phil FRCP

Consultant Gastroenterologist

Chief Investigator

18. Bibliography

1. Mohan N, Edwards ET, Cupps TR, Oliverio PJ, Sandberg G, Crayton H, et al. Demyelination occurring during anti-tumor necrosis factor alpha therapy for inflammatory arthritides. Arthritis Rheum. 2001. p. 2862–9.

2. Abbott. Crohn's disease integrated summary of safety.

3. Lees CW, Ali AI, Thompson AI, Ho G-T, Forsythe RO, Marquez L, et al. The safety profile of anti-tumour necrosis factor therapy in inflammatory bowel disease in clinical practice: analysis of 620 patient-years follow-up. Aliment Pharmacol Ther. 2009;29:286–97.

4. Gupta G, Gelfand JM, Lewis JD. Increased risk for demyelinating diseases in patients with inflammatory bowel disease. Gastroenterology. 2005;129:819–26.

5. Magnano MD, Robinson WH, Genovese MC. Demyelination and inhibition of tumor necrosis factor (TNF). Clin Exp Rheumatol. 2004;22:S134–40.

6. Sierra F, Suarez M, Rey M, Vela MF. Systematic review: Proton pump inhibitor-associated acute interstitial nephritis. Aliment Pharmacol Ther. 2007;26:545–53.

7. Ray S, Delaney M, Muller AF. Proton pump inhibitors and acute interstitial nephritis. BMJ. 2010 Jan 22;341(sep22_4):c4412.

8. Torpey N, Barker T, Ross C. Drug-induced tubulo-interstitial nephritis secondary to proton pump inhibitors: experience from a single UK renal unit. Nephrol Dial Transplant. 2004;19:1441–6.

9. Simpson IJ, Marshall MR, Pilmore H, Manley P, Williams L, Thein H, et al. Proton pump inhibitors and acute interstitial nephritis: report and analysis of 15 cases. Nephrology. 2006;11:381–5.

10. Ruffenach SJ, Siskind MS, Lien YH. Acute interstitial nephritis due to omeprazole. Am J Med. 1992 Oct;93(4):472–3.

11. Ransford RAJ, Langman MJS. Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. Gut. 2002 Oct;51(4):536–9.

12. Geevasinga N, Coleman PL, Roger SD. Rabeprazole-induced acute interstitial nephritis. Nephrology (Carlton). 2005. p. 7–9.

13. Harmark L, van der Wiel HE, de Groot MC, van Grootheest AC. Proton pump inhibitor-induced acute interstitial nephritis. Br J Clin Pharmacol. 2007;64:819–23.

14. González E, Gutiérrez E, Galeano C, Chevia C, de Sequera P, Bernis C, et al. Early steroid treatment improves the recovery of renal function in patients with drug-induced acute interstitial nephritis. Kidney Int. 2008;73:940–6.

15. Gounaris-Shannon N, Benning J, Delaney M, Muller AF. UK experience of proton pump inhibitor related interstitial nephritis. Gut. 2011 Mar 13;60(Suppl 1):A176–A176.

16. Sandborn W, Sutherland L, Pearson D, May G, Modigliani R, Prantera C. Azathioprine or 6-mercaptopurine for inducing remission of Crohn's disease. Cochrane database Syst Rev. 2000 Jan;(2):CD000545.

17. Prefontaine E, Lr S, Jk M, Cepoiu M. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn ' s disease (Review). Cochrane database Syst Rev. 2010;(9).

18. Timmer A, Jwd M, Dj T, Jk M. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis (Review). Cochrane database Syst Rev. 2012;(9).

19. Teml A, Schaeffeler E, Herrlinger KR, Klotz U, Schwab M. Thiopurine treatment in inflammatory bowel disease: clinical pharmacology and implication of pharmacogenetically guided dosing. Clin Pharmacokinet. 2007;46:187–208.

20. Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW. Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther. 2006;24:715–29.

21. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut. 2002;50:485–9.

22. Gisbert JP, Gomollón F. Thiopurine-induced myelotoxicity in patients with inflammatory bowel disease: a review. Am J Gastroenterol. 2008;103:1783–800.

23. Pitchumoni CS, Rubin A, Das K. Pancreatitis in inflammatory bowel diseases. J Clin Gastroenterol. 2010;44:246–53.

24. Bermejo F, Lopez-Sanroman A, Taxonera C, Gisbert JP, Pérez-Calle JL, Vera I, et al. Acute pancreatitis in inflammatory bowel disease, with special reference to azathioprine-induced pancreatitis. Aliment Pharmacol Ther. 2008;28:623–8.

25. López-Martín C, Chaparro M, Espinosa L, Bejerano A, Maté J, Gisbert JP. Adverse events of thiopurine immunomodulators in patients with inflammatory bowel disease. Gastroenterol Hepatol. 2011;34:385–92.

26. Bollen TL, van Santvoort HC, Besselink MG, van Leeuwen MS, Horvath KD, Freeny PC, et al. The Atlanta Classification of acute pancreatitis revisited. Br J Surg. 2008;95:6–21.

27. Connell WR, Kamm MA, Ritchie JK, Lennard-Jones JE. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. Gut. 1993;34:1081–5.

28. Present DH, Meltzer SJ, Krumholz MP, Wolke A, Korelitz BI. 6-Mercaptopurine in the management of inflammatory bowel disease: short- and long-term toxicity. Ann Intern Med. 1989;111:641–9.

29. Gearry RB, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. J Gastroenterol Hepatol. 2005;20:1149–57.

30. Ding L, Zhang F, Liu H, Gao X, Bi H, Wang X, et al. Hypoxanthine guanine phosphoribosyltransferase activity is related to 6-thioguanine nucleotide concentrations and thiopurine-induced leukopenia in the treatment of inflammatory bowel disease. Inflamm Bowel Dis. 2012 Jan;18(1):63–73.

31. Feagan BG, Macdonald JK. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. Cochrane database Syst Rev. 2012 Jan;10:CD000544.

32. Farr M, Scott DG, Bacon PA. Side effect profile of 200 patients with inflammatory arthritides treated with sulphasalazine. Drugs. 1986;32 Suppl 1:49–53.

33. Jick H, Myers MW, Dean AD. The risk of sulfasalazine- and mesalazine-associated blood disorders. Pharmacotherapy. 15(2):176–81.

34. Keisu M, Ekman E. Sulfasalazine associated agranulocytosis in Sweden 1972-1989. Clinical features, and estimation of its incidence. Eur J Clin Pharmacol. 1992;43:215–8.

35. Amos RS, Pullar T, Bax DE, Situnayake D, Capell HA, McConkey B. Sulphasalazine for rheumatoid arthritis: toxicity in 774 patients monitored for one to 11 years. Br Med J (Clin Res Ed). 1986;293:420–3.

36. Chen M, Xia B, Chen B, Guo Q, Li J, Ye M, et al. N-acetyltransferase 2 slow acetylator genotype associated with adverse effects of sulphasalazine in the treatment of inflammatory bowel disease. Can J Gastroenterol. 2007;21:155–8.

37. Jacobson A, Melhus H, Wadelius M. Can mutations in ELA2, neutrophil elastase expression or differential cell toxicity explain sulphasalazine-induced agranulocytosis? BMC Blood Disord. 2004;4:5.

38. Prefontaine E, Lr S, Jk M, Cepoiu M. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn ' s disease (Review). Cochrane database Syst Rev. 2010;(9).

39. Timmer A, Jwd M, Dj T, Jk M. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis (Review). Cochrane database Syst Rev. 2012;(9).

40. Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology. 2000 p. 705–13.

41. Gisbert JP, González-Lama Y, Maté J. Thiopurine-induced liver injury in patients with inflammatory bowel disease: a systematic review. Am J Gastroenterol. 2007;102:1518–27.

42. Chaparro M, Ordás I, Cabré E, Garcia-Sanchez V, Bastida G, Peñalva M, et al. Safety of Thiopurine Therapy in Inflammatory Bowel Disease: Long-term Follow-up Study of 3931 Patients. Inflamm Bowel Dis. 2013;0:1–7.

43. Björnsson ES, Bergmann OM, Björnsson HK, Kvaran RB, Olafsson S. Incidence, presentation, and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology. 2013 Jun;144(7):1419–25, 1425.e1–3; quiz e19–20.

44. Smith M, Blaker P, Patel C, Marinaki A, Arenas M, Escuredo E, et al. The impact of introducing thioguanine nucleotide monitoring into an inflammatory bowel disease clinic. Int J Clin Pract. 2013 Feb;67(2):161–9.

45. Dubinsky MC, Yang H, Hassard P V, Seidman EG, Kam LY, Abreu MT, et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. Gastroenterology. Elsevier; 2002 Apr 1;122(4):904–15.

46. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis. 2009 Nov;29(4):337–47.

47. Aithal GP, Watkins PB, Andrade RJ, Larrey D, Molokhia M, Takikawa H, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 2011;89:806–15.

48 Jharap B, Seinen ML, Boer NKH de, et al. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. Inflamm. Bowel Dis. 2010;16:1541–9.

49. Costantino G, Furfaro F, Belvedere A, et al. Thiopurine treatment in inflammatory bowel disease: Response predictors, safety, and withdrawal in follow-up. J. Crohn's Colitis 2012;6:588–596.

50 Carter MJ, Lobo a J, Travis SPL. Guidelines for the management of inflammatory bowel disease in adults. Gut 2004;53 Suppl 5:V1–16.

51. López-Martín C, Chaparro M, Espinosa L, et al. Adverse events of thiopurine immunomodulators in patients with inflammatory bowel disease. Gastroenterol. Hepatol. 2011;34:385–92.

52. Chande N, Dj T, Jk M. Azathioprine or 6-mercaptopurine for induction of remission in Crohn ' s disease (Review). Cochrane database Syst. Rev. 2013.

53. Fields CL, Robinson JW, Roy TM, et al. Hypersensitivity reaction to azathioprine.; 1998:471–474.

54. Ansari A, Hassan C, Duley J, et al. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment. Pharmacol. Ther. 2002;16:1743–1750.

55 Marinaki AM, Duley JA, Arenas M, et al. Mutation in the ITPA gene predicts intolerance to azathioprine. Nucleosides. Nucleotides Nucleic Acids 2004;23:1393–7.

56. Marinaki AM, Ansari A, Duley JA, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). Pharmacogenetics 2004;14:181–187.

57. Kennedy NA, Rhatigan E, Arnott ID, et al. A trial of mercaptopurine is a safe strategy in patients with inflammatory bowel disease intolerant to azathioprine: an observational study, systematic review and meta-analysis. Aliment Pharmacol Ther. 2013; 38(10):1255–1266.