



Study Protocol (Version 4 10/11/2015)

Research Study to Determine the Genetic Causes of Primary Sclerosing Cholangitis

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1. Background

Epidemiology

Primary Sclerosing Cholangitis (PSC), a condition characterised by progressive inflammation, obstruction and fibrosis of the intra- and extra-hepatic bile ducts of the

liver, presents typically in the fourth or fifth decades leading often to cirrhosis, end stage liver disease and a need for liver transplantation¹⁻⁶. However presentation across all ages, including young children, is well described. In children the label autoimmune sclerosing cholangitis is also used.

The incidence and prevalence of the disease have been validated in 2 large series. In the first, a study from Norway, 17 of 130 000 inhabitants developed PSC over a 10-year period, giving a mean annual incidence 1.3/100,000 and a point prevalence of 8.5/100,000^{7,8}. A second study in Minnesota identified 22 patients with PSC between 1976 and 2000; the age adjusted incidence was 1.25/100,000 for men and 0.54/100,000 for women. Little is known of the effect of race on the incidence and prevalence of primary sclerosing cholangitis, although it is rare in Alaskans⁹. In the USA in children the incidence and prevalence of autoimmune biliary diseases such as PSC are reported as being 0.3 and 2.1 cases per 100,000 children.¹⁰

PSC is associated with Ulcerative colitis. It is estimated that 2 - 26% of patients with inflammatory bowel disease (ulcerative colitis in particular) have PSC; population based studies from Scandinavia are consistent and suggest 2 - 7.6% of patients with ulcerative colitis have PSC¹¹⁻¹⁵. The pattern of ulcerative colitis associated with PSC appears to differ from that without PSC in that rectal sparing is more common, the inflammatory bowel disease is more quiescent and tends to be right sided with backwash ileitis. In general the prevalence of PSC is much lower in Crohn's disease (1%).

Clinical Presentation

The symptoms, when present, are characteristic of cholestasis and include pruritus, jaundice and fatigue, but PSC may be asymptomatic (15 - 45%). Deficiency of fat soluble vitamins is common. Osteoporosis is a common complication¹⁶ and a third of patients have fractures following liver transplant. Patients may have cirrhosis at presentation, although these are more likely to be symptomatic.

Liver failure manifest as deep jaundice, ascites, variceal bleeding¹⁷ and encephalopathy are the ultimate consequence of progressive liver injury and evolution to cirrhosis after 12 years. Recurrent bacterial cholangitis due to biliary outflow obstruction is a serious and common complication⁴.

Patients with PSC face an increased risk of malignancy. Chronic biliary inflammation is associated with dysplasia and with subsequent evolution to cholangiocarcinoma. The lifetime risk of cholangiocarcinoma in PSC is elevated massively, such that between 4 - 20% of cases develop this complication¹⁸⁻²⁰. There is also a heightened risk of hepatocellular carcinoma (2% of patients with PSC and cirrhosis), pancreatic carcinoma²¹ (a 10 - 14 fold increased risk) and gallbladder carcinoma. In addition, the risk of colorectal carcinoma and dysplasia are increased in patients with PSC. The first study to demonstrate this, from Sweden, revealed that the absolute cumulative risk for developing colorectal carcinoma in those with PSC and ulcerative colitis was 9%, 31% and 50% at 10, 20 and 25 years of disease duration; in

contrast, in those with ulcerative colitis alone, the corresponding risk was 2%, 5% and 10% respectively.²²

Diagnosis

There is no specific blood test. Most cases of PSC come to the attention of the clinician because of abnormal liver blood tests. A raised alkaline phosphatase of biliary origin is typical. Anti-nuclear antibody or smooth muscle antibody is detected in 20 - 70% of cases. pANCA is positive in 65 - 95% of cases²³⁻²⁶ and can be detected in up to 25% of first degree relatives.

The diagnosis of PSC is based on a number of modalities of which radiology is undoubtedly the most useful. The biliary tree can be imaged by both ERCP²⁷ and MRCP²⁸⁻³² which reveal multifocal strictures within the biliary tree with associated intervening dilatations. MRCP is non-invasive and the preferred imaging of choice with a reported sensitivity of 88% and 99% specificity. ERCP carries a complication rate which can be as high as 14% in symptomatic patients and 2% in asymptomatic patients.

The role of liver histology can be informative in the diagnosis and clinical management of patients with PSC. However, some studies have argued against this³³. One potential problem with liver biopsy is that regional sampling may occur. In one study cirrhosis was under-estimated in 37% of cases from a cohort of 55 patients biopsied twice on the same occasion. Biopsy may show bile duct injury, with proliferation, periportal inflammation, fibrosis and the almost diagnostic onion skin fibrosis (seen in 13%). Overlapping features with auto-immune hepatitis may be observed.

Differential Diagnosis

Primary sclerosing cholangitis must be distinguished from disorders that cause similar cholangiographic features on imaging (i.e. HIV, gallstones, etc).

Genetics

Primary Sclerosing Cholangitis is thought to develop as a result of the interaction between the human genome and the environment. If we are to begin to understand PSC we must understand the concept that individual genetic variants of the human genome are neither sufficient nor necessary for this complex disease to develop but instead act as disease risk (i.e. susceptibility) factors. PSC, like the vast majority of human diseases, is complex genetically. Complex diseases are multifactorial, the result of interplay between genes and the environment. Thus, the strong correspondence between genotype and disease phenotype characteristics of Mendelian disorders is not present in this complex disease.

Currently, the best means for quantifying the genetic and environmental influences on complex disease is comparison of disease concordance between monozygotic (i.e. identical) and dizygotic (i.e. fraternal twins). Monozygotic twins share 100% of

their DNA, so that disease concordance is suggestive of genetic influence and conversely, discordance illustrates the likely extent of environmental effect. At present there is no peer review study of this kind in PSC.

In addition to twin studies, familial aggregation provides a means to estimate the level of genetic influence in complex diseases. Because family members are more likely to share genetic material among themselves than with the general population, they are also more likely to share genetic characteristics associated with increased disease risk. However, family members (especially close siblings) share environmental exposure that may also contribute to familial disease aggregation.

The risk of disease development in the siblings of affected individuals is defined by the relative risk ratio of a sibling (λ_s). The λ_s is calculated as the prevalence of a complex disease among siblings divided by the prevalence of the disease in the population at large. Generally, the higher this number is, the greater the evidence of a genetic component to disease. However, caution should be taken when considering λ_s values, because they may be misleading due to a substantial shared environment affect or because of inaccurate (or unknown) data on disease prevalence in the population. To date there has been one study of this kind in PSC, which showed that there is a significant increased prevalence of PSC amongst 1st degree relatives with an λ_s of approximately 100.³⁴ This is also supported by several cases of familial PSC³⁵.

A number of case-control studies have been performed to investigate suspected genes of potential significance in PSC. To date, the best evidence for genetic involvement in PSC comes from analysis of the Major Histocompatibility Complex (MHC). This region encodes 252 expressed genes of which 56 are polymorphic. To date 10 of these have been studied.

The HLA A1B8-TNFA*2-DRB3*0101-DRB1*0301-DQB1*0201, DRB3*0101-DRB1*1301-DQB1*0603, and DRB1*1501-DQB1*0602 haplotypes are associated with increased susceptibility to PSC³⁶⁻³⁸ and critically differ from the HLA findings in patients with ulcerative colitis alone³⁹.

In addition, variants of non-HLA genes from the MHC have been reported to be associated with risk of PSC, including a polymorphism in the promoter region of tumour necrosis factor α (-308 G/A)⁴⁰ and the *008 allele of the MICA gene^{41,42}. Finally, several variants in non-MHC genes have been associated with PSC. These include a 32-bp deletion in the chemokine receptor 5 (CCR5) gene⁴³ and homozygosity for the E469 variant of the intracellular adhesion molecule 1 (ICAM1) gene⁴⁴, which was suggested to be protective.

Combinations of killer immunoglobulin-like receptors (KIRs) and HLA class I ligands that reduce natural killer (NK) cell inhibition have also been shown to increase the risk of susceptibility to Primary Sclerosing Cholangitis (PSC). Three hundred and sixty-five Scandinavian PSC patients and 368 healthy controls were genotyped for the presence or absence of genes encoding all KIRs. The KIR gene frequencies were similar among patients and controls. However, the frequency of HLA-Bw4 and

-C2, which are ligands for the inhibitory KIRs 3DL1 and 2DL1 respectively, was significantly reduced in PSC patients as compared with controls. This is of potential interest as two of the HLA risk haplotypes in PSC (carrying DRB1*0301 or DRB1*1501, respectively) are devoid of both of these alleles and also carry the 5.1 variant of the MHC class I chain-related A (MICA) gene, also reported previously to influence PSC susceptibility⁴⁵.

It may be that the genetics of PSC share common features with those of inflammatory bowel disease based on the known association between the two disorders. However in a different study, genetic risk factors in PSC were investigated on the basis of known IBD susceptibility genes. IBD-associated polymorphisms in the CARD15, TLR-4, CARD4, SLC22A4, SLC22A5, DLG5, and MDR1 genes were genotyped. No significant association between any of the investigated genetic IBD risk variants and overall susceptibility to PSC was observed.⁴⁶

Mucosal addressin cellular adhesion molecule-1 (MAdCAM-1) has been implicated in the aberrant homing of intestinal lymphocytes to the liver in primary sclerosing cholangitis (PSC). MAdCAM-1 polymorphisms or the K469E polymorphism of ICAM-1 were examined in PSC patients from Scandinavia. No significant association with PSC was found for any of the MAdCAM-1 or ICAM-1 SNPs.⁴⁷

Finally some of the immunoregulatory genes responsible for individual susceptibility to PSC have been examined. The co-stimulatory receptor gene cluster on chromosome 2q33 encodes both the positive T-cell regulators CD28 and ICOS and the negative regulator CTLA4. The CTLA4 gene has been implicated in several immune-mediated diseases, but it is not known whether PSC is associated with any of these genes. Overall, there were no statistically significant differences between PSC patients and controls in genotype and allele frequencies for the CTLA4 +49AG and CT60 SNPs or for the CD28-A, CD28-B, SARA43, SARA1, SARA31 and SARA47 microsatellite markers.

At the present time the genetic contribution to PSC is therefore recognised but is limited due to a failure to derive a unifying hypothesis and up to now by a candidate gene approach.

The **PSC Genetics Study** began in 2008, with the aim of sourcing DNA from over 3000 patients with Primary Sclerosing Cholangitis. To date over 1800 patients have participated in the study. This is a significant step towards our goal of identifying genes of potential significance in the pathogenesis of this condition.

In April 2015 we were awarded a grant by the NIHR Rare Diseases Translational Research Collaboration (PI Hirschfield), to extend the study. This will enable us to open up the research to paediatric patients, and to undertake deep phenotyping of PSC patients.

2. Methodology

2.1 Aim and Hypothesis

The primary aim of this study is to identify genetic and clinical factors which contribute to the development and outcome of Primary Sclerosing Cholangitis. The study design will be a case control genetic association study, aligned to clinical and laboratory data on patients.

The null hypothesis is that there is no association between genetic and clinical factors and PSC. The alternative hypothesis is that there is an association between genetic and clinical factors and PSC.

A consortium of investigators has been established that are involved directly or indirectly in the clinical care of patients with Primary Sclerosing Cholangitis within the United Kingdom. This includes adult and paediatric clinicians.

Patients are included if the clinician has given a working diagnosis of primary sclerosing cholangitis or autoimmune sclerosing cholangitis.

The Chief Investigator was Dr Simon Rushbrook until April 2015 and is now Dr Gideon Hirschfield of the University of Birmingham. Dr Hirschfield was the principal applicant to the NIHR for RD TRC funding. The sponsor of the study will remain the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.

We plan to collect two blood samples from PSC patients for the following analyses:

2.2 Samples

- Blood or saliva (the latter now taken from paediatric participants only) for DNA extraction for the purposes of a genome wide association, and allied genetic analyses. The goal is that ultimately patient presentation can be correlated with patient genetic data.
- **Second blood sample** for serum extraction for tests to measure markers in the blood associated with PSC including fibrosis markers and IgG4 levels.

2.3 Confirmation of Disease Phenotype

Clinicians will identify patients who are managed as having primary sclerosing cholangitis or autoimmune sclerosing cholangitis.

Patients will be identified as having PSC based on a number of criteria; analysis will be stratified to the clinical phenotype collected. However, a number of supplementary investigations can support or refute the diagnosis; further, the use of

supplementary investigations may help to identify sub groups within the group with a diagnosis of PSC based on MRCP/ERCP/liver biopsy.

2.3.1 Appropriate biliary imaging

The diagnosis of Primary Sclerosing Cholangitis is based on a number of criteria of which perhaps radiological criteria are the most useful.

The biliary tree can be imaged by either Endoscopic Retrograde Cholangio-pancreatography (ERCP) or by Magnetic Resonance Cholangio-pancreatography (MRCP). Within the consortium Professor Lomas with particular expertise in MRCP/ERCP radiology will review the radiographic imaging of each patient to ensure correct phenotyping. Both these imaging modalities show characteristic features which allow correct disease phenotyping.

2.3.2 Liver histology

Providing the patient has had appropriate positive biliary imaging liver biopsy will not be considered an essential criterion for inclusion.

Patients with an abnormal biopsy yet normal biliary imaging will be collected but will be labelled as Small Duct Primary Sclerosing Cholangitis.

2.3.3 Blood tests

LFTs will be recorded, but will not be part of the diagnostic inclusion criteria because whilst a raised alkaline phosphatase of biliary origin is a typical abnormality, 10% of patients with PSC has a normal Alkaline Phosphatase. Positive Anti-nuclear-antibody (ANA) or smooth muscle antibodies (SMA) occur in 20 - 70% of cases. Similarly pANCA is positive in 65 - 95% of PSC. The presence or absence of these will be recorded for each patient, but will not be a diagnostic criterion as they are neither 100% specific or sensitive.

2.3.4 Exclusion of Secondary Sclerosing Cholangitis

All cases must be distinguished from causes of secondary sclerosing cholangitis which include: Congenital Biliary tree abnormalities, previous biliary surgery, bile duct carcinoma, HIV cholangiopathy, Primary Biliary Cirrhosis, Sarcoid, graft-versus-host disease and drug reactions. The individual collaborators at each centre will ensure that secondary causes of an abnormal cholangiogram have been excluded.

2.3.5 Other Criteria

Patients that have received an orthotopic liver transplant for PSC can be considered as potential study participants

The presence of inflammatory bowel disease will be recorded, but will not be an essential criterion for diagnosis.

Age, gender and ethnic origin will be recorded.

Patients will be recruited from throughout the UK. This is to allow the largest possible number of samples. We expect from epidemiological studies that most patients would be Caucasian.

Over time there has been better recognition and understanding of how PSC presents, and its impact across all ages. After discussion with the patient charity (PSC Support), we have secured strong interest and support for our collection to now include children who have presented with PSC. This amendment allows us to work with dedicated, expert paediatric clinicians, to offer young people with an established diagnosis of PSC (also referred to in paediatric practice sometimes as autoimmune sclerosing cholangitis) the chance to take part in this research.

2.4 How will the patients be approached?

The process for recruitment and participation of adult and paediatric patients is outlined on the flow charts in Appendices A and B ('Appendix A – UK PSC Participation Flow Chart – adults v2'; 'Appendix B – UK PSC Participation Flow Chart – children v1'). Existing participants will be invited to re-consent/give further samples/update their clinical status (see section 2.10 and 'Appendix C – UK PSC Participation Flow Chart – Adult re-consenting and resampling').

2.4.1 Adult patients (aged 18 years and over)

Appropriate patients will be identified at the appropriate centres in clinic. Once they have read the Participant Information Sheet (version 6, 10.11.15), they will have the opportunity to ask questions. They will then be asked if they are willing to participate. If they wish to join the study, they will sign the Informed Consent Form (version 3, 10.11.15). Once they have given consent to a study member, they will be asked to do the following:

Provide:

- Two blood samples (one for DNA and one to measure markers in the blood associated with PSC). These samples will be 18mls each (4 tubes) and can be taken at a routine PSC clinic appointment or by their GP.

AND TO:

- Fill in two questionnaires regarding their condition: one about their PSC and their health in general, and one on their experience of itching symptoms (pruritus)
- Return the questionnaires and samples, plus the ICF, to the study team in Cambridge.

In addition a lead clinician at each centre may write to their respective patients and ask if they wish to take part. If the patient wishes to take part, they will be sent a study pack. As before they will be asked to send their blood samples, ICF and questionnaires back to the study team in Cambridge. Note should be made that postage will be free of charge.

2.4.2 Paediatric Patients (aged 0 to 17 years)

Appropriate patients will be identified at dedicated paediatric clinics. The child and their parents/guardian will be introduced to the study at a routine PSC clinic appointment.

Parents/guardians will be given the Parent/Guardian Information Sheet (version 1, 10.11.15) and the child will receive a Participant Information Sheet (PIS), as appropriate to their age group (PIS age 6-10, PIS age 11-17, all version 1, 10.11.15).

The parents/guardian and child will have the opportunity to ask questions. They will then be asked if they are willing to participate in the study. If they wish to join, they will sign the following:

Children aged 0-5 yrs

- The Parent/Guardian signs the Parent/Guardian Informed Consent Form (version 1, 10.11.15)

Children aged 6-15yrs

- The Parent/Guardian signs the Parent/Guardian Informed Consent Form (version 1, 10.11.15)
- The child signs the Child Assent Form (version 1, 10.11.15), if the child is capable of assenting to participate

Children aged 16-17yrs

- The child signs the PSC Informed Consent Form themselves.

They will be asked to do the following:

Provide EITHER:

- Two blood samples (one for DNA and one to measure markers in the blood associated with PSC). These samples will be 2.5mls each for children aged 0-10 years and 5mls each for children aged 11-17 years. Samples will be taken at a routine PSC appointment.

OR

- One blood sample (of 2.5mls for children aged 0-10 years or 5mls for children aged 11-17 years, taken as outlined above) and one saliva sample
- For their parent/guardian to fill in a questionnaire regarding their child's PSC and health in general (UK PSC Paediatric participants questionnaire, v1, 10.11.15). If the child is aged 11 or over, they can complete the questionnaire themselves (UK PSC Participant Questionnaire v2, 10.11.15)
- To return the questionnaire and samples, plus the ICF and Child Assent Form, if completed, to the study team at Cambridge.

2.4.3 Additional recruitment methods

In addition, PSC Support (www.pscsupport.org.uk) and the UK PSC study website (www.uk-psc.com) will promote on their website the details of the study. Patients who wish to take part will then be invited to enter the study, via means of written invitation.

Finally patients that have had a liver transplant for PSC will be identified. We will request that the UKT and Republic of Ireland Audit and Research Commission identify the name of patients with PSC that have had a liver transplant. The lead clinician for that patient will then write to that individual asking them if they wish to take part.

In addition, a detailed summary of the patient's clinical condition will be sent by the referring clinician to ensure the disease phenotype is accurate. On arrival in Cambridge the sample will be given a unique bar-code; the following data will be recorded:

- 1) Age
- 2) Sex
- 3) How the diagnosis was made
- 4) Associated Inflammatory Bowel Disease
- 5) Whether Liver transplantation occurred
- 6) Associated autoimmune conditions
- 7) All information from the questionnaire

2.5 Blood and Saliva donation and DNA storage

The following samples will be collected from patients.

Adults (aged 18+)

- Two blood samples (2 x 18mls; 4 tubes)

Children

EITHER

10

- Two blood samples (2 x 2.5mls for age up to 10 years; 2 x 5mls for age 11-17 years)

OR

- One blood sample (2.5mls for age up to 10 years; 5mls for age 11-17 years) and one saliva sample

These samples will be sent to Cambridge by 1st class post. Here the sample will be processed as follows:

- Blood sample 1 or saliva sample – DNA extracted and stored.
- Blood sample 2 - serum extracted and tests undertaken for markers in the blood associated with PSC

Following analysis, DNA samples will be transferred to an approved Biobank for long-term storage, for use in future, ethically-approved studies. The collected samples will be retained in the Biobank indefinitely.

2.6 Genetic studies

Using DNA from this collection, compared to control datasets available to investigators, genome-wide association studies, as well as deep sequencing/exome sequencing studies, will be performed. Data may be shared as part of international collaborations. All DNA sent out for scientific analysis will have a unique bar code attached to it and no patient details will be included.

2.7 Correlating serum markers with genetic findings and clinical correlates

As outlined above, we will source a second blood sample from participants, for the extraction of serum, to measure markers in the blood associated with PSC. Testing will be carried out for fibrosis markers and IgG4 levels.

Existing participants will be contacted regarding re-consenting to the study and consenting to provide samples, plus further ones in the future, by signing the new Informed Consent Form (version 3, 10.11.15).

2.8 Informing patients of any clinically-actionable genetic findings

If a clinically-actionable genetic abnormality is identified during analysis of a DNA sample, the research team will inform the participant's clinical care team. In turn, the clinical care team will inform the participant and refer them to a clinical geneticist for confirmation of the finding, if the patient so desires. However, we appreciate that some participants might prefer not to know if they have a clinically actionable genetic abnormality. The revised ICF therefore allows the participant to opt out of being informed. Our policy is based on the Genomics England (GEL) initiative.

2.9 Follow-up with existing participants

Patient Resampling

If existing study participants sign the new Informed Consent Form (version 3, 10.11.15), they will provide two blood samples, one for serum extraction and one for DNA extraction. They may also be asked to complete the patient questionnaire again and for their clinician to update the clinician questionnaire. The process for obtaining reconsent and undertaking resampling is outlined in 'Appendix C – UK PSC Participation Flow Chart – Adult Reconsenting and Resampling'.

Patient Recall

To maximise the value of this study, we seek consent to invite participants to take part in future research seeking effective therapies for PSC. The new Informed Consent Form (version 3, 10.11.15) or Parent/Guardian Consent Form for child participants (version 1, 10.11.15), includes consent to be directly contacted and invited to participate in other research studies. Such invitations will be based on information about the patient stored in the research database.

At the time of consent if patients give permission to be recontacted in the future for follow up blood samples, they may be approached at a frequency no greater than annually, to provide one blood sample (18mls in adults; 2.5mls in children aged 0-10 years; 5mls in children aged 11-17 years) for serum extraction, storage in a registered biorepository, and analysis for markers of disease activity and severity, with correlation to clinical course.

2.10 Collaboration with third party investigators

Sharing of clinical and genetic information with third party investigators

The UK-PSC Database contains a large amount of clinical and genetic information about study participants which could be extremely useful to third-party investigators. Now that the UK PSC Study is part of the National Institute for Health and Research (NIHR) Rare Diseases - Translational Research Collaboration (RD-TRC) initiative, we are required to share data with third-party investigators. From an ethical perspective, data sharing is immensely important because it maximises research outputs without subjecting patients to additional research procedures.

Anonymized information from the UK-PSC Database is available to third-party investigators, subject to approval by a Data Access Committee consisting of independent experts appointed by the Study Steering Group.

Sharing of DNA samples with third party investigators

The UK PSC Study DNA collection is a valuable resource for third-party investigators. Now that the study is part of the NIHR RD-TRC, we are required to permit sample sharing. From an ethical perspective, sample sharing is immensely important because it maximises research outputs without subjecting patients to re-sampling. For these reasons, anonymized DNA samples will be made available to third-party investigators, subject to approval by a Data Access Committee, consisting

of members of the study team, plus independent experts appointed by the Study Steering Group and a member of the Information Governance or Data Protection Team at Cambridge University Hospitals NHS Foundation Trust (the sponsor).

Any data exported from the database for third-party investigators will be anonymized, meaning that all identifiable details will be removed, including the NHS or CHI number. However, shared information will be labelled with the unique study identifier. During the study and for ten years after the study has ended, samples and shared information will therefore be 'linked-anonymized' because the study number will be linked to identifiable information retained in the UK-PSC Database. Thereafter, samples and information will be fully anonymized because identifiable information will have been destroyed. Participants in the study will be able to opt out of sharing of clinical data.

Anonymized genetic information will also be deposited and stored indefinitely in the European Genome-phenome Archive (EGA), maintained by the European Bioinformatics Institute (EBI) in Hinxton, Cambridge, UK. Anonymized genetic information stored in the EGA may be shared with legitimate, third-party researchers, in the UK or abroad by managed access. Third-party researchers will be made to sign a legally-binding Data Access Agreement in which they commit to protect the confidentiality of participants and use the genetic data for research purposes only.

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