Establishment of a UK Evans' Syndrome registry and Molecular Investigation of Potential Causative Factors

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STUDY PROTOCOL

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Background: Evans' Syndrome

Evan's Syndrome is a rare autoimmune blood disorder which occurs in both children and adults. The clinical features are anaemia and low blood platelets (thrombocytopenia); the latter leads to bruising and bleeding. The autoantibodies are directed against the patient's own red cells and platelets and are assumed to arise by chance. Evans' syndrome is very similar to an autoimmune platelet disorder called idiopathic thrombocytopenic purpura (ITP) in which antibodies against the patient's own platelets are found, but unlike ITP, the bleeding in Evans' Syndrome is often very severe and difficult to treat, and may be life-threatening. Diagnosis is difficult since there is no diagnostic test (Evans' Syndrome is a clinical diagnosis and one of exclusion). Treatment is also problematic since the disorder does not respond well to standard ITP-type treatment (steroids, immunoglobulin, immunosuppressives) and the side-effects from therapy are often serious.

Treatment comprises

- Corticosteroids
- Intravenous immunoglobulin
- Immunosuppressives e.g. cyclosporin, azathioprine
- Splenectomy

Because Evans' Syndrome is so rare (it is probably 10-100 times less common than ITP which has an incidence of around 10 per 100,000 population) no single hospital sees enough patients to be able to design suitable diagnostic tests, treatments or carry out research into the causes of the disease. What is required is a large series of patients with Evans' Syndrome, and information collected relating to their clinical features, basic lab results, responses to treatment. This type of study requires a registry such as the one proposed here. We have set up two registries for ITP, both UK-wide. These allow all Trusts in the UK to submit anonymous data from patients with ITP into our central database. We hope to accrue 300–500 patients and analyze their data in the hope that it will inform us about which treatments are best, which lab tests are most discriminatory and also something about the cause of the disorder.

There are very few studies in Evans' worldwide largely because the condition is so uncommon. Our department is recognized as the leading UK centre for ITP and related disorders which puts us in an ideal position to carry out this registry.

The questions we wish to answer in Evans' Syndrome using this Registry:

1. How common is Evans' Syndrome in adults and children?
2. Is there a female sex preponderance similar to other autoimmune diseases?
3. What are the typical presenting features: laboratory and clinical?
4. What treatments are being used in the UK and what are their success rates?
5. What causes Evans' syndrome?
6. Could Evans' Syndrome have a genetic component similar to that found in other autoimmune diseases? If so, would examination of polymorphisms* (SNPs) within the immune genes help us understand the disease? This strategy has worked for rheumatoid and many other autoimmune diseases so would appear a reasonable starting point with Evans' Syndrome.
7. Can we use these data to help manage patients better, providing effective treatment and identifying those at risk of severe complications?

[*Polymorphisms: single gene polymorphisms (SNPs) are variations in our individual genes. These base changes occur at roughly every 1000 bases. They are not mutations but simply natural variants. SNPs within specific genes can lead to increased or decreased production of

proteins including those that coordinate the immune response. We already know that in autoimmune disease there is overproduction of some proteins and underproduction of others (cytokines). It is likely therefore that these over/under-productions are genetically determined and the genes most likely to be affected are those intimately involved in antibody and immune regulatory protein production. For this reason we have chosen SNPs within genes involved in coordinating the immune system as the basis for our genetic study.]

Logistics

We are currently running two similar registries and have established the infrastructure for handling samples and data used for the proposed study. All Trusts involved in the Evans' Syndrom Registry would be required to obtain LREC approval, after which they can submit information to the main centre (Royal London). All investigations and information handling will be carried out centrally.

Data type

The clinical and laboratory data will be collected via simple proformas (shown below) which clinicians will complete when they see a patient with Evans' Syndrome. The form is available as a Microsoft Word document and can be completed electronically or via hardcopy. The patient will be given information about the purposes of the Registry (Patient Information Sheet) and asked whether they wish to take part. After signing the consent form 10ml EDTA (standard blood bottle) will be collected from adults and 5ml for children. Anonymity will be maintained for both samples and proformas; no patient names will be used. The participating clinician will send the proforma and blood sample to Dr Provan at The Royal London (Avery labels will be available for all centres with correct postal address for sending samples).

Upon receipt, DNA will be extracted and stored (anonymously) at –70°C in The Department of Experimental Haematology, Royal London Hospital, until required for SNP analysis (below).

The proforma (shown below)

We have intentionally kept this brief and hopefully easy to understand. We have asked what we consider to be the most relevant questions. In all, there are 4 pages (folds into an A5 size if printed double-sided:

Additional information

Any other complications in this patient?

Additional comments

What next?

When complete, please send to Dr Drew Provan using the enclosed label, along with 10ml EDTA blood (5ml for children). First class mail is fine (no need to post on ice). Many thanks for your help with this Registry.

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Evans' Syndrome Registry

Clinical Proforma

Clinical details

Sample date		Referring clinician	
Hospital/Trust			
Pat. No. (e.g. PAS)		DOB	
Sex		Ethnic Group	
Date of presentation			
Age at onset of Evans'			
P/H autoimmune disease			
F/H autoimmune disease			
Any preceding infection?			
Main symptoms at diagnosis			

Investigations

Blood group		Reticulocytes		LDH	
Initial Hb		Platelets			
First line treatment					
Response		Hb		Platelets	
Second line treatment					
Response		Hb		Platelets	
Third line treatment					
Response		Hb		Platelets	
Did the patient undergo splenectomy?					
Response		Hb		Platelets	
Latest count		Hb		Platelets	
General treatment comments					

Other documents

The Patient Information Sheet, Consent Form, and GP letters have already been submitted to the MREC. All will be available electronically for participating centres along with Avery labels (Word document) for posting proformas and samples.

Data store

Data (from the proformas) will be entered into a password-protected FileMaker Pro database designed by Dr Provan (very similar to that used for our other two UK Registries) by our Data Coordinator. This post was created after raising sufficient funding to allow the appointment of a part-time administrator who inputs the data and ensures our data are complete.

Data analysis

Our Trust has a site-wide licence for Dendrite (Dendrite Clinical Systems Ltd, Oxon) which is a computer software package that allows analysis of very large amounts of data such as those described for this study). Dendrite systems have been used in many NHS Trusts for data analyses similar to our proposed Registry. If there are any positive associations between clinical, laboratory or genetic (SNP) parameters Dendrite will determine these. The software will also carry out risk-modelling, looking for associations that might point towards predispositions to, for example, severe bleeding, responses to specific therapies, etc. This type of modelling has been carried out for acute leukaemia very successfully at University College Hospital using Dendrite software.

"Advertising"

UK haematologists will be informed about the Registry via *The British Society of Haematology* newsletter. All UK studies such as this are advertised in this quarterly publication of the BSH which is our National professional association. Patients will also be informed about the Registry via the Evans' Syndrome website, since the patients have helped raise the funding required for the genetic SNP study.

Genetic polymorphism study

Hypothesis

Single nucleotide polymorphisms (SNPs) may play a role in the pathogenesis of Evans' Syndrome. Specific SNPs may be useful markers of severity, chronicity, responses to therapy and overall disease outcome in patients with Evans' Syndrome. SNP studies may enhance our understanding of the cause and pathology of autoimmune disease and potentially may help identify new targets for therapy.

Study end-points

Obtaining demographic (incidence & prevalence), clinical and laboratory data, in addition to SNP profiles from adults and children with Evans' Syndrome, and determining whether there is any association between SNP profile and clinical parameters. Associations such as disease severity, chronicity, clinical behaviour, responses to therapy including splenectomy will be sought.

Eligibility

Prospective and retrospective (Evans' Syndrome is very uncommon, although we have no accurate data to provide the true incidence and prevalence, and we are very keen to accrue as many patients as possible), childhood and adult patients with Evans' Syndrome. Informed consent and LREC approval will be required.

Design

Clinical data will be collected locally and submitted to Dr Provan (proforma) along with EDTA blood sample. DNA will be extracted and archived centrally (Royal London Hospital).

SNP assays have already been established and we will use identical methodology to that in use for the ITP registries.

Polymorphisms chosen for investigation

We are using identical (routine) methods to those for the other ITP registries we are currently running. However, we have include additional genes since we are attempting to look at B and T cells as well as macrophages/antigen-presenting cells and a variety of other genes involved in coordination of the immune response. We have not chosen these blindly, but rather we have based these on studies from a wide variety of other autoimmune diseases.

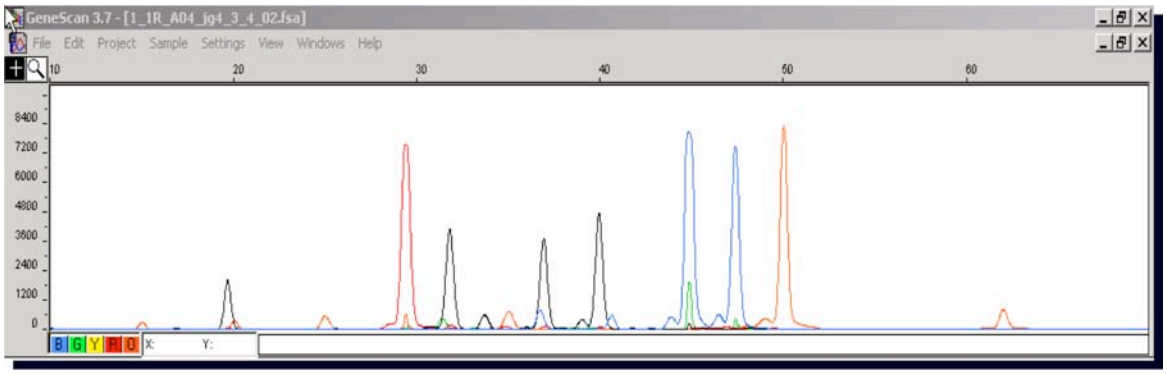
Phase I SNPs		Phase II SNPs	
Cytokines	IL-1	**Cytokines**	IL-5
	IL-2		IL-8
	IL-4		IL-8RA
	IL-4R		IL-8RB
	IL-6		IL-13
	IL-10 (x 3)		IL-15
	IFN-γ		M-CSF
	TNF-α		GM-CSF
	TGF-β (x 2)		G-CSF
Fcγ receptors	FcγRII & III	**Chemokines**	CCR2
			CCR5
Others	NRAMP-1		SDF1
			RANTES
			MIF
		Apoptosis genes	CASP8
			CD95
			CD95L
			BCL2
		T cell signalling	CTLA4
		Others	KIR
			HLA

How much blood is required

10ml EDTA blood from adults and 5ml from children. We can carry out the assays with less DNA but the volumes we have chosen are the ideal quantities. All DNA will be maintained in our Tissue bank and used only by us for this specific study and analysis of the genes described in this document and the MREC form. No material will be passed to third parties for any purposes.

What methods will be used for analysis

The SNP methodology is well-established and we will be using standard methods. This is a DNA-based ABI SNAPshot (PCR) method in a multiplex system. This allows us to analyze 10 SNPs simultaneously for each patient.



The figure above shows 10 distinct SNP tests carried out (in one tube) for a single patient. The results are read off and logged into the database for later analysis.

Control data are already published and in the public domain for all SNPs under investigation. We have a large control DNA bank currently in use for similar studies which has ethical approval (with total anonymity and age, sex and ethnically coded).

Scientific support

We have employed a senior Postdoctoral fellow who is experienced in genomic techniques and routinely carries out SNP analysis. She has previous experience of running these assays from her previous work at The Kennedy Institute and she is proficient in most techniques involving DNA amplification.

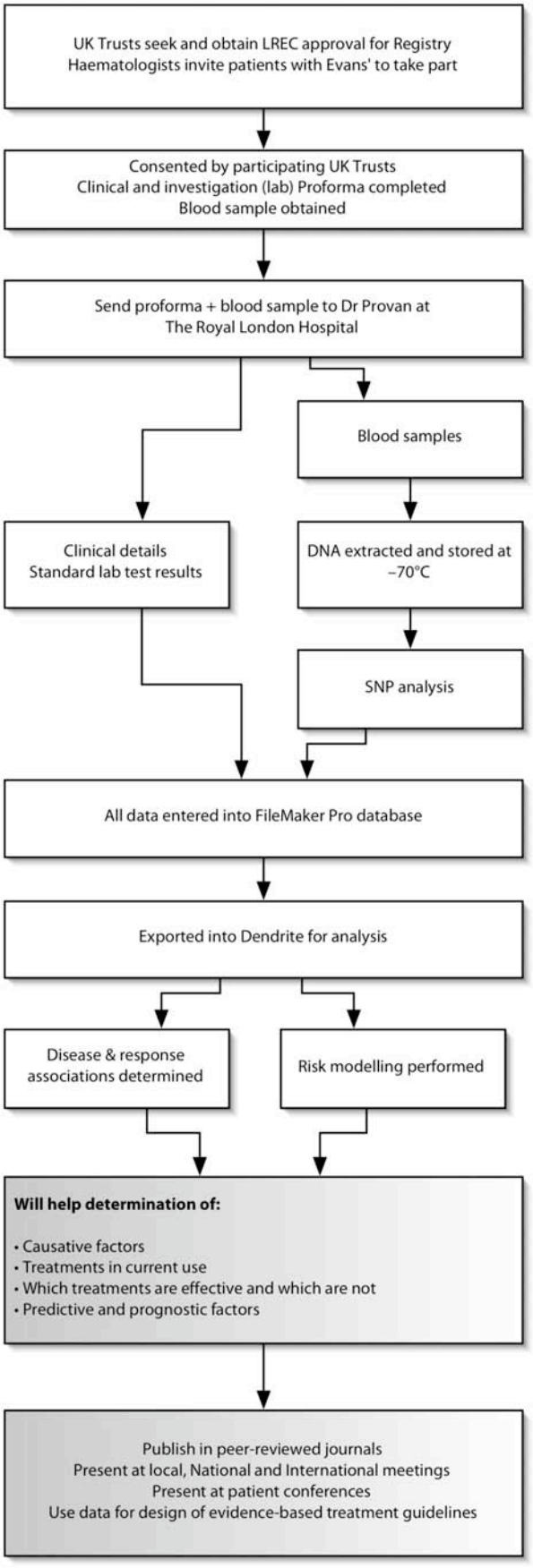
How will the results be disseminated

Complete anonymity will be maintained for all patients and any data published will be similarly anonymous. Data will be written up formally and submitted to peer-reviewed journals. We will also share information with the patients through talks at patient conferences. We would also plan to present data at National and International meetings so that we may share our findings with other haematologists since the whole purpose of the registry is to improve patient care through better diagnosis and treatment. It is hoped that the data generated may be used to design evidence-based practice guidelines for better diagnosis and treatment of patients with Evans' Syndrome. Dr Provan has experience of writing guidelines since he is Chair of the sub-committee of the British Society for Haematology that commissions and writes such guidelines, and was lead writer of the recent ITP treatment guidelines.

Funding

Stationery costs, salary for data analyst and postdoctoral scientist are covered by previous grants or NHS funding. The only funding required is to cover the cost of the SNP analysis and this will be provided by the Evans' Syndrome Patients' Association. We have sufficient funding to carry out the proposed study as described here.

Flow diagram of Registry logistics



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