

Genetic Susceptibility to Rheumatic Heart Disease in New Caledonia: Study Protocol Version 2.0

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1. Project summary

Background: Rheumatic heart disease (RHD) results from an autoimmune response to infection with *Streptococcus pyogenes* (Group A Streptococcus, GAS). Despite being a significant cause of morbidity and mortality in the developing world, control of the disease is limited to antibiotic prophylaxis and there is currently little prospect of developing a GAS vaccine effective in the developing world.

Objective: To identify genetic variants affecting susceptibility to RHD through a case-control association study using a genome-wide and fine-resolution approach. The specific objective of this project will be to recruit, consent and phenotype patients with rheumatic heart disease and peer-nominated controls and collect blood for isolation of DNA.

Study sample: One thousand patients from New Caledonia, Oceania, diagnosed with RHD by echocardiography and a similar number of peer-nominated controls to be recruited from the same population recruited over eighteen months.

Association study: Case-control genome-wide association study genotyping or sequencing single nucleotide polymorphisms (SNPs) and/ or other genetic variants in 1000 cases and 1000 controls which will be followed-up by replication of selected putatively associated variants in further cases and controls recruited in New Caledonia and at other sites and further characterisation of loci of possible association.

Implications: Rheumatic heart disease is unique example of an autoimmune process associated with a known specific pathogen. Therefore, with the potential to reveal genetic variation associated with susceptibility RHD, this study might further our understanding of the complex interaction between human genetics, susceptibility to infection and autoimmunity. In addition the results might generate new approaches to GAS diagnostics, treatments and vaccine development.

2. General Information

a) Project title

Genetic Susceptibility to Rheumatic Heart Disease in New Caledonia

b) Version number

Version 2.0

c) Last updated

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d) Sponsor

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d) Funding

Medical Research Council (UK) Clinical Research Training Fellowship
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e) Name and title of investigators

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3. Background

Humans show marked inter-individual variation in susceptibility to the diseases associated with *Streptococcus pyogenes* (Group A Streptococcus, GAS), which range from invasive infection to post-infectious autoimmune consequences. Worldwide the most significant of these diseases is rheumatic heart disease (RHD). Therefore, to further understanding of the complex interaction between GAS and human populations, we wish to investigate how human genetic variation contributes to susceptibility to RHD.

a) Global burden of RHD

The spectrum of severe disease associated with GAS includes RHD, its precursor, acute rheumatic fever (ARF), streptococcal glomerulonephritis, and invasive infections. There are few reliable data from countries where these diseases are most prevalent but it is estimated that at least 18 million individuals worldwide are affected(1). Rheumatic heart disease has a global prevalence of 15.6 million and is thought to lead to at least 250 000 to 500 000 deaths annually(1), about half as many as attributable to malaria(2). Commissioned by the Bill & Melinda Gates Foundation, the 2007-8 surveys of global public and private investment in research and development for new products for neglected diseases found that funding for ARF, almost exclusively targeted at vaccine development, represented 0.4% of that for malaria(3, 4); other areas such as epidemiologic surveillance of RHD are even more neglected(5).

b) Control of RHD in the developing world

Currently control of RHD is limited to antibiotic prophylaxis(6) and although control would be achieved by immunisation there are major challenges: not only does the development of an effective *S. pyogenes* vaccine require broad coverage(7) but also there is also the risk of induction of autoimmunity(8). To date, only one vaccine candidate has reached clinical trials(9); however, on the basis of available data concerning M protein gene (*emm*) sequence typing, this vaccine is likely to provide poor coverage in the developing world(10).

c) Genetic susceptibility to RHD

Various data suggest susceptibility to rheumatic heart disease is heritable(11). Large family studies from the early twentieth century suggest the precursor to RHD, acute rheumatic fever (ARF), clusters in families with a two-fold increased risk amongst siblings. In a large military study of adults, patients with recurrent ARF had a six-fold increased chance of reporting affected relative. More recently pooled analysis of twin studies find monozygotic twins of index cases at six-fold greater risk, indicating heritability of 60%(12). However genetic studies of human leukocyte antigens and other candidate immune genes have failed to provide convincing associations(13).

4. Rationale

In Pacific Islander populations RHD affects at least 4 in 1000 persons(1). With echocardiography having emerged as a powerful tool for diagnosis of RHD in tropical and low-resource settings(14), we propose a large case-control genome-wide association study (GWAS) of genetic susceptibility to RHD set in New Caledonia in the Pacific. Indeed, GWAS has proved a powerful tool in delineating genetic susceptibility to complex diseases of comparable heritability(15), particularly chronic inflammatory disorders(16).

5. Objectives

The primary aim is to identify genetic variants affecting susceptibility to RHD through a case-control association study using a genome-wide and fine-resolution approach allowing for potential epidemiologic confounders.

6. Experimental Design

a) Study sample

We aim to recruit one thousand patients from New Caledonia, Oceania, diagnosed with RHD by echocardiography, and one thousand peer-nominated controls. Given the epidemiology of the disease in New Caeldonia we anticipate the majority of the patients will be of Pacific Islander ancestry. We will recruit patients from the inpatient wards and outpatient department at the main public hospital in Nouméa, the Centre Hospitalier Territorial Gaston-Bourett, as well as the satellite hospitals around New Caledonia. We estimate XXX patients with RHD are known to the Cardiology services at Centre Hospitalier Territorial Gaston-Bourett.

Patients will be asked to nominate a friend or neighbour (other than a first-degree relative, that is your brother or sister, mother or father, or child) of similar age, sex and ethnicity with no history of RHD willing to participate in the study, who the research team will invite to participate in the study, a process referred to as peer-nomination. This strategy has been used successfully in studies of tuberculosis in rural China (Personal communication, Professor Adrian Hill).

Both patient and control will be consented for enrolment in the genetic studies. From each participant we will document reported ethnicity, location of residence and baseline clinical and demographic details including histories of invasive GAS, ARF, post-streptococcal glomerulonephritis, other significant past medical history (e.g. type II diabetes mellitus), and socio-economic determinants (e.g. occupants per room, household income, maternal education status). Where inadequate echocardiographic data is available we will consent the patients for a further study. In addition a subset of adult volunteers, provisionally selected from those recruited at the Centre Hospitalier Territorial Gaston-Bourett, Nouméa, will undergo to provide controls known definitely not known to have disease ('hypercontrols'). We will

collect a 5 ml blood sample from both cases and controls which will be stored at room temperature in a commercially available DNA stabilization media (e.g. DNAgard®) during transport to and then storage at the clinical diagnostics laboratory at the Centre Hospitalier Territorial Gaston-Bourett, Nouméa, where they will be stored in an air-conditioned room. Later the samples will be transported in batches to Matiaka House, a Fiji Ministry of Health Laboratory in Suva, Fiji, where staff are being trained to extract DNA from blood.

b) DNA processing, micro-array analysis and association analysis

DNA will be extracted from whole blood by an established salting out technique(17) and quantified. A microarray such as the HumanOmniExpress-12 BeadChip (Illumina®, USA) or Human ImmunoChip will be used for genotyping. Additional funding will be available from the Wellcome Trust Centre for Human Genetics for the use of such microarrays.

Samples will be excluded on the basis of quality control measures including call rate, heterozygosity, duplication and relatedness. Single nucleotide polymorphisms will be excluded on the basis of call rate, deviation from Hardy-Weinberg equilibrium (HWE) and minor allele frequency. Multi-dimensional scaling of identity-by-state relationships between study samples and those from the International HapMap project will be used to remove samples with outlying ethnic background. Components identified in this analysis will also be utilised to adjust for potential population structure and cryptic relatedness. Quality control and subsequent statistical analyses will be performed using the PLINK software. Case-control association will be tested using logistic regression, adjusting for components of population structure and any other potential confounding factors as covariates. In order to improve coverage and power, the IMPUTEv2 software will be used to impute variants not present on the HumanCytoSNP-12, but present in the 1000 Genomes project(18). Imputed SNPs will be analysed using the SNPTEST software, with adjustment for covariates as above.

Our group is conducting a study of very similar design in Fiji, where we anticipate recruiting 1200 cases and 1200 controls. The study has been funded by the British Medical Association Josephine Lansdell (2012) grant, awarded to Dr Thomas Parks. The 300 most associated, statistically independent SNPs identified in the New Caledonia study will be followed-up by genotyping in the cases and controls from Fiji using technology such as the Sequenom's MassArray primer extension assay(19) or direct sequencing. Single nucleotide polymorphisms and samples will be assessed for quality in terms of call rate and deviation from HWE and association analyses performed in PLINK as above. The results of the discovery and follow-up stages of the study will be combined via fixed-effects meta-analysis. If a causal variant (or a SNP in complete linkage disequilibrium with a causal variant, $r^2 = 1$) is genotyped in both stages the cohort will give 80% power to detect association at allelic odds ratio of 1.5 or greater alleles with a minor allele frequency of 10% at genome-wide significance (p -value = 5×10^{-7} , (20)). Later, further genotyping and sequencing up to and including whole genome

sequencing will be performed on selected samples to provide greater resolution.

7. Potential implications

We propose that a large, well-designed study employing a genome-wide approach will provide an important insight into the biology of the RHD as has been demonstrated in other common human diseases.(15) While the Wellcome Trust Case Control Consortium and others have taken advantage of existing large-scale collections of DNA, such sample sets do not yet exist in RHD, where genetics research has to date been characterised by studies of poor quality, the majority including less than 100 patients.(11, 13) For us to pioneer studies of genetic susceptibility to RHD, the crucial first step is to establish similar large collections of well-phenotyped patients and it is logical to do so in a setting such as New Caledonia where the disease is endemic and a major public health problem in the context of an established control programme.

Rheumatic heart disease is a unique example of an autoimmune process associated with a known specific pathogen. Therefore, with the potential to reveal genetic variation associated with susceptibility RHD, this study might further our understanding of the complex interaction between human genetics, susceptibility to infection and autoimmunity. Further, insight into RHD pathogenesis gained through studies of genetic susceptibility has significant potential to inform efforts to develop reliable diagnostic tests, therapeutics and vaccines. For example, genetic susceptibility to infection is being pursued in the hope that it may have a major impact on vaccine development, as earlier studies of genetic susceptibility to malaria have shown.(21, 22)

8. Data management and confidentiality

Only the local research team in New Caledonia will know the names of the participants. Once dispatched from the research site each sample and data sheet will be allocated a unique index number such that researchers will not be able to identify participants. Consequently the results of genetic studies will not under any circumstances be conveyed to the participant.

All data will be anonymous and managed in a way that is fully consistent with the terms of consent under which the samples were provided in a manner approved by the relevant research ethics committees. Data will be entered into an electronic database. The original datasheets will be stored in a locked repository and destroyed at the end of the study. The electronic database will be password protected and only individuals directly involved in the project given access.

9. Duration of the project

We will ascertain cases, sample blood and extract DNA between September 2012 and January 2014. Genetic analysis will begin towards the middle of

2012. Following completion of the proposed study, we will, however, retain DNA and genetic data for use in further studies as outlined below.

10. Storage, ownership and governance of future use of the samples

After processing and extraction of DNA of blood samples in the Pacific at the Matiaka House laboratory the sample will be split with a portion stored in the Pacific and the remainder shipped to the Wellcome Trust Centre for Human Genetics, Oxford, UK. Investigators at both institutions will retain both samples and clinical and genetic data for use in further studies related to group A streptococcal disease and RHD as outlined in section 5 of the consent form. Additionally, following publication of initial reports, de-identified genetic data will be made publically available through the European Genome-phenome Archive, as required by research funders and outlined Section 6 of the consent form. Long-term storage, usage and governance of samples and data will be overseen by the local ethics committee alongside, if required, independent experts from the University of Oxford or University of Melbourne to offer to technical advice. For usage of the data other than the study of group A streptococcal disease or RHD within the scope of the ethical approval originally granted or for access to the data in the European Genome-phenome Archive application to the local research ethics committee will be required.

Use of the clinical and experimental data including genetic data from analysis of DNA samples will be at the discretion of the institution where the sample is held within the scope of the work for which ethical approval was granted providing manuscripts are approved prior to publication by the management committee as outlined above.

11. Ethical issues

a) Informed consent

All volunteers will receive written and verbal information and careful counselling prior to participation in the study with specific reference to use and storage of DNA, and inclusion of genetic data for further studies in public databases. Written consent will be collected from each participant on individual consent forms prior to donating blood. During the consent process we will highlight the participants right to withdraw during the enrolment process.

For consent to be valid it must be given competently which can be problematic in specific vulnerable groups of the population. To avoid difficulties we will exclude any individuals unable to give informed consent for any reason including severe illness or learning difficulties. Recruitment of children will require third party consent for children aged five to nine years, third party and child assent for children aged ten to fifteen years and independent consent for all those aged sixteen years or more.

Our participant information sheets specifically outlines both the indefinite storage of samples for further studies and inclusion of electronic data in databases for use by other researchers with permission. We will stress, however, that it will be impossible for the researchers to link information about the participant, genetic data or their sample back to the individual.

Finally in view of the greater emphasis on familial or communal dimension of decision-making and community gatherings, group discussions and consultations, we will encourage to individual participants the scope for them to discuss their decision to consent to the study with partners, family, neighbours, colleagues and other members of the community. To promote such discussion participants will be offered time for deliberation if they wish.

b) Community consultation

We anticipate that the indigenous population will know little of medical research or studies of genetics. Therefore to avoid exacerbating cultural sensitivities or disrupting the relationship between the indigenous community and health and research institutions in New Caledonia we have consulted with representatives of the indigenous community via the Senate in Nouméa. Further, in designing the study we have engaged the support of local doctors, nurses and public health staff who have extensive experience working with local patients from a variety of ethnic backgrounds. Additionally we have sought advice from community representatives in making the project acceptable and the consent materials understandable to the participants, who will predominantly be of Pacific Islander ancestry.

c) Cultural aspects of research

While all cultural groups living in New Caledonia will be offered the chance to participate in the research there are specific cultural considerations to the conduct of the genetics research in indigenous peoples(23). Notwithstanding a difficult history of biomedical research in these settings characterised by unethical practice, which has led to a break down of trust between research and the communities(24), we believe it is vital that indigenous peoples and the populations of developing countries have the opportunity to participate in genetics research particularly given the excess burden of disease and the potential of genetics to assist improvements in diagnostics, prevention and treatment. The extension of the Human Transition Projects to Fiji, for example, in collaboration with Georgia Technical Institute (the first population based genetics study in a Pacific Islander population that we are aware of, <http://www.gibsongroup.biology.gatech.edu/human-transition-projects>) serves as an example of increasing efforts to engage populations in developing countries in genetics research.

In order to reach valid conclusions about the link between disease and genes it is necessary to understand the genetic make up of the population being studied and make comparisons both within the participants and with other populations around the world. This might include, for example, comparison of

the relatedness of different Pacific Islander groups in New Caledonia and elsewhere in the Pacific. We emphasise that we will do these analyses as a necessary step in the understanding the genetic determinants of RHD and not to gain insight into the history or anthropology of the peoples of New Caledonia. Further the study of genetic differences between populations can be useful and was, for example, a necessary step in the lead up to genome-wide associations.(15, 25, 26) To date Pacific Islanders have largely been left out of these studies which our research will begin to address.

d) Clinical Assessment

Where we have access to inadequate echocardiographic data, cases will undergo echocardiography. The result, which will reveal detailed information as to the severity of rheumatic heart disease (RHD) and the risk of complications, will be conveyed, with the patient's consent, to the doctor responsible for their care. We anticipate that this data will be of considerable value for follow-up and treatment decisions but given the cases will be ascertained from an established RHD programme such data should not place an additional burden on local healthcare resources. If a new diagnosis is identified as part of the clinical assessment the patient's doctor will also be informed. Such findings might range from skin disease noted in the clinic room such as streptococcal pyoderma, frequent amongst patients with RHD in the Pacific, to an unexpected finding at echocardiography such as congenital heart disease, in which case referral to local expertise will be recommended.

12. Safety

The only risk to participants is that associated with phlebotomy, which may result in mild tenderness, bruising, light-headedness or rarely vasovagal syncope. There is no risk from echocardiography.

13. Ethical approval

The research proposal will be submitted to the Hospital Ethics Committee at the Centre Hospitalier Territorial Gaston-Bourett, Nouméa, New Caledonia as well as the institution ethics committee at Institut National de la Santé et de la Recherche Médicale, Paris, France. The Oxford Tropical Research Ethics Committee also approved the study (Reference OxTREC 27-11).

14. Reimbursement

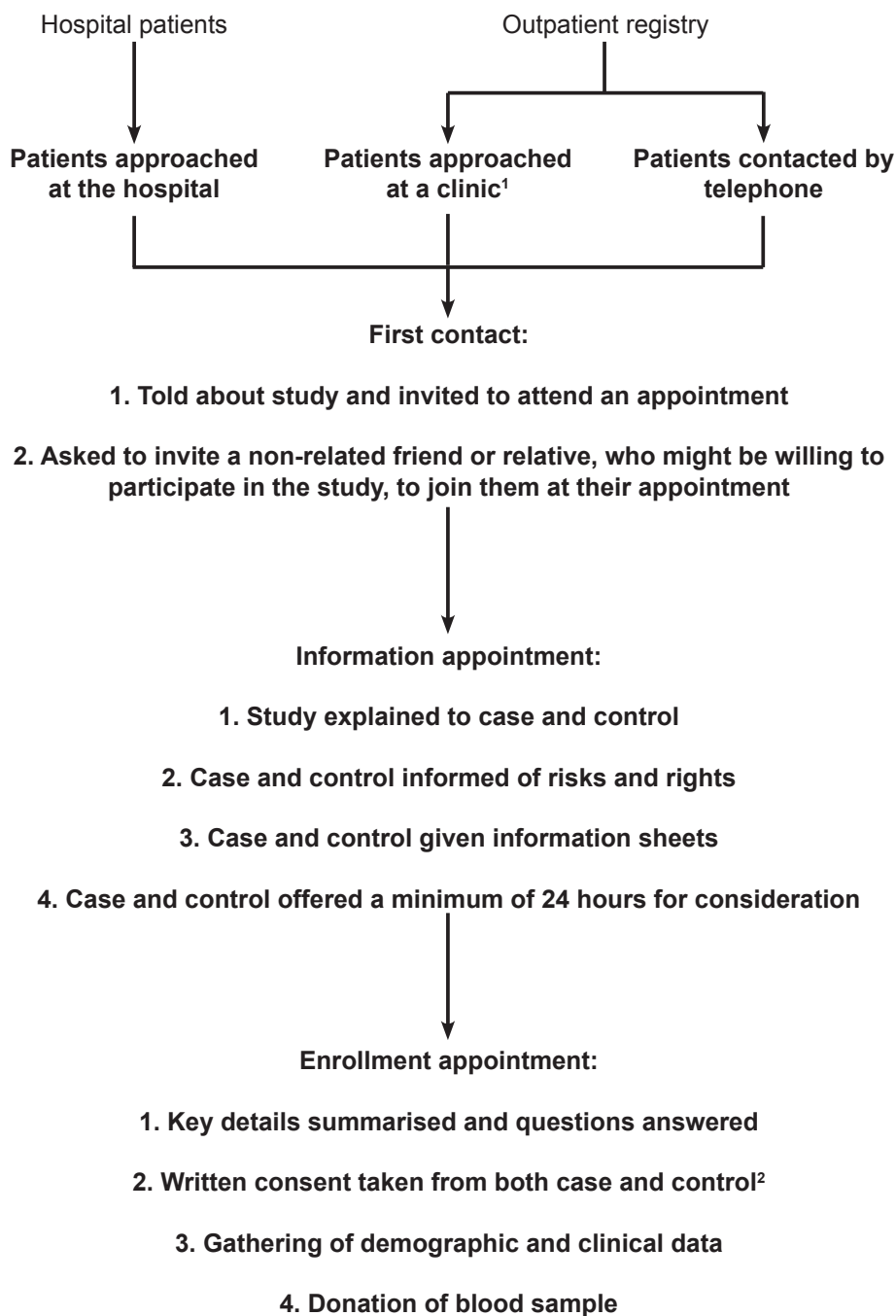
Cases and controls volunteering through the peer-nomination scheme will be compensated for any additional visits to the clinic for travel costs according to a rate to be agreed with the local research ethics committee in New Caledonia.

15. Enrolment process

We will recruit patients from the inpatient ward and outpatient department at the main public hospital in Nouméa, the Centre Hospitalier Territorial Gaston-Bourett, as well as the satellite hospitals around New Caledonia.

We will use a three-stage process for enrolment. First we will make contact with patients either in person at a clinic or on the telephone and invite them to attend an appointment to hear more about the study. At this appointment we will see the patient and their nominated non-related friend or neighbour (see Section 5 for details of the peer-nomination scheme) where we will talk them through the study, provide them each with an information sheet and offer a minimum of 24 hours for consideration. Finally we will see again the patient and their nominated control for completion of written consent. At this point we will gather demographic and clinical data, including a series of questions pertaining to history of ARF, RHD and GAS infections, and donation of a blood sample. We will document echocardiographic data for the patients and where insufficient data is available a repeat echocardiographic study will be performed. Further details are given in Figure 1.

Figure 1. Enrolment process



¹The majority of patients will be recruited through routine follow-up such as administration of secondary prophylaxis.

²At enrollment we will take informed consent from the case and control separately. In order to prevent coercion, a decision by one not to participate will not preclude the other.

16. Budget

EQUIPMENT AND CONSUMABLES

VOLUNTEER AND CASE REIMBURSEMENT	XFP 1,000,000
SAMPLE SHIPMENT TO FIJI	XFP 59,600
ADMINISTRATIVE COSTS	XFP 148,000
ECHOCARDIOGRAPHY COSTS	XFP 1,498,700
LOCAL TRANSPORT	XPF 370,000
DNAGARD® BLOOD BOTTLES	XFP 238,800
VENEPUNCTURE EQUIPMENT	XPF 148,000
SUBTOTAL	

RESEARCH SUPPORT STAFF

RESEARCH NURSE	XPF 8,756,000
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TOTAL	XPF 7,755,700
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17. Informed consent forms and plain language summaries

There are three consent forms in use in this study dependent on age:

Age	Consent	Form(s)
≥ 16 years (adult)	Independent consent	1
10 - 15 years (child)	Third party (parental or guardian) consent AND child assent	2 AND 3
< 10 years (child)	Third party (parental or guardian) consent	3

There are five plain language summaries (PLS) for specific groups:

PLS	Target audience
1	Adult Patient Participant
2	Adult Healthy Volunteers
3	Child Patient Participant (information for parents or guardians)
4	Healthy Child (information for parents or guardians)
5	Information for children (aimed at children aged 10-15 years)

18. References

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