TrypanoGEN: An integrated approach to the identification of genetic determinants of susceptibility to trypanosomiasis

www.trypanogen.net
African Trypanosomiasis Associates with Tsetsefly Distribution

Animal African Trypanosomiasis (AAT)
- *T. Vivax*
- *T. Congolense*
- *T. brucei brucei*

Human African Trypanosomiasis (HAT)
- *T. brucei rhodesiense*
- *T. b. gambiense*
The problem

- HAT is a fatal neglected disease
- Estimated 70,000 human cases/year
- No vaccine
- Drugs are toxic
- Zoonotic - livestock cases estimated cost $1.3bn/year
- Major impact on human and animal health
New tools are needed

- Wide-spread consensus of the need for novel approaches to achieve elimination or sustainable control

  - New control strategies
  - New therapies
The diversity of infection outcomes

Untreated Human Infections by *Trypanosoma brucei gambiense* Are Not 100% Fatal

Vincent Jamonneau1,2*, Hamidou Ilboudo2, Jacques Kaboré2, Dramane Kaba3, Mathurin Koffi4, Philippe Solano1,2, André Garcia5, David Courtin6, Claude Laveissière1, Kouakou Lingue6, Philippe Büscher7, Bruno Bucheton1

1 Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Campus International de Baillarguet, Montpellier, France, 2 Centre International de Recherche- Développement sur l’Élevage en zones Subhumides (CIRDES), Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, Bobo-Dioulasso, Burkina Faso, 3 Institut Pierre Richet, Unité de Recherche «Trypanosomoses», Abidjan, Côte d’Ivoire, 4 Université d’Abobo-Adjame, URES de Daloa, Laboratoire de Génétique Moléculaire et Évolution des Maladies Infectieuses Tropicales, Daloa, Côte d’Ivoire, 5 Institut de Recherche pour le Développement, Unité de Recherche 010, Faculté de Pharmacie, Paris, France, 6 Programme National d’Élimination de la Trypanosomose Humaine Africaine, Abidjan, Côte d’Ivoire, 7 Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium

Abstract

The final outcome of infection by *Trypanosoma brucei gambiense*, the main agent of sleeping sickness, has always been considered as invariably fatal. While scarce and old reports have mentioned cases of self-cure in untreated patients, these studies suffered from the lack of accurate diagnostic tools available at that time. Here, using the most specific and sensitive tools available to date, we report on a long-term follow-up (15 years) of a cohort of 50 human African trypanosomiasis (HAT) patients from the Ivory Coast among whom 11 refused treatment after their initial diagnosis. In 10 out of 11 subjects who continued to refuse treatment despite repeated visits, parasite clearance was observed using both microscopy and polymerase chain reaction (PCR). Most of these subjects (7/10) also displayed decreasing serological responses, becoming progressively negative to trypanosome variable antigens (LiTat 1.3, 1.5 and 1.6). Hence, in addition to the “classic” lethal outcome of HAT, we show that alternative natural progression of HAT may occur progression to an apparently
The diversity of infection outcomes

Phenotype

Susceptible

Stage 2

Stage 1

Seropositives

Self-cure

Asymptomatic/resistant

Parasite detectable in CSF

Parasite detectable in blood or lymph

Treatment

Death

Treatment

Seropositive subjects

Spontaneous cure

Susceptible Asymptomatic/resistant

Phenotype Stage 2 Stage 1 Seropositives Self-cure

time
Research Question/Aim

What are the host genetic determinants of HAT susceptibility/resistance?

Exploit human genetic diversity to find the key to combating the disease:

- In developing new control strategies
- In developing new therapies
Objectives

- Create a network that systematically investigates genetic diversity in relation to HAT in endemic areas across Africa
- To create an extensive biobank of both retrospective and prospective samples with standardised parasitological and clinical metadata
- To generate a database of human genetic variation from different African countries
  - In order to identify loci associated with HAT susceptibility
Hypothesis

HAT per se is controlled by susceptibility/resistance loci and the GWAS that will be carried out in this project will reveal candidate loci for this phenotype.
The TrypanoGEN Biobank

**Existing samples**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>SERO</th>
<th>HAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 1</strong></td>
<td>390</td>
<td>165</td>
<td>1060</td>
</tr>
<tr>
<td><strong>Year 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Minimum required for GWAS**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>SERO</th>
<th>HAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 1</strong></td>
<td>600</td>
<td>400</td>
<td>1200</td>
</tr>
<tr>
<td><strong>Year 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Year 5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Activities**
- Biobanking existing samples
- Ethical agreement
- WGS of 130 controls
- Design custom Omni2.5 Illumina
- Genotyping 2800 individuals
- GWAS
- Candidate gene testing

**Training**
- Ethics
- Sampling/biobanking
- Sequence bioinfo
- NGS bioinfo
- SNPs arrays
- Imputation meth.
- GWAS statistics
- 3rd Generation Seq
- Grantmanship
Our GWAS plan

• To collect biological samples and clinical data from patients and controls.
  • At least 1000 cases
  • At least 1400 controls
• To conduct a GWAS
  • Sequence 130 individuals to obtain local SNPs
    • Focus on four regions:
      Uganda, DRC, Cote d’Ivoire, Guinea
  • Genotype ~half the samples (discovery cohort)
  • Genotype the rest for top 1% hits (validation cohort)
Re-sequencing of African population
N=130 from the 4 centres

Output: To inform SNP imputation

Genotyping
**Discovery cohort**
Stage 2 patients N=600
Asymptomatic controls N=200
Population controls N=500

Output: Top hits

Genotyping of 1% top hits
**Validation cohort**
Stage 2 patients N=400
Asymptomatic controls N=200
Population controls N=900
Case and Control definitions

Cases

• Individuals confirmed as HAT infected by demonstration of Trypanosomes in blood, Lymph or cerebrospinal fluid

Controls

• Living in same villages as cases
• No Trypanosomes demonstrated in any body fluids
• Serologically negative
• Species specific PCRs to confirm negativity
Who/where we are

9 African + 3 European countries

- UK
- France
- Belgium

T.b. gambiense

Potential for epidemics
Acute disease
Capacity building across Africa
Ensuring delivery

ETHICS
Lead: S. MacLean
Deputy: P. Alibu, M. Parker

TRAINING
Lead: I. Sidibe
Deputy: D. Mumba

SAMPLING
Lead: M. Simuunza
Deputy: D. Mumba

BIOBANK
Lead: D. Mumba
Deputy: E. Matovu, I. Sidibe

SEQUENCING
Lead: G. Simo
Deputy: C. Hertz-Fowler

ANALYSIS
Lead: K. Mathurin
Deputy: E. Matovu
Ensuring delivery

Scientific Advisory Board ↔ TrypanoGEN Management Committee

Sample and Data Access Committee ↔ Principal Investigators

1 Expert, 2 Trainee Bioinformaticians

7 PhDs

3 Masters

9 Technicians

ADMINISTRATION

Ethics manager

Project manager

Institute Admin support

Accountant
Areas of potential synergy with other H3 Africa networks

- Ethics
- Training - grantsmanship
  - bioinformatics and GWAS (H3ABionet)
  - ethics workshops
- Sharing data policies
- Shared samples (Kidney Disease)
  SNP database
- Logistics
- Infrastructure building
- Communication across Africa
Developments since the Award

- Visit by WT finance team to Makerere University
  February 2013

- Ethical review (MoH) and recommendation to UNCST

- Ethical approval Granted

- Kick off meeting in Kampala 28-31 May 2013

- Training finance team from participating institutes
Developments since the Award

• Sub-Awards Issued
• Funds transfer just began
• SOPs for sample collection/storage in place
• Sample collection in DRC started (78 cases so-far)
  ➢ Uganda, Cameroon, CIRDES to start October-December
• Piloted with DNA preparation kits
  ➢ Blood
  ➢ Saliva
Streamlining consent documents

- 4 examples on data/sample sharing

<table>
<thead>
<tr>
<th></th>
<th>Approved?</th>
<th>Data</th>
<th>Sample</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uganda</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td>Protocol amendment to specify sample sharing underway</td>
</tr>
<tr>
<td>DRC</td>
<td>Yes</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Zambia</td>
<td>Under Review</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Malawi</td>
<td>Under Review</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>
On-going activities

- Recruit key personnel
  - Study coordinator application under review
  - Expert bioinformatician
- Finalise sub-awards and funds transfer
- Formally recruit identified PhD students
- Workshop on sample processing, storage and DNA extraction

We are challenged by poor intra-network communication!