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

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African Trypanosomiasis Associates with Tsetsefly Distribution

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

Human African Trypanosomiasis (HAT), Sleeping Sickness
- T. brucei rhodesiense
- T. b. gambiense

Tsetse flies live today in moist savanna and woodlands, regions with > 500 mm of rain a year.
Tsetse flies carry a parasite which can infect livestock and people with trypanosomiasis (sleeping sickness).
Tsetse and Cattle Distribution

Tsetse distribution

Cattle distribution
60% of world Arable land is in sub-saharan Africa
80% of that (10m Km²) is tsetse infested
$4 billion/yr production losses by pastoralists
  ➢ Annual calving rates 7% lower in AAT areas
  ➢ Calf mortality 6-10% higher
  ➢ Milk yields 2-26% lower,
  ➢ Oxen 38% less efficient

A big threat to food security!
HAT is a Neglected Tropical Disease (NTD)

Apparently there are only three diseases on that planet!
NTDs shackle the world’s poorest, the bottom billion plus

WHERE THERE ARE NO ROADS.....
there are no doctors
there are no drugs
hunger is greatest and
food security least
incomes are lowest
health information is least
WHERE THE NEED IS GREATEST!
Sleeping Sickness/HAT Importance

- 55m people at risk
- Estimated 70,000 cases/year
- 1.34m DALYs lost to HAT annually
- No vaccine
- Few Drugs
  - High toxicity
  - Drug resistance

T.b.gambiense
97 % of cases

T.b.rhodesiense
3 % of cases
HAT Clinical Presentation

- **Early Haemolymphatic Stage**
  - Intermittent fever
  - Headaches
  - Extreme fatigue
  - Muscle and joint pain
  - Lymphadenopathy

- **Late Meningo-encephalitic Stage**
  - Sleep cycle disruption
  - Confusion, tremors
  - Psychiatric symptoms (aggressiveness, irritability)
  - Coma, systemic organ failure, and death
The diversity of infection outcomes

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

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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 regressions of HAT may occur, progressing to an apparently
The diversity of infection outcomes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 2</td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td></td>
</tr>
<tr>
<td>Seropositives</td>
<td>Asymptomatic/ resistant</td>
</tr>
<tr>
<td>Self-cure</td>
<td></td>
</tr>
</tbody>
</table>

- **Stage 1**: 
  - Parasite detectable in blood or lymph
  - Treatment
  - Seropositive subjects
  - Spontaneous cure

- **Stage 2**: 
  - Parasite detectable in CSF
  - Treatment
  - Death

**time**
Who and where we are

9 African + 3 European countries

UK  France  Belgium

T.b. gambiense

T.b. rhodesiense

Acute disease
Potential for epidemics

African countries:
- Tanzania
- Zambia
- Cote d'Ivoire
- Cameroon
- Guinea
- DRC
- Burkina Faso

European countries:
- UK
- France
- Belgium

Map showing the distribution of T. b. gambiense and T. b. rhodesiense in Africa.
What are the host genetic determinants of disease susceptibility/trypano-tolerance?

Objectives

- 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
The TrypanoGEN Biobank

**Existing samples**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>SERO</th>
<th>HAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>390</td>
<td>165</td>
<td>1060</td>
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</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>Year 5</td>
<td>600</td>
<td>400</td>
<td>1200</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 method
- GWAS statistics
- 3rd Generation Seq
- Grantmanship
Our GWAS and Genotyping plan

- Collect biological samples and clinical data
  - At least 1000 cases
  - At least 1400 controls

- WGS followed by GWAS and SNP Genotyping
  - Sequence 130 individuals to obtain local SNPs
  - Focus on Uganda, DRC, Cote d’Ivoire, Guinea
  - Genotype ~half the samples- discovery cohort
  - Genotype the rest for top 1% hits- validation cohort
Current Status

- All approvals are in place
- Standardised protocols/SOPs across the network
- Field sampling started late 2013
  - We sample adults of consenting age
Key Personnel in Place

- Project Manager
  - Makerere University
- Expert Bioinformatician
  - CIRDES, Burkina Faso
- Trainee Bioinformaticians
  - CIRDES
  - Makerere
- 5 PhD Students
- 6 Technicians
## Included Participants to-date

<table>
<thead>
<tr>
<th>Country</th>
<th>Endemic HAT</th>
<th>Sampled</th>
<th>Cases</th>
<th>Controls</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Cameroon</td>
<td>Gambiense</td>
<td>BC, P</td>
<td>37</td>
<td>52</td>
<td>89</td>
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<tr>
<td>DRC</td>
<td>Gambiense</td>
<td>BC, P</td>
<td>89</td>
<td>113</td>
<td>202</td>
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<td>Ivory Coast</td>
<td>Gambiense</td>
<td>BC, P</td>
<td>107</td>
<td>307</td>
<td>414</td>
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<tr>
<td>Guinea/B. Faso</td>
<td>Gambiense</td>
<td>BC, P</td>
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<td>115</td>
<td>261</td>
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<tr>
<td>Uganda</td>
<td>Gambiense</td>
<td>BC, P</td>
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<td>216</td>
<td>970</td>
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<tr>
<td></td>
<td>Rhodesiense</td>
<td>BC, P, U, S</td>
<td>241</td>
<td>345</td>
<td></td>
</tr>
<tr>
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<td>Rhodesiense</td>
<td>BC, P, U, S</td>
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<td>148</td>
<td>228</td>
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<tr>
<td>Zambia</td>
<td>Rhodesiense</td>
<td>BC, P, U, S</td>
<td>15</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>883</strong></td>
<td><strong>1301</strong></td>
<td><strong>2184</strong></td>
</tr>
</tbody>
</table>

B=Buffy Coat; P=Plasma; U=Urine; S=Saliva
Sequencing

- 60 Samples in Pipeline at Liverpool
  - 30 from Uganda
  - 30 from Guinea
- First sequences have been obtained
- 60 samples in Transit to Liverpool
  - 30 each from DRC and Ivory Coast
- Expect to send 100 samples from NW Uganda to be sequenced by GDAP
G1/G2 Alleles in a *T. b. rhodesiense* Focus in Uganda: An off-shoot study

- Uganda is major focus for *T. b. rhodesiense*
  - Are G1/G2 present in the *T. b.rh* endemic foci?
  - Are they protective?

- 200 Blood samples from confirmed *T.b.rhodesiense* patients
- 200 Blood samples from non-infected controls

![Map of Uganda with T. b. rhodesiense patients and controls indicated](image)
Genotyping

PCR exon 5 on APOL1 gene which contains SID (and G1 and G2)

Design RFLP to identify G1 and G2 mutations
G1 S342→G342 destroys a HindIII site
G1 I384 → M384 creates a NSPI site
G2 6bp deletion destroys a MluCI site

Confirm any positives by sequencing

APOL1
398 AA

Signal peptide
Pore-forming domain
Membrane-addressing domain
SRA-interacting domain

G1
rs73885319
rs60910145
p.Ser342Gly  p.Ile384Met

G2
rs71785313
p.Asn388_Tyr389del
G1 and G2 are present in Uganda

Allele distribution in Uganda (control population)
3.3% G1
7.2% G2

Geographic distribution consistent with reported distributions from across Africa
% Patients with 1 or more G1 allele

T.b. rhodesiense patients

OR = 0.95
No evidence for a protective effect of G1 APOL1 allele in the field
% Patients with 1 or more G2 allele

Controls

T.b.rhodesiense patients

14.4% G2

3.8% G2

OR = 0.23

G2 is associated with a 4x reduced risk of *T.b.rhodesiense* infection
i.e. G2 does not completely eliminate the risk
Challenges

- Differences in Ethical clearances obtained
  - Some sites only collect blood and serum
  - Others can extend to urine and saliva
  - 1-4 Essential phenotypes missing for some sites
    (weight, height, medications, smoking/alcohol history)

- Infrastructure limitations

- Coping with elaborate finance reporting procedures
  - Fieldwork at some sites lagging far behind

- Ebola has halted work in Guinea
Welcome to Uganda