Genomics for Undiagnosed Infectious Disease Evaluation and Diagnosis in Northern Australia (GUIDED-North Australia)

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This approach will enable the validation of genomics methods and provide a ‘proof-of-principle’ that a metagenomics approach can be used to diagnose fevers of unknown origin.
Genomics for infectious disease diagnosis

- Detection and diagnosis of infectious diseases currently relies on a best guess of likely causative agents with specific testing for those agents.
- The use of metagenomics for diagnostics is becoming more realistic with the declining costs of next generation sequencing (NGS).

Genomics for infectious disease diagnosis

• In a review of 340 patients presenting to the Cairns Hospital over a 3 year period, a diagnosis was not made in 57% of patients and there was an average of 4 tests requested for specific agents (range 1 to 20) (Susilawati et al. *Int J Infect Dis*, 2014; 27:59-64)

• A review of research projects in Asia investigating undifferentiated fevers found that undiagnosed cases ranged from 8 - 80% (Susilawati et al. *Southeast Asian J Trop Med Public Health*, 2014; 45:719-726)

• A more agnostic diagnostic approach to detect unexpected or novel pathogens is needed to both improve our diagnostic accuracy and provide early warning for diseases with epidemic potential
Research aim

To assess the capacity and sensitivity of deep sequencing for broad-scale characterisation of pathogens associated with fever.
Fever outcomes

Undifferentiated fever
- Nonspecific clinical features
- A lack of clue from initial lab findings

Acute undifferentiated fever
- AUF, ≤ 21 days
  - Diagnosed
  - Undiagnosed

Fever of unknown origin
- FUO, > 21 days
  - Resolved
  - Prolonged
Sample processing

- Nucleic acid extraction
  - DNase treatment
  - cDNA synthesis

- Whole genome amplification
  - PCR clean-up
  - Primer removal

- Sent to AGRF
  - Library synthesis
  - Sequencing (HiSeq)
Results

- 4-14 million reads per sample.
- Up to 93% are human reads.
- Most non-human reads are unclassified.
- Bacteriophage is common.
- Various microbes found in every sample.
- Positive calls were made on pathogen for which there were supporting reads and clinical and lab findings.
Case Report
May 2013

Undiagnosed case #1 (57 yo F)
- Fever, headache, muscle pain, vomiting, diarrhoea
- Hypotensive
- Multiorgan failure, septic shock
- Died

Undiagnosed case #2 (55 yo M)
- Fever, headache, muscle pain
- Diarrhoea, rash
- Fluid resuscitation, ceftriaxone, doxycycline, vancomycin
- Intubated
- Improved condition
- Home
History

- 2 weeks prior, the couple performed some yard work, both had mosquito bites
- No pets, livestock or other animal exposure
- Live in bush land in Babinda, a tick-infested area
- O/E: fever >39°C, tick eschar NOT found
Investigation

Cytomegalovirus
Scrub typhus
Spotted fever
M. pneumoniae
S. pneumoniae
Legionella sp.

Epstein Barr virus
Q fever
Barmah Forest virus
Ross River virus
Dengue virus
Respiratory viruses
Malaria
N. meningitidis
Leptospira
### Deep sequencing results

<table>
<thead>
<tr>
<th>Case #1</th>
<th>No. reads</th>
<th>Case #2</th>
<th>No. reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F, died)</td>
<td></td>
<td>(M, recovered)</td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>4472</td>
<td>A. baumannii</td>
<td>1947</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>2514</td>
<td>Rickettsia africai</td>
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<tr>
<td>L. monocytogenes</td>
<td>13</td>
<td>Dengue virus 1</td>
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<td>Rickettsia africai</td>
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<td>Rickettsia conorii</td>
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</tr>
<tr>
<td>Rickettsia rickettsii</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dengue virus 1</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Undiagnosed case #3

- 40 yo man with 3 days history of fever, muscle pain and nausea
- Recent travel to Torres Strait islands
- **Rash** on trunk, chest and extremities
- **Eschar** on his upper left arm
- Possible contact with ticks
Undiagnosed case #3

- Elevated liver transaminases (ALT: 177 u/L, AST: 164 u/L)
- Low PLT (108x10^9/L), low WCC (3.4x10^9/L), elevated CRP (97 mg/L).
- Urinalysis: NAD, blood culture (-)
- Malaria screening (-)
- Serology (-) for Flavivirus, dengue, Ross River, Barmah Forest
- Serology (-) for Q fever and leptospirosa
- PCR (-) for SFG Rickettsia and scrub typhus
- He was given IV Doxycycline, then switched to orals prior discharge. He had marked clinical improvement during admission.

Deep sequencing: 11 reads of *O. tsutsugamushi*
GUIDED-North Australia

• Multicentre study: Royal Darwin Hospital and Cairns Hospital

• We aim to evaluate a genomics approach for the diagnosis of acute febrile illnesses of moderate to severe intensity
  • Determine the optimum conditions for sample collection and processing for genomic analysis
  • Determine the most efficient, clinically relevant, analysis pipeline

• The study will provide a ‘proof-of-principle’ that a metagenomics approach can be used to diagnose fevers of unknown origin

• The findings from this project could be extended to investigation of other syndromes such as meningitis/encephalitis, septic arthritis, and infections of other sterile sites
Project protocols

• Patients who meet the following inclusion criteria will be invited to participate:
  • 1) 16-65 years of age;
  • 2) Fever duration >24 hours to 21 days;
  • 3) Documented temperature is at least 38°C or history of fever associated with symptoms of feeling cold or shivering;
  • 4) Patients from whom detection of a causative agent is considered likely (samples will be collected, with informed consent, from all patients in this category). However, only patients with a laboratory confirmed aetiology will be included for downstream metagenomics analysis);
  • 5) Have an undifferentiated fever score of 5 or more (see Susilawati et al., 2014);
  • 6) Willing to provide acute and convalescent blood samples.

• Exclusion criteria:
  • 1) Immunosuppressed;
  • 2) Suspected nosocomial infection.

• Participant recruitment will focus on patients in whom the detection of a causative agent is likely and subsequently confirmed in the hospital laboratory.
Project protocols

• Serum, EDTA and RNA blood tubes will be collected from each patient to determine the ideal sample for metagenomic analysis.

• There will be target enrollment of 15 patients per site. The clinical investigators will monitor the confirmed diagnoses progressively to optimise the diversity of diagnoses to reflect the diseases typically seen in Northern Australia.

• In the event of over-enrollment of any one particular diagnosis (e.g. Dengue or Melioidosis) enrollment of patients in whom those diagnoses are suspected, may be ceased.

• Whole genome amplification will be achieved using commercial kits and the products will be sent the Australia Genome Research Facility (AGRF) for sequencing.
Project protocols

• Library preparation will be performed using TruSeq Nano DNA Library Preparation kit protocol (Illumina) and paired-end (PE) sequencing will be conducted on the Illumina MiSeq instrument

• Bioinformatics analysis to identify pathogens associated with fever will be performed using an existing in-house custom pathogen identification pipeline developed by investigator Field
  • Pipeline consists of three main steps; data QC and cleanup, de novo assembly, and taxonomic assignment

• Once a final optimised version of the pipeline is implemented the results from the bioinformatics pipeline will be compared with conventional diagnosis to measure sensitivity, specificity, and negative and positive predictive values
Current progress

• Ethics proposals have been submitted to the Human Research Ethics Committees (HRECs) for the Cairns and Darwin Hospitals

• Conditional approval has been received from the HREC of the Northern Territory Department of Health and Menzies School of Health Research. A letter of clarification has been received by the Far North Queensland HREC.

• The project partners are currently drafting responses to address the minor issues raised by the two HRECs

• Initiation of sample collection is expected soon after ethics approval from the HRECs