Role of next generation whole genome sequencing and clinical relevance

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WGS - Clinical Impact

Whole genome sequencing (WGS) and resistance genotyping

Predictive information to manage MDR/XDR-TB treatment
- 9 – 18 months
- Requires Good clinical utility
  - give best possible evidence-based advice to clinicians
  - likely resistance/sensitivity
  - likely MICs
  - likely cross-resistance between different members of drug families

Needs good predictive genotype-phenotype correlation data

Evidence base – tbdreamdb, literature, in house 2nd line DST + WGS
<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>Gene product/ function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td><em>katG, inhA, ahpC, kasA</em></td>
<td>catalase peroxidase, enoyl acp reductase, alkyl hydroperoxide reductase, β-ketoacyl-ACP synthase</td>
</tr>
<tr>
<td>Rifampicin</td>
<td><em>rpoB</em></td>
<td>RNA polymerase β subunit</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td><em>pncA</em></td>
<td>pyrazinamidase</td>
</tr>
<tr>
<td>Ethambutol</td>
<td><em>embA, embB, embC</em></td>
<td>arabinosyl transferase</td>
</tr>
<tr>
<td>Streptomycin, Kanamycin, AMK,CAP</td>
<td><em>rrs, whiB7, rpsL, tlyA, gidB, eis promoter</em></td>
<td>16S ribosomal RNA, promoter eis and tap ribosomal subunit 12, methyltransferase, 16S methyltransferase, acetyltransferase</td>
</tr>
<tr>
<td>Cycloserine, Prothionamide</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Genotypic tests depend on strength of associations

High confidence calls

e.g. INH$^R$  

  kat$G$ or inh$A$ SNPs

Rifampicin$^R$  

  rpo$B$ SNPs in RRDR – codons 507-533

Quinolone$^R$  

  gyr$A$ SNPs in QRDR

More Complex Associations - hard to make predictions

e.g.

  KAN$^R$  

  rrs  A1401G & C517T plus eis promoter G-10A or C-14

  CAP$^R$  

  rrs  A1401G, C1402T, G1158T + possible eis C-12T

  AMK R  

  rrs  A1401G + eis promoter

Difficulties:

  Cross resistance
  no DST data for new SNPs
  DST and MICs
  strain variation
  compensatory and secondary mutations
WGS – Clinical use at St George’s?

XDR-TB patients
MGIT positive cultures

TTP = 12 days
3 mL = 150ng DNA yield

WGS IonTorrent
clinically timely turnaround time – 24 hours
high cost but high value test - ~£180 per genome

Informs treatment options
DST PRIOR to Tx - weeks
Clinical perspective - often too little, too late

- 20-100ng DNA
- 1.8million reads
- mean 128 bp
- M.tb genome = 4.4Mb
- > 35x coverage (perfect reads)
XDR-TB clinical case

Genotype Results - PCR for gyrA, gyrB and pncA
reference strain H37Rv

• SNPs in gyrA
  *269nt GCG→GTG codon 90, Alanine to Valine

Evidence to call

**gyrA**  A90V mutation
- significant associated with MIC of 1- 2 µg/ml for moxifloxacin
  ([www.tbdreamdb.com](http://www.tbdreamdb.com)) multiple publications with frequency data.
  Wilby et al 2012  i.e. predictive evidence

• **WT gyrB**

**Treatment informed:**

• Moxifloxacin dose increased to 600 mg
• Theoretically achieves in vivo MIC sufficient for treatment
  ~ 2ug/ml

XDR-TB clinical case

• \textit{pncA} \quad \text{AAT} \rightarrow \text{ATT} \quad \text{nt 335} = \text{codon 112} = \text{stop codon at 118}.

Treatment informed:

• Pryazinamide – stopped -
• Weight of evidence of SNP and DST for PZA is unreliable
Some SNPs found in XDR-TB clinical case

<table>
<thead>
<tr>
<th>Gene</th>
<th>Resistance</th>
<th>H37Rv Position</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>gyrA</td>
<td>FLQ</td>
<td>7,582</td>
<td>Asp94Gly</td>
</tr>
<tr>
<td>gyrA</td>
<td>FLQ</td>
<td>7,585</td>
<td>Ser95Thr</td>
</tr>
<tr>
<td>rpoB</td>
<td>RIF</td>
<td>761,155</td>
<td>Ser450Leu*</td>
</tr>
<tr>
<td>rpsL</td>
<td>SM</td>
<td>781,822</td>
<td>Lys88Arg</td>
</tr>
<tr>
<td>rrs</td>
<td>AMI;SM</td>
<td>1,473,246</td>
<td>A1400G</td>
</tr>
<tr>
<td>fabG1</td>
<td>ETH;INH</td>
<td>1,673,425</td>
<td>-15 C/T*</td>
</tr>
<tr>
<td>katG</td>
<td>INH</td>
<td>2,154,724</td>
<td>Arg463Leu</td>
</tr>
<tr>
<td>katG</td>
<td>INH</td>
<td>2,155,168</td>
<td>Ser315Thr*</td>
</tr>
<tr>
<td>pncA</td>
<td>PZA</td>
<td>2,288,847</td>
<td>Gly132Asp</td>
</tr>
<tr>
<td>accD6</td>
<td>INH</td>
<td>2,521,428</td>
<td>Asp229Gly</td>
</tr>
<tr>
<td>embA</td>
<td>EMB</td>
<td>4,243,221</td>
<td>-12 C/T</td>
</tr>
<tr>
<td>embB</td>
<td>EMB;INH;RIF</td>
<td>4,247,513</td>
<td>Tyr334His</td>
</tr>
</tbody>
</table>

Genotype matched the XDR phenotype

* Matched Hain test
Rif$^R$ SNPs – cross-resistance

• 1 high level high confidence Rif$^R$ mutation present in rpoB gene:

  – 761155CT  S450L  = S531L (ec nomenclature)
  – reported phenotype: MICs:
    • >8mg/ml rifampicin
    • 4->8mg/ml rifabutin
    • >8mg/ml rifapentin
Report for clinicians: need for assessment of evidence

<table>
<thead>
<tr>
<th>Results</th>
<th>Good evidence of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluoroquinolone resistance:</td>
</tr>
<tr>
<td></td>
<td>1 high level high confidence (FLQ^{R} ) mutation present in (gyrA):</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 other (gyrA) mutations, for which there is evidence they do not affect function. No SNPs in (gyrB)</td>
</tr>
<tr>
<td></td>
<td>Isoniazid resistance:</td>
</tr>
<tr>
<td></td>
<td>1 high level high confidence (INH^{R} ) mutation present in (katG) gene:</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
XDR-TB clinical cases

• Early prediction of TB drug resistance would:
  • allow faster and more effective treatment
  • improve patient outcomes
  • reduce onwards transmission - ↓ time of infectivity
  • reduce the chance of further resistance developing
  • cost-effective compared to total care package ~£1000/day
  • Genotype matched the XDR phenotype.

Genomics
Direct impact on treatment selection
Role in clinical management and public health
WGS Point of Care- Are we there yet?

PoC - possibly in 3 – 5 years
- need to prepare for technology advances
- clinically timely – hours (PoC)
  – days (WGS)
  – weeks (DST)
- multiplex R+ gene PCR sequencing or hybridisation
  *viz* Hain or GeneXpert

MinIon
Oxford Nanopore Technologies Ltd

QuantuMDx  Q-POC or Q-SEQ nanowires
Where Next?

- Need phenotypic DSTs – maintain capacity
  Leonid Heifets 2010 The second coming of the white plague.

- Rapid sequencing direct from specimens

- Database for routine clinical use
  e.g. TBDReaMDB, WIKI & reportable front end

- Regional and global frequencies for accurate prediction
  - large scale WGS
Proposal – a wiki for sharing evidence
(stoker.neil@gmail.com)

• **tbresist.org** – may change address

• Sharing of expertise
  - SNP evidence
  - DST details – methods, MICs, interpretation

• **Evidence base of phenotype-genotype correlations**

• permanent / temporary (data can be moved)
Strain – SNP list → wiki SNP list → individual SNP evidence summary → summary and review of individual paper

- Important to add details of phenotypic testing
- Is anyone interested in extending this beyond SGUL?

Summary of Takiff et al. 1994


Abstract

Notes on this paper:
- TB: not known
- Takiff: not known
- this was the first report of the cloning
- they only look at ciprofloxacin, and
- reported CIP MIC in sensitive strains: 0.25-1.0 ug/ml
- phenotypic methods: radiometry as described by Siddiqui et al 1981
- Expt 1: in vitro selection of ciprofloxacin-resistant mutants in BCG/Mtb
  - mutants isolated (a) resistant to 1.0 ug/ml CIP, (b) 2.0 ug/ml CIP. None isolated with higher
New database for *Mtb* genomes?

- Initiative by people with multiple genomes planned (at this meeting)

- combine with DST Ab$^R$ data $\rightarrow$ powerful tool for predicting SNP function

- **meeting this lunchtime here to discuss**
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