New approaches for the molecular epidemiology of HIV and tuberculosis

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What we need from an epidemiological marker?

Is it able to answer the question under investigation?

- Can I distinguish source of infection?
- Can I distinguish recent transmission?
- Is a clone global or local?

- Other considerations: price, tech transfer, standard, throughput, low/high-tech
Fig. 2.1 Different time-space scales for tracking epidemic clones

M. Hallin et al 2012.
Application to infectious diseases

NEW HIV INFECTIONS BY REGION

- 88k Eastern Europe and Central Asia
- 110k Sub-Saharan Africa
- 25k Middle-east & North Africa
- 94k Latin America
- 1.5m Asia and Pacific
- 350k Asia and Pacific
- 12k Caribbean
- 2.1m Global

SOURCE: UNAIDS Gap Report 2014

HIV

9 million cases
1.5m deaths

TB

Comas & Gagneux, Plos Pathogens 2009

HIV-1 Gene Map: drug resistance

HXB2 strain

Partial PR/RT sequence, 1302 bp

- 1806 patients
- 2004-2014
Can we use the same region for transmission cluster identification in Valencia?

HIV SubTypes

Typing

Transmission cluster ID
- Clade phylogenetic support
- Genetic distances
- Local and Global control strains
Cluster size distribution for type B

Cluster 113 cases unique to Valencia
Whole genome for the epidemiology and diagnoses of tuberculosis

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Slow and Steady is not an option!

Decades old tools
Inaccurate epidemiological models
Knowledge gaps on host-pathogen

Transmission is on-going!
Contributes to latent reservoir -> future epidemics

Dye 2013

Estimated transmission rate

Low-burden
High-burden

10-20%
50-70%

Current rate
Possible with current technology
Beyond current technology
Modernizing DOTS to accelerate TB eradication

2010 - Expert MTB/RIF

2013 – Bedaquiline
- Short-course treatment
- Universal (sensitive and resistant TB)

2013 – MVA85A
- Resistance to infection
- Avoid active disease
- Avoid transmission

New drugs

New vaccines
- Stop transmission
- Evaluate interventions
- Better epi models

New diagnostics
- Point of care
- Cheap
- Easy access
- Active/latent

New epidemiology
- Whole genome sequencing
Mycobacterium tuberculosis
Genoma (4.4 millones nucleótidos)

Single Nucleotide Polymorphisms (SNP)
Large sequence polymorphisms (deletions)
Insertion sequence IS6110

DR locus

SPOLOGTYPES
Strain 1
Strain 2
Strain 3

VNTR loci
MIRU GENOTYPE
4 3 2 5
What can we expect from genomes?
A new molecular epidemiology for a new DOTS

- Low-resolution
- Group-based
- Retrospective

- High-resolution
- Individual-based
- Prospective
Genomic epidemiology of TB

Tuberculosis Genomics Unit

Regional Public Health

Hospitals microbiological units
Bencktop sequencers

TB eHealth Valencia

- Clinical
- WGS
- Follow-up
- Microbial
- Contact tracing
- Demographic

TB control

Illumina MiSeq
(example: low-burden country)

- Only 20 samples/run
- Fast-turn around (from sample to data in 1 week)
- In-house library and sequencing
- ~85 euros (in-house)/~200 euros (external)
- Prospective
(window: 1 year)

86 Transmission events

70% with direction
30% no direction

50 Transmission Group
21 missed index case
Confirmed contacts by Public Health (16%)
Negative sputum index cases (20%)
WGS an example: Epidemiological and clinical case

Study Case 2009

Relapse 2013

On-going 2016

Household contact (brother) 2013

In collaboration with Hospital General de Valencia

Dra. Reme Guna
Microbiology

Molecular probes

Relapse

Sputum smear

Treatment

Phenotypic or Genotypic Drug Resistance

No

RIF, INH

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide; E, Ethambutol
Complexity of outbreaks

1. MDR diagnosed by WGS

2. Brother infected between 1st and 2nd

3. WGS molepi -> epi
Within patient diversity associated to drug resistance

- Three drug resistance mutations not described before!
  - Personalized medicine for TB? Is it feasible?
  - How to interpret heteroresistance clinically?
A glimpse to the future....
From the bench to the bedside

ReSeqTB
Relational Sequencing TB Data Platform

Contributed Data

WGS
- Genotypic data
- Phenotypic data
- Clinical trial data
- DR study data
- Surveillance data
- Original WGS SNP reports
- New SNP reports from Unified Pipeline

Unified Pipeline

Expert Panel Review

RDST Consortium

CURATED AND AGGREGATED DATA
- Genotypic Data
- Phenotypic Data
- Clinical Data
- Drug Resistance Data
- SNP Reports

ANALYTICS TOOLS
- “R” Statistical Analysis
- Misc. Integrated Analysis

RECOGNITION
- Individual Recognition
- Institutional Recognition
- Global Impact

Bill & Melinda Gates Foundation

CRITICAL PATH INSTITUTE
FINDS
Critical Path to TB Drug Regimens
World Health Organization
Stop TB Partnership
New Diagnostics Working Group

PUBLIC HEALTH
RESEARCH
MONITORING
OUTBREAKS
DISEASE PREVENTION
COMMUNICATION
RISK
SURVEILLANCE
HEALTH PROMOTION
ANALYSIS
Genome sequencing from sputum samples?

Time to diagnosis = 2-5 months
Time to epidemiology = 6-7 months
Genome sequencing from sputum samples?

Time to diagnosis AND epidemiology: 1/2 month

Sp X ad

Pro X g resistance

Molepi

No PH intervention

<1 month

1 day

1 day

1 day
Conclusions and open questions

- HIV PR/RT as diagnostic, surveillance, epi marker > reduce costs, strengthen collaborations
  - High-resolution network of TB transmission and infer missing/unsampled cases
  - WGS TB can be used for DR diagnostics

- Interpretation of genetic networks and within host diversity for epi?
  - Incorporate epidemiological data
  - DR diagnostics and heteroresistance?
Agradecimientos

• TB Genomics Unit @ CSIC-IBV
  • Amparo Broseta
  • Luis Villamayor
  • Galo Adrian Goig
  • Irving Cancino
  • Álvaro Chiner
  • Victoria Furió

• HIV work
  • Fernando González Candelas
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  • Juan Ángel Patinño
  • Hospital Clínico Valencia
  • Hospital General Valencia
  • Unidad prevención SIDA y otras ITS

• Microbiology Units Valencia Region Hospitals

• Valencia Region Public Health
  • Francisco González Morán
  • Hermelinda Vanaclocha

• Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana

• Ministerio de Economía y Competitividad

• Universitat de València

• Generalitat Valenciana
Beyrer et al. 2012. The Lancet. Figure. Molecular epidemiology of HIV subtypes in MSM, 2007–11

Wertheim 2013. JID. Figure. Large international transmission clusters

von Wyl et al. 2011. JID. Figure. Swiss microepidemics for subtype A.

Bartha et al. 2013. eLife. Figure. Use of HIV sequencing for GWAS analyses
TDO: tratamiento directamente observado

6 millones de vidas!

No infectado → Latente → STOP TB → Resistente Ab

Recuperación

Transmisión
6 million lives saved!

Uninfected → Latent → Stop TB → Recovery

Drug resistance

Transmission
From genomic data to surveillance

1. Detect cluster specific SNPs

   - Detect cluster specific SNPs
   - TRAP
   - Rv0102
   - Targeted gene
   - T
   - R1
   - Strain B
   - No amplification
   - Strain other than B

GCP

S1

R1

171 bp

NoB noF

B F

TRAP design

Validation

2. Develop SNP specific assays

   - TRAP

   - Isolates 2013-2014
   - Strain F
   - pre-2013 Isolates
   - Strain B

   - SNP-based surveillance of highly transmissible strains

   - Low-cost approaches to de-centralize analyses

   Pérez-Lago et al. (CMI2015)

3. Detect on-going transmission

   - TRAP

   - Isolates 2013-2014

   - Strain F

   - Strain B
Y en el presente...

TB hechos

Más de 1.4 millones muertes
Alrededor de 9 millones de casos
Un tercio de la población mundial infectada
Casos resistentes en todo el mundo
€5.8 mil millones de Euros a la EU

WHO Global Tuberculosis Report 2015
**HIV patients Naïve**

- **NNRTIs**
- **PIs**
- **EIs**
- **IIs**

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**Mutations in the Reverse Transcriptase Gene Associated with Resistance to Reverse Transcriptase Inhibitors**

<table>
<thead>
<tr>
<th>Nucleoside and Nucleotide Analogue Reverse Transcriptase Inhibitors (nRTIs)*</th>
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<td>Multi-nRTI Resistance: 69 Insertion Complex (affects all nRTIs currently approved by the US FDA)</td>
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<th>Multi-nRTI Resistance: 151 Complex (affects all nRTIs currently approved by the US FDA except tenofovir)</th>
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Identification of drug resistance

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**Microbiology**

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**Sputum smear**

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**Treatment**

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**Microbiology DST**

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**Molecular probes**

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*The patient is MDR-TB! Diagnosed **ONLY** by whole genome sequencing!*

**No** Phenotypic or Genotypic **Drug Resistance**

R, Rifampicin; H, Isoniazid; Z, Pyrazinamide; E, Ethambutol
Within patient diversity

Patient 8

P8-R1M1 (22)

P8-R2M1 (25)

P8-R2M2 (21)

P8-R3M3 (23)

P8-R2M3 (24)

P8-R1M2 (27)

P8-R2M2 (26)

Patient I

PI-R1M1 (26)

PI-R1M2 (27)

PI-R1M1 (29)

PI-R1M1 (28)

Patient H

PH-R2M2 (15)

PH-R2M3 (18)

mv1

PH-R1M1 (20)

PH-R3M1 (16)

PH-R1M1 (14)

Diversity transmitted?

Impact on drug resistance?

Impact on epidemiological links?

Lymph node

Urine

Respiratory

Blood

Pérez-Lago et al. JID 2014
Spatial epidemiology: foci of transmission
A new molecular epidemiology for a new DOTS

A high resolution network of tuberculosis transmission will:

• Differentiate between host, pathogen and environment factors
• Intervention tool in combination with faster turn-around times
• Measure impact of interventions