Mycobacterial and host genomics in transmission of leprosy

The origin of leprosy and its future
Genomics – definition & terms

• Systematic analysis of complete genetic content of an organism by DNA sequencing and bioinformatics

• Underway for >30Y, succession of whole genome sequencing (WGS) technologies

• DNA – 4 bases or nucleotides, 2 strands; $10^3 = 1$Kb; $10^6 = 1$Mb; $10^9 = 1$Gb
M. leprae - genome decay

Mycobacterium leprae
3,268,203 bp

M. leprae - genome decay

MB case Tamil Nadu

Cole et al. 2001 Nature
Gross features of *M. leprae* genome

- 1,614 genes
- 1,433 genes common with *M. tub*
- >1,310 pseudogenes
- ~1,500 genes "deleted"
- ~160 *M. lep* "specific" genes
- Mosaic, ~65 segments = *M. tub*

*M. leprae* has undergone reductive evolution

Rep-DNA involved
So why won’t it grow?

- Few anabolic lesions
- Starved - lost many catabolic functions
- Energy deficient - deleted *nuo* operon
- Iron limited? No siderophores

Is *M. leprae* starving at the banquet?
A second genome - Br4923

- 21 bp bigger than TN
- 7 InDels (5 Ins, 2 Dels), 1 x 8 bp Del in rep-DNA
- More in VNTR
- 156 SNP, 38 in rep-DNA, 78 informative
Is *M. leprae* variable?

- Leprosy spectrum: TT < - > LL
- Microarrays
- Comparative genomics
- Repeat copy number
- SNP typing

Monot et al. 2005 Science 308:1040
SNP are very rare 1:28,400 bp

A. Tamil Nadu

Br4923

B. 14 676 1 642 875 2 935 685

SNP-type 1

SNP-type 2

SNP-type 3

SNP-type 4

SNP are very rare 1:28,400 bp
Old and new SNP-type

SNP-Type4

SNP-Type3

RNC1

SNP-Type2

SNP-Type1

M

K

J

I

H

G

F

E

D

1378

9

2462
SNP-based phylogeography

Strong geographical association

Alex Gt – NEG / Romans - POS / Indians - NEG
BERING - NEG

Hidden challenges
Advances in instrumentation

ABI373
ca. 1995
50 Kb

ABI3730XL
ca. 2003
500 Kb

Illumina HiSeq
ca. 2011
500 Gb
Increased sensitivity, reduced cost

ABI373
ca. 1995
>50 μg
TN genome
>$3,000,000

ABI3730XL
ca. 2003
>5 μg

Illumina
HiSeq
ca. 2011
<5 ng

Today
$1,000
Advances in IT: Assembly time

Cray
ca. 1996
14 days

MicroVax
ca. 2003
3 days

Laptop
ca. 2011
1 day
Next generation sequencing technologies

Fragmentation
- Hydrodynamic shearing
- Enzymatic (e.g., Fragmentase or Ion Xpress)
- Nebulization
- Forced acoustic shearing
- Ultrasonication

Tagging
- Blunt-end fragments
- Size selection
- Adaptors

Amplification
- End repair
- Transposase + adaptors

Sequencing
- Emulsion PCR and enrichment
- Solid-phase bridge amplification

Leprosy in the USA

• ~150 cases/year, mostly immigrants or US citizens who lived in endemic areas

• Autochthonous cases
  ➢ Never left US
  ➢ No known contact with a leprosy patient

  - Local sources? Environment/animal reservoir?
  - Increased risk of leprosy due to armadillo exposure.
Leprosy is rare ...but armadillos aren’t!

High population densities: 30-50 M armadillos in South Range expansion is 10X that of other mammals (4-10 Km per year)
Nine-banded armadillos highly susceptible to *M. leprae*

- Low body temperature (30-33° C)
- Long life span (10-15 y) allows sufficient multiplication of *M. leprae*
- Infection rates up to 20% among adults in some areas

Armadillos as large natural reservoir
Sequencing *M. leprae* from wild armadillo

- Wild armadillo strain & 3 from US patients (all subtype 3I).
- Comparison with whole genomes from India, Brazil, Thailand, & America (1A,4P,1A,1D).
- Revealed 49 SNPs and one InDel restricted to US.
- Four 3I differed in 1 - 8 SNP
- Analyzed 50 US patients & 33 wild armadillos by SNP-VNTR genotyping.
Minimum spanning tree

Elsewhere in US

South

* Foreign residence

Truman et al. 2011 NEJM 364: 1626-33
Lesson for disease control

• Leprosy can be a zoonosis
• Difficult to eliminate zoonotic sources
• Proof for non-human reservoirs

Are there others?
Ancient leprosy

- Endemic in Europe
- 19,000 leprosaria in C12th
- Disappeared after middle ages
- Why?

1175 English Church Council: “lepers shall not live among the healthy”
Medieval leprosy

Sample: 3077; Location: Sigtuna, Sweden; Age: cal AD 1032-1155

Sample: SK8; Location: Winchester, UK; Age: cal AD 1010-1160

Sample: SK2; Location: Winchester, UK; Age: cal AD? (11-17th century)

Sample: Refshale_16; Location: Refshale, Denmark; Age: cal AD 1046-1163

Sample: Jørgen_625; Location: Odense, Denmark; Age: cal AD 1293-1383

Universities of Surrey, Tübingen & Kiel

Schuenemann et al. 2013
Science 341, 179-83
DNA capture enrichment

Enrichment applied successfully to many samples but in one case it wasn’t required!

Blue – *M. leprae*

Green - Host
Ancient *M. leprae* genome

- Abundant DNA
- Good quality
- Genome assembled *de novo*
- Mycolic acids also present
- After >600 years
Phylogenetic trees

And East Africa?
Lessons from the past

- Medieval *M. leprae* did not lose virulence
- Same 3I strain exists today in Americas
- At least two strains in circulation 3I & 2F
- Link between Europe & Middle East

**How long can *M. leprae* survive in environment?**
Molecular drug susceptibility testing (MDST)

Accurate & effective but laborious
New applications of WGS

• Capture arrays to replace PCR-based MDST
• Will provide drug susceptibility & genome sequence for same cost & effort
• Epidemiology at single nucleotide resolution
• Can be used to probe environment

Provide deeper insight into transmission
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