Genetic research of leprosy

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Outline

1 Background
2 GWAS1
3 GWAS2
4 Candidate gene analysis
5 Pharmacogenomics: DIHS
6 Acknowledgement
Part1: Background
Leprosy

- Chronic infectious disease
  caused by *Mycobacterium leprae*
  *in susceptible individuals*

- Affects skin and peripheral nerves
  leading to irreversible nerve damage
Etiology of Leprosy

Mycobacterium leprae

Susceptible background
About 200,000 new cases registered annually
Mainly in the developing countries, such as India, China, Brazil......
### Global Leprosy Situation, 2012

<table>
<thead>
<tr>
<th>WHO Region* – Région de l’OMS*</th>
<th>No. of new cases detected – Nombre de nouveaux cas dépistés</th>
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<tbody>
<tr>
<td></td>
<td>2004</td>
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<tr>
<td>African – Afrique</td>
<td>46,918</td>
</tr>
<tr>
<td>Americas – Amériques</td>
<td>52,662</td>
</tr>
<tr>
<td>South-East Asia – Asie du Sud-Est</td>
<td>298,603</td>
</tr>
<tr>
<td>Eastern Mediterranean – Méditerranée orientale</td>
<td>3,392</td>
</tr>
<tr>
<td>Western Pacific – Pacifique occidental</td>
<td>6,216</td>
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<tr>
<td><strong>Total</strong></td>
<td>407,791</td>
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</table>

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2012, 87, 317–328

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<table>
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<td>Angola</td>
<td>2 109</td>
<td>1 877</td>
<td>1 078</td>
<td>1 269</td>
<td>1 184</td>
<td>937</td>
<td>1 076</td>
<td>508</td>
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<tr>
<td>Bangladesh</td>
<td>8 242</td>
<td>7 882</td>
<td>6 280</td>
<td>5 357</td>
<td>5 249</td>
<td>5 239</td>
<td>3 848</td>
<td>3 970</td>
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<tr>
<td>Brazil – Brésil</td>
<td>49 384</td>
<td>38 410</td>
<td>44 436</td>
<td>39 125</td>
<td>38 914</td>
<td>37 610</td>
<td>34 894</td>
<td>33 955</td>
</tr>
<tr>
<td>China – Chine</td>
<td>1 499</td>
<td>1 658</td>
<td>1 506</td>
<td>1 526</td>
<td>1 614</td>
<td>1 597</td>
<td>1 324</td>
<td>1 144</td>
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<tr>
<td>Democratic Republic of the Congo –</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>République démocratique du Congo</td>
<td>11 781</td>
<td>10 369</td>
<td>8 257</td>
<td>8 820</td>
<td>6 114</td>
<td>5 062</td>
<td>5 049</td>
<td>3 949</td>
</tr>
<tr>
<td>Ethiopia – Éthiopie</td>
<td>4 787</td>
<td>4 698</td>
<td>4 092</td>
<td>4 187</td>
<td>4 170</td>
<td>4 417</td>
<td>4 430</td>
<td>NA</td>
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<tr>
<td>India – Inde</td>
<td>260 063</td>
<td>169 709</td>
<td>139 252</td>
<td>137 685</td>
<td>134 184</td>
<td>133 717</td>
<td>126 800</td>
<td>127 295</td>
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<tr>
<td>Indonesia – Indonésie</td>
<td>16 549</td>
<td>19 695</td>
<td>17 682</td>
<td>17 723</td>
<td>17 441</td>
<td>17 260</td>
<td>17 012</td>
<td>20 023</td>
</tr>
<tr>
<td>Madagascar</td>
<td>3 710</td>
<td>2 709</td>
<td>1 536</td>
<td>1 644</td>
<td>1 763</td>
<td>1 572</td>
<td>1 520</td>
<td>1 577</td>
</tr>
<tr>
<td>Mozambique</td>
<td>4 266</td>
<td>5 371</td>
<td>3 637</td>
<td>2 510</td>
<td>1 313</td>
<td>1 191</td>
<td>1 207</td>
<td>1 097</td>
</tr>
<tr>
<td>Myanmar</td>
<td>3 748</td>
<td>3 571</td>
<td>3 721</td>
<td>3 637</td>
<td>3 365</td>
<td>3 147</td>
<td>2 936</td>
<td>3 082</td>
</tr>
<tr>
<td>Nepal – Népal</td>
<td>6 958</td>
<td>6 150</td>
<td>4 235</td>
<td>4 436a</td>
<td>4 708a</td>
<td>4 394 a</td>
<td>3 118 a</td>
<td>3 184</td>
</tr>
<tr>
<td>Nigeria – Nigéria</td>
<td>5 276</td>
<td>5 024</td>
<td>3 544</td>
<td>4 665</td>
<td>4 899</td>
<td>4 219</td>
<td>3 913</td>
<td>NA</td>
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<tr>
<td>Philippines</td>
<td>2 254</td>
<td>3 130</td>
<td>2 517</td>
<td>2 514</td>
<td>2 373</td>
<td>1 795</td>
<td>2 041</td>
<td>1 818</td>
</tr>
<tr>
<td>South Sudan – Soudan du Sud</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1 799</td>
</tr>
</tbody>
</table>

Prevalence

2000 new cases in China

50 new cases in Shandong Province
Scientific Questions for leprosy control

No primary prevention for leprosy

- vaccine is not available so far
- *Mycobacterium leprae can not be cultured in vitro*
- No measures to screen for susceptible individuals of leprosy among populations in endemic areas
<table>
<thead>
<tr>
<th>Regions/genes</th>
<th>Population</th>
<th>MLS/SNPs</th>
<th>Samples</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>10p13</td>
<td>India</td>
<td>4.09</td>
<td>224 families</td>
<td>Nature Genetics (Siddiqui MR, et al. 2001)</td>
</tr>
<tr>
<td>6q25</td>
<td>Vietnamese</td>
<td>4.31</td>
<td>86 families</td>
<td>Nature Genetics (Mira MT, et al. 2003)</td>
</tr>
<tr>
<td>6q21.32/17q22</td>
<td>Brazil</td>
<td>3.23/2.38</td>
<td>71 families</td>
<td>Genes Immun (Miller EN, et al. 2004)</td>
</tr>
<tr>
<td>PARK2/PACRG</td>
<td>Vietnamese, Brazil</td>
<td>PARK2_e01 (22599) -rs104007</td>
<td>197 families, 587 sporadic cases and 388 controls</td>
<td>Nature (Mira MT, et al. 2004)</td>
</tr>
<tr>
<td>LTA</td>
<td>Vietnamese, Brazil, India</td>
<td>LTA+80</td>
<td>298 families, 571 sporadic cases and 562 controls</td>
<td>Nature Genetics (Alcais A, et al. 2007)</td>
</tr>
</tbody>
</table>

Few of these associations have been replicated
• GWAS Advent in the year 2005
• Infectious disease is not immune to GWAS
• NG 2010 09
• Several GWAS on infectious disease conducted so far
  Heptitis B, L, TB, M, HIV and Malaria
Part2: GWAS I
### Table 1. Baseline Characteristics of the Case Patients and Controls, According to Cohort.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Genomewide Association Study</th>
<th>Replication Study 1</th>
<th>Replication Study 2</th>
<th>Replication Study 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case patients</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No.</td>
<td>706</td>
<td>2164</td>
<td>304</td>
<td>786</td>
<td>3960</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>65.5</td>
<td>66.5</td>
<td>58.7</td>
<td>54.9</td>
<td>62.8</td>
</tr>
<tr>
<td>Mean age at onset of leprosy (yr)</td>
<td>21.8</td>
<td>21.4</td>
<td>26.5</td>
<td>26.2</td>
<td>23.3</td>
</tr>
<tr>
<td>Sex (no.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>562</td>
<td>1742</td>
<td>221</td>
<td>535</td>
<td>3060</td>
</tr>
<tr>
<td>Female</td>
<td>144</td>
<td>422</td>
<td>83</td>
<td>251</td>
<td>900</td>
</tr>
<tr>
<td>Clinical subtype of leprosy (no.)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Multibacillary</td>
<td>305</td>
<td>918</td>
<td>166</td>
<td>379</td>
<td>1768</td>
</tr>
<tr>
<td>Paucibacillary</td>
<td>397</td>
<td>1081</td>
<td>124</td>
<td>357</td>
<td>1959</td>
</tr>
<tr>
<td>Disabled due to leprosy (no.)‡</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>570</td>
<td>947</td>
<td>149</td>
<td>353</td>
<td>2019</td>
</tr>
<tr>
<td>No</td>
<td>90</td>
<td>654</td>
<td>150</td>
<td>340</td>
<td>1234</td>
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<tr>
<td>Family history of leprosy (no.)§</td>
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<tr>
<td>Familial</td>
<td>185</td>
<td>165</td>
<td>62</td>
<td>80</td>
<td>492</td>
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<tr>
<td>Sporadic</td>
<td>521</td>
<td>1799</td>
<td>193</td>
<td>574</td>
<td>3087</td>
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<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No.</td>
<td>1225</td>
<td>4373</td>
<td>709</td>
<td>873</td>
<td>7180</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>34.9</td>
<td>63.0</td>
<td>40.4</td>
<td>43.9</td>
<td>48.0</td>
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<td>Sex (no.)</td>
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<tr>
<td>Male</td>
<td>670</td>
<td>2913</td>
<td>300</td>
<td>590</td>
<td>4473</td>
</tr>
<tr>
<td>Female</td>
<td>555</td>
<td>1460</td>
<td>409</td>
<td>283</td>
<td>2707</td>
</tr>
</tbody>
</table>
PCA and QQ plot

706 cases, 1225 controls (1931 samples)

\[ \lambda_{GIC} = 1.034 \]
PCA and QQ plot

706 cases, 515 controls _ well matched (1220 samples)
Validation stage

• Totally, 93 SNPs were selected to be validated.

• Validation samples

  – sample1: 1742 cases, 4373 controls (North Han)

  – sample 2: 221 cases, 709 controls (South Han

  – sample3: 535 cases, 873 controls (South Minority)
<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Gene</th>
<th>MAF</th>
<th>aGWAS</th>
<th>Replication 1 (P, OR, 95% CI)</th>
<th>Replication 2 (P, OR, 95% CI)</th>
<th>Replication 3 (P, OR, 95% CI)</th>
<th>Combined_all (P, OR, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs602875</td>
<td>6</td>
<td>HLA-DR/DQ</td>
<td>0.32</td>
<td>3.47E-04</td>
<td>0.58, 0.43–0.79</td>
<td>0.64, 0.59–0.71</td>
<td>0.85, 0.65–0.93</td>
<td>0.77, 0.64–0.93</td>
</tr>
<tr>
<td>rs424908</td>
<td>8</td>
<td>RIPK2</td>
<td>0.42</td>
<td>1.23E-03</td>
<td>1.54, 1.18–2.01</td>
<td>1.32, 0.68–0.80</td>
<td>1.21E-02, 0.69–1.11</td>
<td>1.21E-02, 0.71–0.96</td>
</tr>
<tr>
<td>rs6478108</td>
<td>9</td>
<td>TNFSF15</td>
<td>0.46</td>
<td>4.55E-04</td>
<td>1.80E-11, 1.18–1.43</td>
<td>0.87, 0.65</td>
<td>3.95E-02, 1.02–1.66</td>
<td>3.29E-01, 0.65–1.12</td>
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<tr>
<td>rs3088362</td>
<td>13</td>
<td>LRRK2</td>
<td>0.25</td>
<td>9.37E-03</td>
<td>3.62E-03, 0.49–0.91</td>
<td>1.87, 1.53</td>
<td>1.39E-03, 0.79–0.95</td>
<td>1.26, 0.86–1.18</td>
</tr>
<tr>
<td>rs3764147</td>
<td>13</td>
<td>CCDC122</td>
<td>0.26</td>
<td>2.00E-06</td>
<td>6.64E-23, 1.38–2.53</td>
<td>1.7, 1.40–1.67</td>
<td>4.69E-04, 1.22–2.09</td>
<td>1.11E-02, 1.05–1.51</td>
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<tr>
<td>rs9302752</td>
<td>16</td>
<td>NOD2</td>
<td>0.29</td>
<td>1.42E-09</td>
<td>2.8, 1.49–2.62</td>
<td>1.46E-37, 1.49–2.62</td>
<td>7.29E-06, 1.36–2.22</td>
<td>4.54E-08, 1.32–1.82</td>
</tr>
</tbody>
</table>

Seven susceptibility genes were validated
## Results of subtype analysis

### Table 3. Heterogeneity Analysis of the Five Single-Nucleotide Polymorphisms (SNPs) Found to Be Differentially Associated with Clinical Subtypes.

| SNP      | Chromosome | Position | Gene    | Multibacillary Leprosy (N = 1768) | Paucibacillary Leprosy (N = 1959) | P Value for Heterogeneity
|----------|------------|----------|---------|----------------------------------|----------------------------------|-----------------------------
|          |            |          |         | P Value | Odds Ratio (95% CI) | P Value | Odds Ratio (95% CI) |                       |
| rs3764147| 13         | 43355925 | C13orf31| 7.47×10⁻⁶² | 1.93 (1.79–2.09) | 5.11×10⁻²¹ | 1.44 (1.33–1.55) | 5.44×10⁻¹⁰          |
| rs10507522| 13        | 43377000 | C13orf31| 2.99×10⁻³⁸ | 0.60 (0.54–0.65) | 9.40×10⁻¹⁰ | 0.77 (0.71–0.84) | 2.57×10⁻⁶           |
| rs9302752| 16         | 49276604 | NOD2    | 8.87×10⁻⁴¹ | 1.73 (1.60–1.88) | 1.44×10⁻¹⁹ | 1.44 (1.33–1.55) | 3.98×10⁻⁵           |
| rs42490  | 8          | 90847650 | RIPK2   | 1.35×10⁻¹⁰ | 0.69 (0.64–0.75) | 9.54×10⁻⁸  | 0.82 (0.76–0.88) | 8.23×10⁻⁴           |
| rs1491938| 12         | 38931897 | LRRK2   | 2.26×10⁻⁶  | 0.81 (0.75–0.89) | 2.96×10⁻¹  | 0.96 (0.88–1.04) | 8.55×10⁻⁴           |
Conclusions

We performed the first GWAS of leprosy and identified 7 susceptibility genes.

**NOD2-mediated Signaling Pathway**

**HLA-DQ/DR, NOD2, RICK2, TNFSF15, LRRK2, CCDC122 and C13orf31**

Genomewide Association Study of Leprosy

Fu-Ren Zhang, M.D., Ph.D., Wei Huang, Ph.D., Shu-Min Chen, M.D., Ph.D., Liang-Dan Sun, M.D., Ph.D., Hong Liu, M.D., Yi Li, Ph.D., Yong Cui, M.D., Ph.D., Xiao-Xiao Yan, M.D., Hai-Tao Yang, M.D., Rong-De Yang, M.D., Tong-Sheng Chu, M.D., Chi Zhang, M.D., Lin Zhang, M.D., Jian-Wen Han, M.D., Gong-Qi Yu, B.S., Cheng Quan, M.D., Yong-Xiang Yu, B.S., Zheng Zhang, M.D., Ben-Qing Shi, M.D., Lian-Hua Zhang, M.D., Hui Cheng, M.D., Chang-Yuan Wang, M.D., Yan Lin, M.D., Hou-Feng Zheng, M.D., Xi-An Fu, M.D., Xian-Bo Zuo, M.S., Qiang Wang, M.D., Heng Long, M.D., Yi-Ping Sun, M.D., Yi-Lin Cheng, M.S., Hong-Qing Tian, M.D., Fu-Sheng Zhou, B.S., Hua-Xu Liu, M.D., Ph.D., Wen-Sheng Lu, M.D., Su-Min He, M.D., Wen-Li Du, B.S., Min Shen, B.S., Qi-Yi Jin, B.S., Ying Wang, Ph.D., Hui-Qi Low, B.S., Tantoso Erwin, B.S., Ning-Han Yang, B.S., Jin-Yong Li, M.D., Xin Zhao, M.D., Yue-Lin Jiao, M.D., Li-Guo Mao, M.D., Gang Yin, M.D., Zhen-Xia Jiang, M.D., Xiao-Dong Wang, M.D., Jing-Ping Yu, M.D., Zong-Hou Hu, M.D., Cui-Hua Gong, M.D., Yu-Qiang Liu, M.D., Rui-Yu Liu, M.D., De-Min Wang, M.D., Dong Wei, M.D., Jin-Xian Liu, M.D., Wei-Kun Cao, M.D., Hong-Zhong Cao, M.D., Yong-Ping Li, M.D., Wei-Guo Yan, M.D., Shi-Yu Wei, M.D., Kui-Jun Wang, M.D., Martin L. Hibberd, Ph.D., Sen Yang, M.D., Ph.D., Xue-Jun Zhang, M.D., Ph.D., and Jian-Jun Liu, Ph.D.

ABSTRACT
Part3: GWAS II
Much more genetic susceptibility factors remain to be discovered.


Expanded GWAS of leprosy_GWAS II

706 leprosy cases + 1225 healthy controls

4,367 population
(10 controls+1,012 AD+1,139 psoriasis+1,082 SLE+1,124 vitiligo cases)

Select novel loci

Replication in 3 independent cohorts

mRNA analysis of susceptibility loci

only for maximize statistical power to select novel loci
Discovery stage

✓ Samples:

- 706 leprosy cases and 5,581 controls
  (1225 healthy controls and 4367 population controls)

✓ SNPs:

- 1,701,673 SNPs (genotyped + imputed)

- IMPUTE version 1 and the HapMap reference data (HapMap phase II, CHB+JPT data)
- Individual genotypes with probability less than 90% were excluded
- SNPs with impute info score less than 80%, MAF less than 1% and missing rate greater than 10% of genotypes were dropped
All the SNPs reported have been removed.

Top 22 SNPs were selected for replications (located in 21 novel loci, \(P < 2.0 \times 10^{-5}\)).
Discovery stage

- TLR1 was identified as susceptibility loci of leprosy by GWAS in India population.
- Rs5743618 and rs17616475 in TLR1 gene were selected for further validation.

Totally, 24 SNPs located in 22 novel loci were followed up in the replication samples.

## Replication stage

**Samples:**

<table>
<thead>
<tr>
<th></th>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethnicity</td>
<td>Sample size</td>
</tr>
<tr>
<td>Replication 1b</td>
<td>Han</td>
<td>2,307</td>
</tr>
<tr>
<td>Replication 2c</td>
<td>Han</td>
<td>273</td>
</tr>
<tr>
<td>Replication 3d</td>
<td>Minority</td>
<td>721</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>4,007</td>
</tr>
</tbody>
</table>
Genotyping analyses of the three replication samples were conducted by using the Sequenom MassArray system.
Results

No supporting evidence was identified in TLR1 gene.
Interaction analysis

The interaction between *NOD2* (rs9302752) and *RIPK2* (rs40457) loci were identified (*P*=0.0011).

The protective association at rs40457 is only significant in the subjects of carrying the TT genotype of rs9302752 (*P*=3.03×10^{-14})
mRNA expression analysis of two identified genes

- We investigate the expression of the confirmed genes in the FFPE tissue of leprosy using branched DNA (bDNA) technology

- Samples:
  - 36 leprosy cases
  - 32 controls

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![Bar charts showing the expression levels of IL23R and RAB32 in leprosy and control samples.](image-url)
Discussion

- Rs3762318 is located within a LD region of 150kb.
- The identification of IL23R has firmly established the involvement of innate immunity in the pathogenesis of leprosy.
- The marked similarity between leprosy and Crohn’s disease susceptibility.
Discussion

- Rs2275606 was located within a 250-kb LD block where C6orf103 and RAB32 reside.
- The identification of RAB32 has provided new biological insight by highlighting the potential involvement of autophagocytosis in the host defense against *M. leprae* infection.
Conclusion

- Two new loci as susceptibility genes of leprosy were identified,
- Suggesting the potential involvement of autophagocytosis
- Particularly, the $IL23R$ association extends previous observations between leprosy and Crohn’s disease susceptibility genes.
Identification of two new loci at IL23R and RAB32 that influence susceptibility to leprosy

Furen Zhang, Hong Liu, Shumin Chen, Huiqi Lu, Liangdan Sun, Yong Cui, Tongsheng Chu, Yi Li, Xi'an Fu, Yongxiang Yu, Gongqi Yu, Benqing Shi, Hongqing Tian, Dianchang Liu, Xiulu Yu, Jinghui Li, Nan Lu, Fangfang Bao, Chunyaing Yuan, Jian Liu, Huaxu Liu, Lin Zhang, Yonghui Sun, Mingfei Chen, Qing Yang, Haitao Yang, Rongde Yang, Lianhua Zhang, Qiang Wang, Hong Liu, Fuguang Zuo, Haizhen Zhang, Chiea Chuen Khor, Martin I. Hibberd, Sen Yang, & Xuejun Zhang

We performed a genome-wide association study with 706 individuals with leprosy and 5,581 control individuals and replicated the top 24 SNPs in three independent replication samples, including a total of 3,301 individuals with leprosy and 5,299 control individuals from China. Two loci not previously associated with the disease were identified with genome-wide significance: rs2275606 (combined \( P = 3.94 \times 10^{-14} \), \( OR = 1.30 \)) on 6q24.3 and rs3762318 (combined \( P = 3.27 \times 10^{-11} \), \( OR = 0.69 \)) on 1p31.3. These associations implicate IL23R and RAB32 as new susceptibility genes for leprosy. Furthermore, we identified evidence of interaction between the NOD2 and RIPK2 loci, which is consistent with the biological association of the proteins encoded by these genes (NOD2-RIPK2 complex) in activating the NF-\( \kappa \)B pathway as a part of the host defense response to infection. Our findings have expanded the biological functions of IL23R by uncovering its involvement roles for host genetic factors in human susceptibility to infection and in the progression of infectious diseases.

Leprosy is a chronic granulomatous infectious disease caused by Mycobacterium leprae that affects both the skin and peripheral nerves. Although the prevalence of leprosy has declined dramatically since the introduction of multidrug therapy in the 1980s, more than 200,000 new cases are reported globally each year, and leprosy remains a major public health problem, especially in China and India. Family studies and population epidemiological surveys have clearly demonstrated a substantial contribution of host genetics to the susceptibility of individuals to leprosy, with estimated heritability of up to 57% (ref. 4). However, there is a great need to identify the genetic basis of leprosy, which is compounded by the lack of suitable animal hosts for M. leprae and the difficulty of culturing it in vitro; both limitations have greatly hindered research on the mechanisms underlying leprosy.

In 2009, we performed a GWAS of leprosy and identified six suscepti-
Part 4: candidate gene analysis

1: Leprosy and IBD
Genome wide association study of leprosy

NOD2, TNFSF15, LRRK2, CCDC122/C13orf31, HLA-DR, RIPK2

Genome wide association study of leprosy

IL23R and RAB32(2011)
Inflammatory bowel disease (IBD)

IBD: a chronic relapsing inflammatory disorder of the gut.

The cause of IBD is unknown but is now widely accepted to be multi-factorial.

(Crohn's disease, Ulcerative colitis,

- An interplay of environmental risk factors and immunologic changes would trigger the onset of the disease in a genetically susceptible host.
- IBD: mainly in developed countries. The incidence each year in Europe is up to 322/100,000
Recent GWAS analyses of CD and UC have corroborated that nearly one-third of their susceptibility loci are shared.
## Common characteristics shared between leprosy and IBD

<table>
<thead>
<tr>
<th>Disease</th>
<th>Etiology</th>
<th>Immune characteristic</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leprosy</td>
<td>Susceptibility background of host</td>
<td>Mycobacterium leprae</td>
<td>Multibacillary: Th2 paucibacillary: Th1</td>
</tr>
<tr>
<td>IBD</td>
<td>Susceptibility background of host</td>
<td>Unknown, Mycobacterium avium-intracellular infection has been found in the intestinal mucosa of CD disease.</td>
<td>UC: Th2 CD: Th1</td>
</tr>
</tbody>
</table>
3 susceptibility genes of leprosy, 5 were shared by leprosy and CD.
A Common Genetic Fingerprint in Leprosy and Crohn's Disease?

Erwin Schurr, Ph.D., and Ph.D.

Card15 Polymorphisms in the Immunopathogenesis of Crohn’s Disease and Mycobacterial Infectious Diseases
Subjects

- 12 CD GWASs and 7 UC GWASs through the Catalog of published GWAS (total 155 SNPs within 118 genes)
- SNPs within MHC region and 15 SNPs with the 5 known leprosy susceptibility genes were excluded.
- After exclusion, 133 SNPs,(75 CD-associated SNPs, 54 UC-associated SNPs, 4 IBD) were selected
- 119 SNPs were successfully designed and genotyped.
# Samples

## Table 1. Summary of the Four Independent Leprosy Samples Used in the Current Study

<table>
<thead>
<tr>
<th>Cases</th>
<th>Ethnicity</th>
<th>Sample Size</th>
<th>Mean Age</th>
<th>Mean Age at Onset</th>
<th>Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Han</td>
<td>1,504</td>
<td>66.22</td>
<td>22.96</td>
<td>1,251/253</td>
</tr>
<tr>
<td>Sample 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Han</td>
<td>1,154</td>
<td>66</td>
<td>23.57</td>
<td>934/220</td>
</tr>
<tr>
<td>Sample 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Han</td>
<td>1,165</td>
<td>62.36</td>
<td>32.73</td>
<td>846/319</td>
</tr>
<tr>
<td>Sample 4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Chuang</td>
<td>334</td>
<td>54.76</td>
<td>24.68</td>
<td>224/110</td>
</tr>
<tr>
<td>Miao</td>
<td>277</td>
<td>53.03</td>
<td>27.88</td>
<td>193/84</td>
<td></td>
</tr>
<tr>
<td>Yizu</td>
<td>236</td>
<td>54.37</td>
<td>26.71</td>
<td>166/70</td>
<td>182</td>
</tr>
<tr>
<td>other</td>
<td>301</td>
<td>56.90</td>
<td>26.84</td>
<td>212/89</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4,971</td>
<td>62.63</td>
<td>26.19</td>
<td>3,826/1,145</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls</th>
<th>Sample Size</th>
<th>Mean Age</th>
<th>Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Size</td>
<td>1,502</td>
<td>65.25</td>
<td>1,254/248</td>
</tr>
<tr>
<td>Han</td>
<td>2,605</td>
<td>57.01</td>
<td>1,313/1,292</td>
</tr>
<tr>
<td>Sample 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>648</td>
<td>42.09</td>
<td>275/373</td>
</tr>
<tr>
<td>Chuang</td>
<td>310</td>
<td>45.6</td>
<td>195/115</td>
</tr>
<tr>
<td>Miao</td>
<td>190</td>
<td>41.48</td>
<td>152/38</td>
</tr>
<tr>
<td>Yizu</td>
<td>182</td>
<td>43.77</td>
<td>147/35</td>
</tr>
<tr>
<td>other</td>
<td>66</td>
<td>42.18</td>
<td>26/40</td>
</tr>
<tr>
<td>Total</td>
<td>5,503</td>
<td>55.71</td>
<td>3,362/2,141</td>
</tr>
</tbody>
</table>

<sup>a</sup>Samples collected in Shandong, Anhui, and Jiangsu provinces (eastern China).
<sup>b</sup>Samples collected in Shandong, Anhui, and Jiangsu provinces (eastern China).
<sup>c</sup>Samples collected in Yunnan, Guizhou, and Fujian provinces (eastern China).
<sup>d</sup>Samples collected in Yunnan, Guizhou, and Fujian provinces (southern China).
Stage 1

1,504 cases vs 1,502 controls

119 SNPs

Stage 2

19 SNPs

(P<0.05)

1,154 cases vs 2,605 controls (North Han)
1,165 cases vs 648 controls (South Han)
1,148 cases vs 748 controls (South Minority)

Two associations were discovered (rs2058660 and rs6871626)

Design

Sequenom MassARRAY system
## Results:

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Gene</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2058660</td>
<td>IL18RAP/IL18R1</td>
<td>4.57E-19</td>
</tr>
<tr>
<td>rs6871626</td>
<td>IL12B</td>
<td>3.95E-18</td>
</tr>
</tbody>
</table>
Regional Association Plots of rs2058660

rs2058660 on 2q12.1 (p = 4.57 × 10^{-19}; odds ratio [OR] = 1.30)
Regional Association Plots of rs6871626

rs6871626 on 5q3 (p = \(3.95 \times 10^{-18}\); OR = 0.75)
The directions of 17 SNPs located in the common regions of leprosy and IBD

- Totally, 7 genes were shared between leprosy and IBD
- Only 3 SNPs were genotyped both in leprosy and IBD samples.

- **Current study**
- **Previous study**
- **Directions**
  - opposite
  - consistent

It is intriguing to see that the two susceptibility loci showed opposite associations between leprosy and IBD.
Discussion

It is intriguing to see that the two susceptibility loci showed opposite associations between leprosy and IBD.

With the growing of inflammatory diseases, the infectious diseases will die out?
Conclusion:

- The discovery of the two susceptibility loci has implicated **IL18RAP/IL18R1 and IL12B** as susceptibility genes for leprosy.

- And also highlighted the important role of **IL12/IL18-mediated transcriptional regulation of IFN-γ** production in the development of leprosy.

- Together with previous findings, our study has further demonstrated the shared genetic susceptibility basis between inflammation and infectious diseases.
Identification of \textit{IL18RAP/IL18R1} and \textit{IL12B} as Leprosy Risk Genes Demonstrates Shared Pathogenesis between Inflammation and Infectious Diseases

Hong Liu,1,2,3,4,11 Astrid Irwanto,5,6,11 Hongqing Tian,1,4,11 Xi’an Fu,2,3 Yongxiang Yu,2,3 Gongqi Yu,2,3 Huiqi Lou,5 Tongsheng Chu,2,3 Yi Li,5 Benqing Shi,1,4 Mingfai Chen,2,3 Yonghu Sun,2,3 Chunying Yuan,2,3 Jiabao You,2,3 Fangfang Bao,2,3 Jinghui Li,2,3 Jian Liu,2,3 Huaxu Liu,2,3 Dianchang Liu,2,3 Xiulu Yu,2,3 Lin Zhang,2,3 Qin Yang,2,3 Na Wang,2,3 Guiye Niu,2,3 Shanshan Ma,2,3 Yan Zhou,2,3 Chuan Wang,2,3 Shumin Chen,2,3 Xuejun Zhang,7,8 Jianjun Liu,2,5,9,12 and Furen Zhang1,2,3,4,10,12,*

Of eight leprosy susceptibility loci identified by genome-wide association studies, five have been implicated in Crohn disease, suggesting a common genetic fingerprint between leprosy and inflammatory bowel disease (IBD). Here, we conducted a multiple-stage genetic association study of 1.53 IBD susceptibility loci in multiple leprosy samples (totaling 4,971 leprosy cases and 5,503 controls) from a Chinese population and discovered two associations at rs2058660 on 2q12.1 (p = 4.57 × 10^{-19}; odds ratio [OR] = 1.30) and rs6871626 on 8q33.3 (p = 3.95 × 10^{-18}; OR = 0.75), implicating \textit{IL18RAP/IL18R1} and \textit{IL12B} as susceptibility genes for leprosy. Our study reveals the important role of IL12/IL18-mediated transcriptional regulation of IFN-\(\gamma\) production in leprosy, and together with previous findings, it demonstrates the shared genetic susceptibility between infectious and inflammatory diseases.

Genome-wide association studies (GWASs) have offered a powerful and unbiased approach for the identification of susceptibility genes for complex diseases. As of September 2011, a total of 1,617 susceptibility loci have been identified in 249 complex traits (see Web Resources for the Catalog of Published GWASs). The enormous progress of GWASs has led to the revelation of biological connections between some clinically unrelated diseases through the identification of shared risk variants; such connections include associations between \textit{IL23R} (MIM 607562) variants and leprosy (MIM 609888),1 Crohn disease (CD [MIM 266600]),2 ulcerative colitis (UC [MIM 266600]),3 psoriasis (MIM 177900),4 and ankylosing spondylitis (AS [MIM 106300]),5 as well as the association between \textit{PTPN2} (MIM 176887) variants and CD and final mucosa. Recent GWAS analyses of CD and UC have corroborated that nearly one-third of their susceptibility loci are shared.8 Leprosy is a chronic infectious disease caused by \textit{Mycobacterium leprae} and affects both the skin and the peripheral nerves. Although leprosy and CD are distinct clinical entities, they both belong to chronic inflammatory diseases, and there is some suggestive evidence that they share pathogenic mechanisms. For example, the formation of granuloma is an important clinical hallmark of both leprosy and CD. In addition, it has also been suggested that the development of CD, at least in some individuals, might be triggered by mycobacterial infection.9,10 \textit{Mycobacterium avium} subspecies \textit{paratuberculosis} has been cultured and identified from the intestines and blood of CD-affected individuals.11 It has...
Part 4: candidate gene analysis

2: Association study of TOLL and CARD with leprosy susceptibility
TLRs and leprosy susceptibility

• TLR1, TLR2 and TLR4 variants have been reported to be associated with leprosy in Indian, Nepalese and African populations.

CARD and leprosy susceptibility

- Our GWAS of leprosy identified five genes directly implicated in the NOD2-mediated regulatory node of innate immunity.

- NOD2 is a member of the CARD-containing protein family. CARD6 and CARD9 were also involved in the Gene-Interaction Network.
TLRs and CARD participate in the activation of NF-κB.
To further investigate the role of TLRs and CARDs in leprosy, we performed a large three-stage candidate gene-based association study of TLRs and CARDs in leprosy samples of Chinese Han population.
Subjects

Genotyping analyses
- Case: 2442
- Control: 8082

branched DNA
- Case: 36
- Control: 32
## Sample summary of 2,442 cases and 8,082 controls

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Mean age</th>
<th>Mean age at onset</th>
<th>Males* (%)</th>
<th>Sample size</th>
<th>Mean age</th>
<th>Males* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Validation Set 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,504</td>
<td>66.22</td>
<td>20.96</td>
<td>83%</td>
<td>1,502</td>
<td>65.25</td>
<td>84%</td>
</tr>
<tr>
<td><strong>Validation Set 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>938</td>
<td>65.05</td>
<td>24.14</td>
<td>81%</td>
<td>5,827</td>
<td>49.82</td>
<td>65%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,442</td>
<td>65.78</td>
<td>22.2</td>
<td>82%</td>
<td>8,082</td>
<td>52.42</td>
<td>68%</td>
</tr>
</tbody>
</table>
Top SNP (rs233100) as well as the confirmed SNP (rs2735591) within the LD block of 300Kb on 1p22.3 region were labeled out.
## Summary of the association results of rs2735591

<table>
<thead>
<tr>
<th>SNP</th>
<th>rs2735591</th>
<th>MAF cases</th>
<th>MAF controls</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>Q-testc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Position</td>
<td>85,517,060</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor/Major Allele</td>
<td>T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test allelea</td>
<td>T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>BCL10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GWAS 1,220 samples (Stage 1)b</td>
<td>0.33</td>
<td>0.29</td>
<td>1.66×10⁻²</td>
<td>1.24 (1.04-1.48)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Validation Stage 2</td>
<td>0.36</td>
<td>0.30</td>
<td>2.18×10⁻⁶</td>
<td>1.32 (1.17-1.48)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Validation Stage 3</td>
<td>0.32</td>
<td>0.29</td>
<td>4.58×10⁻³</td>
<td>1.17 (1.05-1.3)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Combined Validation Stage 2 and 3</td>
<td>0.34</td>
<td>0.30</td>
<td>1.27×10⁻⁷</td>
<td>1.27 (1.14-1.33)</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>Combined Stage 1-3</td>
<td>0.34</td>
<td>0.29</td>
<td>1.03×10⁻⁹</td>
<td>1.24 (1.16-1.33)</td>
<td>0.954</td>
<td></td>
</tr>
</tbody>
</table>
Expression analysis of *BCL10* in skin tissues. A significantly lower expression of *BCL10* was observed in the lesions of patients than the normal skin tissues of healthy controls (P = 3.83 × 10^{-5}).
The protein encoded by *BCL10* contains a caspase recruitment domain (CARD) and plays an important role in the activation of NF-κB, an important regulator of immune response against the infection by *M. leprae*.
Conclusion

- Our discovery implicates BCL10 as the new susceptibility gene for leprosy, highlighting the important role of both innate and adaptive immune responses in leprosy.

- We did not observe supporting evidence for the association of TLRs with leprosy susceptibility in Chinese population, which suggests the genetic heterogeneity of leprosy susceptibility between ethnic populations.
An association study of TOLL and CARD with leprosy susceptibility in Chinese population

Hong Liu¹,²,³, ⁴, Fangfang Bao⁵, ⁶, Astrid Irwanto⁵,⁶, Xi’an Fu³, ⁴, Nan Lu³, ⁴, Gongqi Yu¹,³, Yongxiang Yu¹,³, Yonghu Sun¹,³, Huiqi Low⁵, Yi Li⁵, Herty Liang⁵, Chunying Yuan¹,³, Jinghui Li¹, ³, Jian Liu¹, ³, Mingfei Chen¹, ³, Huaxu Liu¹,³, Na Wang¹,³, Jiebao You¹,³, Shanshan Ma¹,³, Guiyue Niu¹,³, Yan Zhou¹,³, Tongsheing Chu¹,³, Hongqing Tian²,³, Shumin Chen¹,³, Xuejun Zhang²,³,¹⁰, Jianjun Liu¹, ³,  ⁶, ⁷, ⁸, ⁹, ¹⁰ and Furen Zheng¹,²,³, ⁴, ⁸, ⁹, ¹⁰

¹Shandong Provincial Institute of Dermatology and Venerology, Shandong Academy of Medical Sciences, Shandong, China. ²Shandong Provincial Hospital for Skin Diseases, Shandong University, Shandong, China. ³Shandong Provincial Key Lab for Dermatovenerology, Shandong, China. ⁴Shandong Provincial Medical Center for Dermatovenerology, Shandong, China. ⁵Human Genetics, Genome Institute of Singapore, A*STAR, Singapore. ⁶Saw Swee Hock School of Public Health, National University of Singapore, Singapore. ⁷School of Life Sciences, Anhui Medical University, Anhui, China. ⁸Institute of Dermatology and Department of Dermatology at No.1 hospital, Anhui Medical University, Anhui, China and ⁹Shandong Clinical College, Anhui Medical University, Anhui, China and ¹⁰State Key Laboratory Incubation Base of Dermatology, Ministry of National Science and Technology, Anhui Medical University, Anhui, China

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Previous genome-wide association studies (GWASs) identified multiple susceptibility loci that have highlighted the important role of TLR (Toll-like receptor) and CARD (caspase recruitment domain) genes in leprosy. A large three-stage candidate gene-based association study of 30 TLR and 47 CARD genes was performed in the leprosy samples of Chinese Han. Of 4363 SNPs investigated, eight SNPs showed suggestive association ($P < 0.01$) in our previously published GWAS datasets (Stage 1). Of the eight SNPs, rs2735591 and rs4889841 showed significant association ($P < 0.001$) in an independent series of 1504 cases and 1502 controls (Stage 2), but only rs2735591 (next to BCL10) showed significant association in the second independent series of 938 cases and 5827 controls (Stage 3). Rs2735591 showed consistent association across the three stages ($P > 0.05$ for heterogeneity test), significant association in the combined validation samples ($P_{combined} = 5.54 \times 10^{-5}$ after correction for 4363 SNPs tested) and genome-wide significance in the whole GWAS and validation samples ($P = 1.03 \times 10^{-5}$, OR = 1.24). In addition, we demonstrated the lower expression of BCL10 in leprosy lesions than normal skins and a significant gene concatenation between BCL10 and the eight previously identified leprosy loci that are associated with MHC, a major comp...
Part 5: pharmacogenetics

DDS induced hypersensitivity syndrome (DHS)
Dapsone (4-4’-diaminodiphenylsulfone, DDS) was first synthesized in 1908\(^1\), which is both an antibiotic and an anti-inflammatory agent.

- Infectious diseases (e.g., leprosy, malaria, actinomycetoma and *Pneumocystis jirovecii* pneumonia in individuals with HIV infection)

- Chronic inflammatory diseases characterized by the infiltration of neutrophils or eosinophils (e.g. dermatitis herpetiformis, linear IgA dermatosis)
Background

About 0.5-3.6% of individuals treated with DDS suffer drug hypersensitivity syndrome, which was noted by Lowe in 1949 and termed “dapsone hypersensitivity syndrome” (DHS) in 1951, whose mortality rate has been reported to be 11-13%.

Currently, no tests are available to predict the risk of DHS.
Design

Discovery stage
- 39 DHS cases
- 950 non-DHS leprosy cases
- 1944 healthy controls

Validation stage
- 38 DHS cases
- 1109 non-DHS leprosy cases

HLA Imputation
- Illumina_660K Beadchips
- 454-Roche sequencing
- Sequenom platform

Hidden challenges
Results

Results of genomewide association analysis.

Results of genomewide association analysis.
Results
Results

Receiver-operating curve for an additive prediction model of DHS

AUC, area under the curve with 95% CI = 0.84-

Overall sensitivity is 85.53%, whereas specificity is 85.69%.
Part 6: Acknowledgement
Acknowledge

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Jinghu Li
Fangfang Bao
Jiaibao You
Jian Liu
Zhenzhen Wang
Hongqin Tian
Benqing Shi
Xiuwu Yu
Tongsheng Chu
Shumin Chen
Lin Zhang

Prof. Jianjun Liu
Ms. Astrid Irwanto
Ms. Yi Li
Hui-Qi Low
Jia-Nee Foo
Herty Liany

Prof. Xuejun Zhang
Prof Sen Yang
Liangdan Sun
Yong Cui

Prof Paul I.W. de Bakker
Sara L. Pulit

Shenjian Ping
Liangbin Yan
Guocheng Zhang

Lianhua Zhang
Zhonghe Wei
Wen-Bin Wu
Zuo-Sheng Liu

Hidden challenges
Thank you for your attention!