The International Serious Adverse Events Consortium (iSAEC)
Unraveling the genetic basis of drug induced liver injury

Sally John co-chair scientific management committee

May 14th, 2010
San Juan
Presentation overview

- Overview of the iSAEC
- DILI focused research
  - Flucloxacillin
  - Augmentin
  - Anti-TB
  - Zileuton
- What have we learned
- What is next for SAEC DILI research
iSAEC’s Mission

“The SAEC will identify and validate DNA-variants useful in predicting the risk of drug induced serious adverse events.”

Arthur Holden (CEO and Chairman)

http://www.saeconsortium.org/
Phase 2 membership

Top 5 SAEs

External Collaborators/Contributors

Spanish DILI

Columbia University

EUDRAGENE  DILIGEN

United States Department of Veterans Affairs

Spanish DILI

Marshfield Clinic Research Foundation

Duke

University of Dundee

International SAE Consortium
Why focus on DILI

- Drug-induced liver injury (DILI) is an important cause of serious liver disease
- Important cause of attrition of novel medicines
- Established academic networks that could provide cases for analysis
Phase 1 DILI research network

Clinical Cohort Sourcing

- Diligen
  - 214 Cases
- EUDRAGENE
  - 105 Cases
- Spanish DILI
  - 53 Cases
- Scotland
  - 46 Cases
- SAEC Members
  - ~70 Cases
  - ~700 Controls

Global DACC
- Columbia University

Global SAEC GT Core
- EA, Inc.

WGKT to date
- Discovery Cases → ~500
- Population Controls → ~700
- WTCCC Controls → ~4800
## DILI case profile

<table>
<thead>
<tr>
<th>Total cases</th>
<th>505</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>% Female</td>
<td>296 (59%)</td>
</tr>
<tr>
<td>% Male</td>
<td>219 (41%)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>White/European</td>
<td>473</td>
</tr>
<tr>
<td>Other</td>
<td>32</td>
</tr>
<tr>
<td><strong>Country/origin for Europeans</strong></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>208</td>
</tr>
<tr>
<td>Spain</td>
<td>93</td>
</tr>
<tr>
<td>USA</td>
<td>65</td>
</tr>
<tr>
<td>Scotland, UK</td>
<td>44</td>
</tr>
<tr>
<td>France</td>
<td>42</td>
</tr>
<tr>
<td>Italy</td>
<td>21</td>
</tr>
</tbody>
</table>
## Total Phase 1 DILI case recruitment

<table>
<thead>
<tr>
<th>Drug (group)</th>
<th>Diligen</th>
<th>EUDRA</th>
<th>ScotInd</th>
<th>Malaga</th>
<th>Abbott</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coamoxiclav</td>
<td>78</td>
<td>25</td>
<td>13</td>
<td>58</td>
<td>0</td>
<td>174</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>70</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>76</td>
</tr>
<tr>
<td>Coamoxi or Fluclox</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Zileuton</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>25</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>IRPE</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>44</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>89</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>226</strong></td>
<td><strong>106</strong></td>
<td><strong>44</strong></td>
<td><strong>58</strong></td>
<td><strong>71</strong></td>
<td><strong>505</strong></td>
</tr>
</tbody>
</table>
DILI controls strategy

- Country (or PCA) and sex-matched population controls at ~2:1 (control:case) ratio
  - Drawn from GSK Population Reference Sample (POPRES)
- Draw on publicly available Illumina whole-genome genotype data to supplement
  - WTCCC (UK) ~4800 controls
  - Improve statistical power for modest effects
Genetic structure of European subjects: 457 cases, 650 POPRES controls (post QC)
In *HLA-B*\textsuperscript{*}5701 carrier cases, rs10937275 in *ST6GAL1* on chromosome 3 also showed genome-wide significance (OR = 4.1, $P = 1.4 \times 10^{-8}$).
Augmentin liver injury study

145 Cases

56 Cases

iSAEC/DILIN Collaboration
201 Genotyped Cases
Augmentin sample

<table>
<thead>
<tr>
<th></th>
<th>Diligen</th>
<th>EUDRA</th>
<th>Malaga</th>
<th>DILIN</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>77</td>
<td>19</td>
<td>49</td>
<td>56</td>
<td>201</td>
</tr>
<tr>
<td>Gender</td>
<td>M</td>
<td>39</td>
<td>11</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>38</td>
<td>8</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Age</td>
<td>Mean</td>
<td>61</td>
<td>68</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>StdErr</td>
<td>14</td>
<td>14</td>
<td>19</td>
<td>14</td>
</tr>
</tbody>
</table>

659 POPRES controls
Augmentin case characteristics

- Cholestatic
- Mixed
- Hepatocellular
- Unknown

Count

UK | EUDRA | Malaga | DILIN

Count

UK | EUDRA | Malaga | DILIN

- Possible
- Probable
- Highly Probable
Augmentin case-control matching
Augmentin GWAS results

- 201 cases versus 532 controls
- Logistic regression under additive model, with covariates:
  - Gender
  - PCA scores (1 and 2)
Augmentin MHC association

<table>
<thead>
<tr>
<th>Top SNP</th>
<th>Chr:Mbp</th>
<th>P-value</th>
<th>OR (95% CI)</th>
<th>P-value conditioned on rs3135388 (DR2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9274407</td>
<td>6:32.74</td>
<td>4.8e-14</td>
<td>3.1 (2.2-4.2)</td>
<td>0.00011</td>
</tr>
</tbody>
</table>

P-value: 3.2 (1.8-5.8)

Dr2 tag
Augmentin MHC association
Conditioned on top class II SNP

<table>
<thead>
<tr>
<th>Top SNP</th>
<th>Chr:Mbp</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2523822</td>
<td>6:29.94</td>
<td>1.3e-9</td>
<td>2.3 (1.7-2.9)</td>
</tr>
</tbody>
</table>

Position on Chromosome 6 (Mbp)
Augmentin MHC association
Conditioned on top class I and II SNPs
Augmentin top SNPs and HLA alleles

<table>
<thead>
<tr>
<th>SNP</th>
<th>Tagged Alleles</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9274407</td>
<td><em>DR2</em></td>
<td>In LD with <em>DR2</em></td>
</tr>
<tr>
<td>rs3135388</td>
<td><em>DR2</em></td>
<td>Reported to be associated by two previous studies;</td>
</tr>
<tr>
<td>rs2523822</td>
<td><em>HLA-A</em>0201</td>
<td>Not reported in previous studies</td>
</tr>
</tbody>
</table>

\[ r^2 \sim 0.9 \]
\[ r^2 \sim 0.77 \]
\[ r^2 \sim 1 \]

\[ *DR2* = DRB1*1501–DQB1*0602 \]
Augmentin liver injury patterns

**B*1801**

<table>
<thead>
<tr>
<th>N</th>
<th>Description</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Spanish_hepatocellular</td>
<td>7.8</td>
</tr>
<tr>
<td>34</td>
<td>Spanish_mixed/cholestatic</td>
<td>2.1</td>
</tr>
<tr>
<td>29</td>
<td>nw–EU_hepatocellular</td>
<td>2.8</td>
</tr>
<tr>
<td>108</td>
<td>nw–EU_mixed/cholestatic</td>
<td>0.63</td>
</tr>
</tbody>
</table>

**DQB1*0402**

<table>
<thead>
<tr>
<th>N</th>
<th>Description</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Spanish_hepatocellular</td>
<td>1.3</td>
</tr>
<tr>
<td>34</td>
<td>Spanish_mixed/cholestatic</td>
<td>0.57</td>
</tr>
<tr>
<td>29</td>
<td>nw–EU_hepatocellular</td>
<td>8.2</td>
</tr>
<tr>
<td>108</td>
<td>nw–EU_mixed/cholestatic</td>
<td>3.6</td>
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</tbody>
</table>
Augmentin conclusions

- **DR2** association is confirmed, however…
  - *DQB1*0602 has a larger effect in Spanish than nw-EU
  - *DQB1*0402 is strongly associated only in nw-Eu and perhaps stronger in hepatocellular cases

- **rs2523822** within class I is a novel association, significant in both sub-populations, however…
  - In nw-EU it is a near-perfect tag of *A*0201; association not distinguishable
  - In Spanish it is a poor tag of *A*0201, which is not significantly associated

- **B*1801** is nominally associated in Spanish, especially in hepatocellular cases

- Cannot determine likely causal variants
  - Investigation in additional populations likely required to sort out
Anti-TB: 14 cases, 291 controls

Intergenic, between ASAH1 and NAT1
Zileuton case summary
Derived from a single open label safety surveillance study
Cases with maximum on treatment ALT ≥ 3 × ULN

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>White</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Black</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>2</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>48 ± 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max ALT (xUNL)</td>
<td>7.8 ± 6</td>
</tr>
</tbody>
</table>

Zileuton liver injury results
58 cases, 207 controls

**Overall**

- MDH1 Malate dehydrogenase OR = 4.6
- HMGCR OR = 5.0

**MHC**
iSAEC DILI data release

- Complete phase I Diligen, EUDRAGENE, Dundee and Spanish DILI cases
  - Released in 2009
- Includes demographic, clinical and Illumina 1M genotype data
Phase 1 Experience
What we’ve learned

- Common variants with a significant impact on the risk of serious adverse events can be identified in small case sample sizes (<50) using a GWAS approach
- Most genetic risk factors are drug specific, but not all drug specific DILI shows compelling associations.
- The iSAEC and related work has demonstrated an important role for the MHC in the pathology of DILI
  - Emphasized the influence of genetic variants in immune response in addition to ADME genes
- Several common risk alleles that are shared across drugs and/or SAEs are emerging (e.g. HLA-B*5701, HLA-DRB1*1501, UGT1A1*28) that will provide insights into SAE mechanisms
- Common genetic risk factors (alone) do not accurately predict patient risk for rare SAEs
Phase 2 Opportunities
Where we’re going

- **Drug specificity** — Build up drug-specific cohorts to identify new genetic risk factors and investigate instances of cross-drug effects

- **Population specificity** — Actively pursue non-Europeans with SAEs due to Augmentin and Carbamazapine to study influence on genetic risk

- **Rare variants** — Initiate genome-wide resequencing pilots to explore the role rare variants and potentially improve predictive values

- **Biological mechanism** — Conduct and foster *in silico, in vitro* and *in vivo* experiments to improve mechanistic understanding; adapt plans accordingly
Acknowledgments - Phase 1

Scientific Management Committee

- Co-chairs: Matt Nelson (GSK) & Sally John (Pfizer)
- Anahita Bahthena (Abbott)
- Steve Lewitzky (Novartis)
- Joe Walker (Daiichi Sankyo)
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- Michael Dunn (Wellcome Trust)

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- Munir Pirmohamed (Liverpool)

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- Aris Floratos
- Paola Niccoletti
- Andrea Califano

iSAEC DILI Members

- Ann Daly & Diligen (Newcastle)
- Mariam Molokhia & EUDRAGENE (London)
- Maribel Lucena and Camille Stephens (Malaga)
- John Dillon (Scotland)

DILIN

- Paul Watkins (Hamner)
- Bob Fontana (Michigan)
## Genetic Influence on DILI Risk
### Known Examples

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reaction Details</th>
<th>Prev</th>
<th>Risk Allele</th>
<th>Freq.</th>
<th>Rel Risk</th>
<th>PPV</th>
<th>1 - NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ximelagatran</td>
<td></td>
<td>0.08</td>
<td>HLA-DRB1*0701</td>
<td>0.08</td>
<td>4</td>
<td>0.22</td>
<td>0.055</td>
</tr>
<tr>
<td>Augmentin</td>
<td></td>
<td>&lt;0.001</td>
<td>HLA-DRB1<em>1501&lt;br&gt;A</em>0201/B*1801</td>
<td>0.15</td>
<td>4</td>
<td>5.7e-4</td>
<td>5.7e-5</td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
<td>0.15</td>
<td>CYP2E1*1 &amp; 2</td>
<td>0.13</td>
<td>7</td>
<td>0.59</td>
<td>0.084</td>
</tr>
<tr>
<td>Lapatinib</td>
<td></td>
<td>0.09</td>
<td>HLA-DQA1<em>0201 (HLA-DRB1</em>0701)</td>
<td>0.08</td>
<td>9</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Lumiracoxib</td>
<td></td>
<td>0.013</td>
<td>HLA-DRB1*1501</td>
<td>0.15</td>
<td>13</td>
<td>0.039</td>
<td>0.0030</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td></td>
<td>&lt;0.001</td>
<td>HLA-A*3303</td>
<td>0.07</td>
<td>36</td>
<td>1.2e-3</td>
<td>3.5e-5</td>
</tr>
<tr>
<td>Tranilast</td>
<td></td>
<td>0.12</td>
<td>UGT1A1*28</td>
<td>0.30</td>
<td>48</td>
<td>0.23</td>
<td>0.0048</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td></td>
<td>&lt;0.001</td>
<td>HLA-B*5701</td>
<td>0.04</td>
<td>81</td>
<td>0.0022</td>
<td>2.8e-5</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Neutropenia</td>
<td>0.20</td>
<td>UGT1A1*28</td>
<td>0.30</td>
<td>28</td>
<td>0.36</td>
<td>0.013</td>
</tr>
<tr>
<td>Mercaptopurine</td>
<td>Neutropenia</td>
<td>0.12</td>
<td>TPMT*2/3A/3B/3C</td>
<td>0.05</td>
<td>9.0</td>
<td>0.77</td>
<td>0.086</td>
</tr>
<tr>
<td>Abacavir</td>
<td>Hypersensitivity</td>
<td>0.04</td>
<td>HLA-B*5701</td>
<td>0.04</td>
<td>&gt;1000</td>
<td>0.50</td>
<td>5.0e-4</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>SJS/TEN</td>
<td>0.003</td>
<td>HLA-B*1502</td>
<td>0.04</td>
<td>&gt;1000</td>
<td>0.038</td>
<td>3.8e-5</td>
</tr>
</tbody>
</table>