The Role of Genotype in Selectively Enriching Patients for Clinical Studies

Developing Treatments for Dry Age-Related Macular Degeneration (AMD):
A Workshop

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AMD-associated Genes/Loci

**Chromosome 1**
- *CFH*
- *CFHR1/CFHR3*

**Chromosome 10**
- *ARMS2/HTRA1*

**Minor Gene Associations**
- *GWAS 2013*
- *CFB/C2*
- *APOE*
- *LIPC*
- *CFI*
- *C3*

‘Interactive Triad’
5.1% (161 of 3,166) of AMD cases grades 1B-4C from the combined Iowa/Utah/Melbourne cohort & 7.6% (60 of 444) of neovascular AMD cases in the combined NHS & HPFS cohorts carry no risk at \textit{CFH} (Chr1), \textit{ARMS2/HTRA1} (Chr10), or \textit{C3} (Chr6)
Age-related Macular Degeneration

AMD Phenotypes

Diverse clinical phenotypes of both early-stage (‘drusen’) & late-stage (GA, CNV, PPCV, RAP) AMD exist
Age-related Macular Degeneration

Macular ‘Drusen’

‘Drusen’ are often ‘lumped’ into a single category without consideration of genotype, histological phenotype, etc.
Age-related Macular Degeneration

In addition to clinical phenotypes, there are diverse histological, pathological, & likely functional phenotypes.
Age-related Macular Degeneration

Neovascular Disease

The same is true for atrophic & neovascular phenotypes
A refined understanding of genotype-phenotype associations in AMD will be crucial for the development of effective, gene-directed therapeutics. Let’s not lose effective drugs for the wrong reasons!!
• Assessment of genetic ‘outliers’ is providing a refined understanding of genotype-phenotype-pathway associations in AMD

• There is no evidence to support a direct biological interaction between AMD-associated Chr1 & Chr10 gene products
Chr1- & Chr10-directed Biology

Ethnicity-based Associations

- AMD in Ghanaian Africans is characterized by macular drusen; neovascularization is rare (*paucity of ch10 risk alleles*)
- AMD in Asians is primarily neovascular; macular drusen are uncommon (*paucity of ch1 risk alleles*)
### Chr1 & Chr10 Associations

**Utah, Iowa & Melbourne Cohorts (~6,000 samples)**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Chr1 (no risk @ Chr10)</th>
<th>Chr10 (no risk @ Chr1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All AMD</td>
<td>0.594, 0.397 p=4.47E-25</td>
<td>0.410, 0.203 p=5.67E-18</td>
</tr>
<tr>
<td>Early AMD (1b-3)</td>
<td>0.568, 0.397 p=5.36E-10</td>
<td>0.235, 0.203 p=0.3703</td>
</tr>
<tr>
<td>GA</td>
<td>0.565, 0.397 p=3.17E-05</td>
<td>0.398, 0.207 p=2.72E-05</td>
</tr>
<tr>
<td>CNV</td>
<td>0.613, 0.397 p=3.56E-22</td>
<td>0.465, 0.203 p=4.86E-22</td>
</tr>
</tbody>
</table>

Values represent case/control frequencies followed by p-values.

- ‘GA’ & ‘CNV’ associate independently with both chromosome 1 & 10 risk variants & haplotypes (phenotypes?)
- Macular drusen are not significantly associated with chromosome 10-directed AMD
Chr1- & Chr10-directed Phenotypes

Clinical

Phenotypes of cases with Chr1- & Chr10-directed AMD exhibit distinct characteristics
Chr1-directed Phenotypes (Pre-GA/CNV)

Homozygous Risk at Chromosome 1 (464-10)

‘Typically’ characterized by large soft, coalescing drusen/PEDs, as compared to Chr10-directed phenotypes
Chr10-directed Phenotypes (Pre-GA/CNV)

Homozygous Risk at Chromosome 10 (560-11)

‘Typically’ characterized by fewer & smaller macular drusen, as compared to Chr1-directed phenotype
## Chr1- & Chr10-directed Phenotypes

### Utah, Iowa & Melbourne Patient Cohorts

<table>
<thead>
<tr>
<th>Grade</th>
<th>CC/GG Risk @ Chr1</th>
<th>TT/TT Risk @ Chr10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected (0)</td>
<td>23%</td>
<td>7%</td>
</tr>
<tr>
<td>Early (1B-3)</td>
<td>34% (45% AREDS grade 3)</td>
<td>17% (8% AREDS grade 3)</td>
</tr>
<tr>
<td>GA (4A)</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td>CNV (4B)</td>
<td>34%</td>
<td>60%</td>
</tr>
<tr>
<td>GA &amp; CNV (4C)</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>Ave Age Initial CNV</td>
<td>78.6 years</td>
<td>72.8 years</td>
</tr>
</tbody>
</table>

- Drusen are more common in Chr1 patients (phenotype?)
- CNV & GA are more prevalent in Chr10 patients
- Average age of initial CNV is younger in Chr10 patients
Chr1- & Chr10-directed AMD

Geographic Atrophy

Chr1- & Chr10-directed geographic atrophy may also be distinct…stay tuned
• The biological manifestations of ‘Chr1-directed’ AMD are also distinct from those of ‘Chr10-directed’ AMD
  ✓ Gene expression (patient samples & eye repository)
  ✓ Serum biomarkers (patient samples)
  ✓ Histology (eye repository)
There is marked histological variation between chromosome 1- and chromosome 10-directed AMD.

Morphometric data generated from >700 pairs of human donor eyes.

Multivariate regression model corrected for gender & age.

<table>
<thead>
<tr>
<th>Feature</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macular drusen</td>
<td>1.8 x 10(^{-5}) 0.023</td>
</tr>
<tr>
<td>Thickened Bruch’s membrane</td>
<td>0.0027   0.43</td>
</tr>
<tr>
<td>RPE ‘spheres’</td>
<td>0.0071   0.11</td>
</tr>
<tr>
<td>Sub-RPE BLD (grade 3)</td>
<td>0.44     1.8 x 10(^{-12})</td>
</tr>
<tr>
<td>Choriocapillaris ghosts</td>
<td>0.44     2.6 x 10(^{-5})</td>
</tr>
<tr>
<td>Choroidal fibrosis</td>
<td>0.24     0.0053</td>
</tr>
<tr>
<td>Basal linear deposits</td>
<td>0.52     0.054</td>
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Age-related Macular Degeneration

‘Take Home’ Messages

• New insights into the underpinning biology of AMD suggest that it is multiple, distinct biological diseases rather than a single, complex trait disease

• A refined understanding of genotype-phenotype associations will be critical to the identification of gene-directed pathways & targets, the development of therapeutics & the design of clinical trials

• Major implication – an individual may have more than one disease
The levels of C5 & C5b-9 at the macular RPE-choroid interface are low &/or non-existent in a subset of donors with AMD.