Genetic Epidemiology of Monogenic and Polygenic Diseases – Retinitis Pigmentosa and Myopia

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Genetics of Human Disease

- Modes of inheritance
- Genetic Testing for Monogenic Disease
  - Retinitis Pigmentosa
  - Ethical Issues
- Genetic Epidemiology of Polygenic Disease
  - Myopia
Identifying Disease Genes
The Utility Pathway

Disease/Susceptibility
Gene Discovery

Biochemistry & Function
Animal Models
Human Syndromes
Genetic Epidemiology

Understanding Disease Processes / Identifying Targets

Protein Structure/ Function
Pathophysiology
Genotype-Phenotype

Clinical Genetics & Molecular Medicine
Diagnosis
Prognostication
Counselling
Intervention
# Categories of Disease Causation

adapted from Ward RH 1979: Social Biology 27:87-100

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mendelian</strong></td>
<td></td>
</tr>
<tr>
<td>Single-gene Inborn errors of metabolism</td>
<td></td>
</tr>
<tr>
<td>Chromosomal</td>
<td></td>
</tr>
<tr>
<td>- aneuploidy</td>
<td>Down’s syndrome</td>
</tr>
<tr>
<td>- structural</td>
<td>Prader-Willi syndrome</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>musculoskeletal/neurodegenerative syn</td>
</tr>
<tr>
<td><strong>Complex</strong></td>
<td></td>
</tr>
<tr>
<td>Multifactorial</td>
<td></td>
</tr>
<tr>
<td>- high heritability</td>
<td>cleft lip / palate, neural tube defects</td>
</tr>
<tr>
<td>- low heritability</td>
<td>coronary artery disease, asthma etc</td>
</tr>
<tr>
<td>Infectious pathogens</td>
<td>viral, bacterial, fungal, protozoal</td>
</tr>
<tr>
<td>Environmental</td>
<td>physical (radiation, trauma), chemical (drug, pollutant, occupational), nutritional</td>
</tr>
</tbody>
</table>
## Morbidity of Genetic Disease

<table>
<thead>
<tr>
<th>TYPE</th>
<th>LIFETIME FREQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal</td>
<td>3.8 per 1000</td>
</tr>
<tr>
<td>Single gene</td>
<td>20 per 1000</td>
</tr>
<tr>
<td>Multifactorial</td>
<td>646 per 1000</td>
</tr>
</tbody>
</table>

Emory & Rimoin (1997)
Segregation of autosomal dominant trait (one parent affected)

(Both sexes are equally at risk)
Segregation of autosomal recessive trait (both parents carriers)

Carrier parents

Affected

Carriers

Normal

(Both sexes are equally at risk)

Risk 25%
Segregation of X-linked recessive trait (father affected)

Segregation of X-linked recessive trait (mother carrier)

Risk: if female 100% carrier
If male 100% normal

Risk: if female 50% carrier
If male 50% affected
Deviations from Mendelian inheritance

- Incomplete penetrance
- De novo (new) mutations
- Variable expression
- Incomplete lyonization
- Mosaicism
- Imprinting
- Uniparental disomy
- Triplet repeats and anticipation
- Contiguous gene syndromes
- Mitochondrial inheritance
- Digenic
<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>-29 A→G</td>
<td>15,17</td>
</tr>
<tr>
<td>-88 C→T</td>
<td>17-21</td>
</tr>
<tr>
<td>IVS1 110G→A</td>
<td>30</td>
</tr>
<tr>
<td>CD 39 C→T</td>
<td></td>
</tr>
<tr>
<td>IVS1 1 G→A</td>
<td></td>
</tr>
<tr>
<td>IVS2 1 G→A</td>
<td></td>
</tr>
<tr>
<td>IVS1 5 G→C</td>
<td></td>
</tr>
<tr>
<td>CD 8-10</td>
<td></td>
</tr>
<tr>
<td>CD 44-12</td>
<td></td>
</tr>
<tr>
<td>CD 41/42-TCTT</td>
<td>58,59</td>
</tr>
<tr>
<td>CD 17 A→T</td>
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</tr>
<tr>
<td>CD 41/42-TCTT</td>
<td>120</td>
</tr>
<tr>
<td>CD 41/42-TCTT</td>
<td>128</td>
</tr>
<tr>
<td>FS 6/8/9,16</td>
<td></td>
</tr>
<tr>
<td>FS 39</td>
<td></td>
</tr>
<tr>
<td>FS 41/42,44</td>
<td></td>
</tr>
<tr>
<td>FS 71/72</td>
<td></td>
</tr>
<tr>
<td>NS 151</td>
<td>120</td>
</tr>
<tr>
<td>NS 127</td>
<td>126</td>
</tr>
<tr>
<td>FS 1,132</td>
<td>153</td>
</tr>
<tr>
<td>FS 94,109,114,123,126</td>
<td>156</td>
</tr>
<tr>
<td>Hb Tac FS 147</td>
<td>157</td>
</tr>
<tr>
<td>Hb Cranston FS 145</td>
<td>157</td>
</tr>
</tbody>
</table>

Nature Reviews | Genetics
Gene-Gene Interaction

Secondary modifiers

Proteolysis → Excess α-chains

Primary gene defect

γ-chains (HbF, α2γ2)

Inclusion bodies
Ineffective erythropoiesis, haemolysis

Anaemia

Bone disease
Iron loading
Jaundice
Infection

Tertiary modifiers

VDR
ESR1
Collagen

HFE

UGT1

Co-selection
HLA-DR
TNF
ICAM1

Nature Reviews | Genetics
Genetics of Human Disease

- Modes of inheritance
- Genetic Testing for Monogenic Disease
  - Retinitis Pigmentosa
  - Ethical Issues
- Genetic Epidemiology of Polygenic Disease
  - Myopia
<table>
<thead>
<tr>
<th>DISEASE</th>
<th>AD</th>
<th>AR</th>
<th>XL</th>
<th>Mit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bardet-Biedl syndrome</td>
<td>5(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital amaurosis</td>
<td>4(3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cone or cone-rod dystrophy</td>
<td>6(2)</td>
<td>2(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital stationary night blindness</td>
<td>1(1)</td>
<td>2(2)</td>
<td>3(1)</td>
<td></td>
</tr>
<tr>
<td>Macular degeneration</td>
<td>9(3)</td>
<td>1(1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>2(0)</td>
<td>1(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>11(5)</td>
<td>13(7)</td>
<td>6(2)</td>
<td></td>
</tr>
<tr>
<td>Syndromic or systemic retinopathy</td>
<td>3(2)</td>
<td>11(6)</td>
<td>1(0)</td>
<td></td>
</tr>
<tr>
<td>Usher syndrome</td>
<td>9(2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other retinopathy</td>
<td>8(3)</td>
<td>8(5)</td>
<td>9(7)</td>
<td>4(4)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>119</td>
<td>mapped</td>
<td>(56 cloned)</td>
<td></td>
</tr>
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</table>
Autosomal Dominant RP Family
<table>
<thead>
<tr>
<th>Category</th>
<th>OMIM no.</th>
<th>Mode</th>
<th>Chromosomal Localisation</th>
<th>Candidate genes/proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP1</td>
<td>180100</td>
<td>AD</td>
<td>8p11-q21</td>
<td>Not known</td>
</tr>
<tr>
<td>RP2</td>
<td>312600</td>
<td>XL-R</td>
<td>Xp11.23</td>
<td>Not known</td>
</tr>
<tr>
<td>RP3</td>
<td>312610</td>
<td>XL-R</td>
<td>Xp21.1</td>
<td>GTPase regulator (RPGR)</td>
</tr>
<tr>
<td>RP4</td>
<td>180380</td>
<td>AD/AR</td>
<td>3q21-q24</td>
<td>Rhodopsin (RHO)</td>
</tr>
<tr>
<td>RP6</td>
<td>312612</td>
<td>XL-R</td>
<td>Xp21.3-p21.2</td>
<td>Not known</td>
</tr>
<tr>
<td>RP7</td>
<td>179605</td>
<td>AD</td>
<td>6p21.1-cen</td>
<td>Peripherin/rod degeneration slow (RDS)</td>
</tr>
<tr>
<td>RP9</td>
<td>180104</td>
<td>AD</td>
<td>7p15.1-p13</td>
<td>Not known</td>
</tr>
<tr>
<td>RP10</td>
<td>180105</td>
<td>AD</td>
<td>7q31-q35</td>
<td>Not known</td>
</tr>
<tr>
<td>RP11</td>
<td>600138</td>
<td>AD</td>
<td>19q13.4</td>
<td>Not known</td>
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<tr>
<td>RP12</td>
<td>600105</td>
<td>AR</td>
<td>1q31-q32.1</td>
<td>Not known</td>
</tr>
<tr>
<td>RP13</td>
<td>600059</td>
<td>AD</td>
<td>17p13.3</td>
<td>Not known</td>
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<tr>
<td>RP14</td>
<td>600132</td>
<td>AR</td>
<td>6p21.3</td>
<td>Tubby-like protein (TULP1)</td>
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<tr>
<td>RP15</td>
<td>300029</td>
<td>XL-D</td>
<td>Xp22.13-p22.11</td>
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<tr>
<td>RP17</td>
<td>600852</td>
<td>AD</td>
<td>17q22</td>
<td>Not known</td>
</tr>
<tr>
<td>RP18</td>
<td>601414</td>
<td>AD</td>
<td>1p13-q23</td>
<td>Not known</td>
</tr>
<tr>
<td>RP19</td>
<td>601718</td>
<td>AR</td>
<td>1p21-p13</td>
<td>ABCR</td>
</tr>
<tr>
<td>RP20</td>
<td>180069</td>
<td>AR</td>
<td>1p31</td>
<td>Retinal pigment epithelium (RPE65)</td>
</tr>
<tr>
<td>RP21</td>
<td>601850</td>
<td>AD</td>
<td>(9q34)</td>
<td>Mitochondrial genome MTTS2</td>
</tr>
<tr>
<td>RP22</td>
<td>602594</td>
<td>AR</td>
<td>16p12.3-p12.1</td>
<td>Not known</td>
</tr>
<tr>
<td>RP24</td>
<td>300155</td>
<td>XL</td>
<td>Xp26-q27</td>
<td>Not known</td>
</tr>
<tr>
<td>RP25</td>
<td>602772</td>
<td>AR</td>
<td>6q14-q21</td>
<td>Not known, GABAR clusters</td>
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<tr>
<td></td>
<td>180071</td>
<td>AR</td>
<td>5q31.2-q34</td>
<td>Phosphodiesterase α subunit, PDEA</td>
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<tr>
<td></td>
<td>180072</td>
<td>AR</td>
<td>4p16.3</td>
<td>Phosphodiesterase β subunit, PDEB</td>
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<tr>
<td></td>
<td>123825</td>
<td>AR</td>
<td>4p12-cen</td>
<td>cGMP Gated Channel α subunit, CNCG1</td>
</tr>
<tr>
<td></td>
<td>180721</td>
<td>digenic</td>
<td>11q13</td>
<td>Rod Outer Segment Protein. ROM1</td>
</tr>
</tbody>
</table>

**Retinitis Pigmentosa Loci**
2-Step Diagnosis

- Linkage Analysis to exclude uninvolved loci
- DNA Sequencing to identify pathogenic mutation
<table>
<thead>
<tr>
<th>Candidate Gene</th>
<th>Locus</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopsin (RHO)</td>
<td>3q21-q24</td>
<td>RHO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D3S3606</td>
</tr>
<tr>
<td>Rod cGMP Phosphodiesterase α subunit (PDEA)</td>
<td>5q31.2-q34</td>
<td>D5S2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D5S434</td>
</tr>
<tr>
<td>Rod cGMP Phosphodiesterase β subunit (PDEB)</td>
<td>4p16.3</td>
<td>D4S227</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D4S3038</td>
</tr>
<tr>
<td>Rod cGMP gated Channel α subunit (CNCG1)</td>
<td>4p14-q13</td>
<td>D4S3002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D4S1536</td>
</tr>
<tr>
<td>Peripherin / rds (RDS)</td>
<td>6p</td>
<td>RDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D6S1582</td>
</tr>
<tr>
<td>Rod Outer Membrane Protein 1 (ROM1)</td>
<td>11q13</td>
<td>D11S480</td>
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<tr>
<td></td>
<td></td>
<td>D11S4076</td>
</tr>
</tbody>
</table>
Gene of Phosphodiesterase A

2 cM

Idiogram of Chromosome 5

Genetic Map at Phosphodiesterase A gene locus

Sequence of D5S434 marker

Electrophoresis Gel Separation
Linkage Analysis with STR Markers

Markers

- D5S2013
- D11S1536
- D3S3606
- D4S3002
- D5S434
- D4S3036
- RHO
- D4S3002
- D4S227
Linkage and Haplotypes for Rho

A269G  RHO  A5145G  A5510G

~ 2 kb  ~ 3 kb  0.36 kb  < 0.4 cM

RHO

118  142  142  142  118  142  142  142  142  142

118  118  142  142  142

118  142  142  142  142
Linkage and Haplotypes for Rho
Linkage and Haplotypes for Rho

A269G  RHO  A5145G  A5510G  D3SS3606

~ 2 kb  ~ 3 kb  0.36 kb  < 0.4 cM
Significance of Linkage

- LOD (Log of Odds Ratio)

\[
\text{LOD} = - \log \left( \frac{p \text{ of observing given genotypes if there is no linkage}}{p \text{ of observing given genotypes if there is linkage}} \right)
\]

- Threshold of significance for gene discovery is 3.0
- LOD score for RHO = 0.6, D3S3606 = 2.86
Sequencing of Q344ter RHO Mutation

Affected

GGAGACGAGCNAGGTGGCCCC

GGGCCACCTNGCTCGTCTCC

Unaffected

GGAGACGAGGCCAGGTGGCCCC

GGGCCACCTGGCTCGTCTCC

Forward

Reverse
Rhodopsin Mutation Sites
Rapid diagnosis of Q344ter mutation with BstNI
LET'S JUST BE FRIENDS

IT'S MY DNA... ISN'T IT?
Some Molecular Genetic Tests

- Thalassaemias, Hbopathies
- Cystic fibrosis
- Duchenne and Becker muscular dystrophy
- Fragile X
- Huntingdon’s disease
- Mitochondrial diseases
- Angelman and Prader-Willi syndromes (15q11-13)
- Familial breast cancer (BRCA1, BRCA2)
## Genetic Testing and Counselling

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MONOGENIC</th>
<th>COMPLEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene penetrance</td>
<td>Hi</td>
<td>Lo or variable</td>
</tr>
<tr>
<td>Phenotype</td>
<td>Severe, consistent</td>
<td>Highly variable</td>
</tr>
<tr>
<td>Env factors</td>
<td>Usually minor</td>
<td>Potentially sig.</td>
</tr>
<tr>
<td>Goal of gene testing</td>
<td>Presymptomatic diagnosis</td>
<td>Susceptibility assessment</td>
</tr>
<tr>
<td>Primary Indication</td>
<td>Affected 1(^{\circ}) relatives</td>
<td>Multiple affected relatives</td>
</tr>
</tbody>
</table>

Genetic tests and counselling

- Non-genetic (biochemical etc) screening
- Confirmatory diagnosis
- Carrier testing
- Prenatal / Antenatal testing
- Pre-implantation diagnosis
- Presymptomatic testing
- Population screening
Prognostication & Intervention

- Counselling
- Behavioural, diet modification
- Early diagnosis
- Pharmacological prevention, treatment
- Pharmacogenetics and individualised use of medication based on efficacy and side effects
- Surgical
- Gene therapy
Limitations of genetic testing

• Gene not identified (localised) nor cloned (sequenced)
• Many possible mutations (allelic heterogeneity) or many possible genes (genetic heterogeneity)
• Family samples not available or informative (for linkage)
• Poor predictive value (susceptibility genes, poor genotype-phenotype correlation, phenotypic heterogeneity)
• Laboratory assay unavailable, costly or difficult
• No therapy available
Ethical Legal Social Implications

- Confidentiality / privacy, right to know or not know or not act
- Informed consent
- Access to health care
  - life, medical insurance
- Costs, Patenting, Licensing
- Employment
- Individuality
- Genetic determinism
Genomics and Society

- Genomics may change our lives:
  - Social stratification (employment, marriage, emigration)
  - Genetic / Genomic engineering
  - Cloning
  - Euphenics (modifications in phenotype) rather than eugenics
  - Discrimination against individuals and groups
Genetics of Human Disease

• Modes of inheritance
• Genetic Testing for Monogenic Disease
  – Retinitis Pigmentosa
  – Ethical Issues
• Genetic Epidemiology of Polygenic Disease
  – Myopia
Polygenic/Multifactorial Inheritance

from VP Johnson, C Christianson
Genes & Environment

DISEASE

“Environmental”

Non-genetic

Multifactorial “Complex”

Polygenic

Oligogenic

Digenic

Monogenic

“Aetiological spectrum”

“Genetic”
Genes & Environment

DISEASE
- Non-genetic
- Multifactorial “Complex”
- Polygenic
- Oligogenic
- Digenic
- Monogenic

“Environmental”

“Identical”

STUDY SAMPLE
- MZ twins & clones
- Time course, trends
- Pure-bred strains
- Sibs & DZ twins
- Relatives
- Clan
- Isolated population
- Dialect group
- Ethnic group
- Humans
- Comparative biology

Aetiological spectrum

Genetic diversity

“Genetic”

“Diverse”
Complex Diseases

**“Environmental”**

- **Non-genetic**
- **Multifactorial “Complex”**
- **Polygenic**
- **Oligogenic**
- **Digenic**
- **Monogenic**

**Aetiological spectrum**

**Common, prevalent**

Genetic contribution not well characterised

Poor correlation between phenotype and genotype:

- Reduced penetrance
- Genetic heterogeneity
- Pleiotropy (Phenotypic heterogeneity)
- Multiple genes: oligogenic, polygenic
- Gene-Environment interaction
- Gene-Gene interaction (epistasis):
  - additive, multiplicative
Gene-Environment Interaction

• Definition:
  – a different effect of an environmental exposure on disease risk in persons with different genotypes, or
  – a different effect of a genotype on disease risk in persons with different environmental exposures

Gene-Environment Interaction

Model A - PKU (AR), serum Phe, mental retardation, intermediate variable, intrauterine exposure in maternal PKU

Model B - XP(AR) UV, skin cancer

Model C - Porphyria variegata (AD), photosensitivity, skin blistering, barbiturates

Model D - G6PD deficiency (XR), fava beans, drugs, haemolytic crisis

Model E - α1 anti-trypsin deficiency smoking, chronic obstructive lung disease
Scope of Genetic Epidemiology

Population Dynamics
Mutation Genotype Phenotype
Environment
Complex Diseases

- Multifactorial, non-Mendelian, polygenic
- Common, genetic contribution not characterised
- Poor correlation between phenotype and genotype
  - Reduced penetrance
  - Genetic heterogeneity
  - Pleiotropy (Phenotypic heterogeneity)
  - Multiple genes: oligogenic, polygenic
  - Gene-Environment interaction
  - Gene-Gene interaction (epistasis): additive, multiplicative
Identifying Disease Genes

Candidate Genes

Genetic Epidemiology

REVERSE GENETICS

Postional Cloning

Pathophysiology

Chromosomal abnormalities

Animal Models

Syndromes

Candidate Genes

Disease Susceptibility Genes
Genetic Epidemiology
Stages in identifying disease genes

- RESEARCH QUESTION
  - Disease characteristics?
  - Familial clustering?
  - Genetic or Environmental?
  - Mode of inheritance?
  - Disease susceptibility loci?
  - Gene? Mutation?

- METHOD/APPROACH
  - Descriptive epidemiology
  - Family aggregation studies
  - Twin/Adoption studies
  - Segregation analysis
  - Linkage analysis
  - Association studies

- OBJECT
  - Species
  - Population
  - Family
  - Genetic locus
  - Gene
  - Mutation
Singapore MyGen Study

DMRI-SERI-NMRC

• Aim
  – To identify genetic loci and candidate genes for susceptibility to severe myopia in Singaporean Chinese

• Objectives
  – Recruit and ascertain 250 sib-pairs and their parents
    • severe/pathological myopia <6D in adults
    • early-onset myopia in children
  – Obtain DNA samples from these 1000 individuals
  – Genotype 300 markers throughout genome
  – Perform sib-pair linkage analysis, 2-stage parallel association and mutation studies
**Singapore MyGen Study**

*DMRI-SERI-NMRC*

- **Hypothesis:**
  - Myopia is a syndrome of complex multifactorial diseases caused by environmental and visual risk factors acting on a genetically susceptible background

- **Research Question:**
  - Is Myopia Genetic?