Human genetic variation and therapeutic development

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Pfizer Global Research & Development

Human genetic variation

• Most traits that are of significant public health impact are attributable to a complex interaction of genetic and environmental effects
  – Susceptibility to common diseases
  – Progression and severity of chronic disease
  – Response to treatments
    • Efficacy
    • Adverse drug reactions (ADRs)

Asthma and allergies
Cardio-vascular disease
Schizophrenia
Obesity
Type 2 diabetes
Depression
Common cancers
Rheumatoid arthritis
Osteoarthritis

Public databases archive data describing genetic variation

• A genetic polymorphism is a variant that occurs at a frequency >1% in the population
• It is estimated there are > 10 million SNPs in the human genome
• There has been an unprecedented increase in the number of SNPs identified and submitted to public databases
  – NCBI dbSNP
  – HapMap project
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dbSNP

- Build 125 contains 10,430,753 unique rs numbers

Drug discovery pipeline

Implications of understanding human genetic variation
Most medicines are efficacious for 40-60% of patients.

- Efficacy
- Tolerability of side effects
- No Efficacy and tolerable side effects
- No Efficacy and intolerable side effects

Human variation and therapeutic development

100 discovery projects
Identify potential disease targets
Target prioritization/validation/selection
Screening the common allotype of the target protein
Test for human variation impact on the drug discovery pipeline
Target identification
Defining targets
Identification, prioritization and validation

Products
A good target will play a key role in disease pathology

- Genetic approaches can be used to identify novel targets or validate potential targets.

**TNFα**
- A target for rheumatoid arthritis (RA)
- TNFα is expressed at high levels in affected tissues of RA patients
- TNFα polymorphisms are associated with RA

- Blocking the action of TNFα in vitro and in vivo attenuates the immune response

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**Family and population based methods**

- **Linkage**
  - Linkage may extend over 10 cM
  - Requires 350 markers to scan the genome
  - Mendelian disease genes
  - Low power to detect common low penetrance alleles

- **Association**
  - Association extends only a few kB
  - Requires >100K markers per genome scan
  - Greater power to detect alleles that influence complex traits

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**Genetic association studies**

- Gene A: two allelic forms
- Genotype cases and controls

- Compare allele/genotype frequencies between cases and controls
Evidence for association

<table>
<thead>
<tr>
<th></th>
<th>+ risk</th>
<th>-risk</th>
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<tbody>
<tr>
<td>Case</td>
<td>192</td>
<td>214</td>
</tr>
<tr>
<td>Control</td>
<td>147</td>
<td>339</td>
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</tbody>
</table>

Odds ratio > 1

ch squared = 27.27  p < 0.00001

Odds Ratio = 2.07; 95% CI (1.6 - 2.7)

Genes that confer low genetic relative risks (GRR) are hard to find

<table>
<thead>
<tr>
<th>Genes</th>
<th>MAF</th>
<th>p</th>
<th>N</th>
<th>GRR</th>
</tr>
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<tbody>
<tr>
<td>ADAM3</td>
<td>20%</td>
<td>0.05</td>
<td>61</td>
<td>2.8</td>
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<tr>
<td>DRO4</td>
<td></td>
<td>0.0001</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>PTPN2</td>
<td></td>
<td></td>
<td>295</td>
<td>1.6</td>
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<tr>
<td>PRDH</td>
<td></td>
<td></td>
<td>840</td>
<td></td>
</tr>
<tr>
<td>PFABY</td>
<td></td>
<td></td>
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<td>1.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>5717</td>
<td></td>
</tr>
</tbody>
</table>

GRR = 1.2

Which SNPs to type?

Candidate gene: angiotensin converting enzyme ACE

http://www.hapmap.org
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Haplotype tagging SNPs
10 SNPs from the androgen receptor (AR) gene on the X chromosome genotyped in 92 healthy males
There are 2^{10} possible haplotypes that could be observed
4 common haplotypes account for all the variability observed

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>ATTTGCGTGG</td>
<td>10%</td>
</tr>
<tr>
<td>GCCCCACAGG</td>
<td>73%</td>
</tr>
<tr>
<td>ATTGTGGCC</td>
<td>15%</td>
</tr>
<tr>
<td>GCCATAAGG</td>
<td>2%</td>
</tr>
</tbody>
</table>

LD, haplotype estimation and tagging SNPs
http://www.hapmap.org
http://www.broad.mit.edu/mpg/haploview/

The "druggable" genome
- Annotated genome sequence
- 2-3000 tractable gene targets
  - Kinases
  - Proteases
  - CPGRs
  - Ion channels
- Sample size >1000
  - Asthma
  - Arthritis
  - Depression
  - Obesity
  - Schizophrenia

Roses AD et al., Drug discovery today 2005 10: 177-189
Whole genome by association

- Identify novel targets
- Define key pathways
- Identify genetic predictors of clinical outcome
- Increase the predictive value of genetic factors by considering gene-gene interactions

Clinical trials

Predicting efficacy and safety

Phase 1

First in human studies understanding drug ADME properties
Azathioprine (AZA) and chronic inflammatory disease

- Thiopurine methyltransferase (TPMT) metabolises azathioprine into both active and inactive metabolites
- Genotyping patients for 3 polymorphisms in the TPMT gene may predict those at greater risk of adverse events e.g., bone marrow toxicity
- Will this be useful in the clinic?

Variation and metabolism I

- Codeine treatment for headache
  - Codeine needs to be metabolised to morphine by the liver enzyme CYP2D6
  - The drug is ineffective in those people who cannot metabolise the drug properly (10% of Caucasians)
  - The gene encoding CYP2D6 is polymorphic
    - Over 30 mutant forms of the enzyme are described
    - These variations can predict efficacy

CYP gene family variation

- CYP (Cytochrome P450) oxidases are enzymes that metabolize compounds
- Six CYP enzymes account for 90% of drug metabolism
- Patients can carry mutations or duplications in one or more CYP genes
- As a consequence, individuals may be a range of poor to ultra-rapid metabolizers
- Poor metabolizers may develop side effects
- Ultra-rapid metabolizers may not respond to treatment
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Variation and metabolism II

- AmpliChip CYP450 Test
- Provides comprehensive coverage of gene variations in CYP2D6 and CYP2C19 genes
- For use by physicians in individualizing treatment selection and dosing for drugs metabolized through these genes

http://www.roche-diagnostics.com/products_services/amplichip_cyp450.html

Phase 2, 3 and in the clinic

“Will it work?”

Atypical anti-psychotics and schizophrenia

- Drug response heterogeneous both for efficacy and ADRs such as rapid weight gain
  - DMEs and drug targets
  - Dopaminergic, serotonergic, and glutamatergic pathways

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Whole genome by association

Wish list for pharmacogenetics

- Sequence of the human genome
- Knowledge about polymorphisms
- A haplotype map of the genome
- Accurate, inexpensive genotyping technologies
- Populations accurately measured for the outcome of interest

Genotyping methods must be high-throughput

Criteria for success of WGA:

- 100K array captures a mean of 32% of common genetic variation in the European population
- 500K array captures >80% of common genetic variation in the European population
- Rare variation (MAF<5%) is not well captured using current technologies

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Criteria for success of WGA: magnitude of effect size and multiple testing

- Two stage approach
  - Replication cohorts
  - Joint analysis
- Meta-analysis
- Collaboration

Genotype 500K SNPs
1000 cases 10000 controls

- P > 0.05
  - Type II error
  - Not associated markers
- P < 0.05
  - Reject the null hypothesis
  - Associated markers
- P > 0.05
  - Type I error

- P < 0.05
  - Accept the null hypothesis

Non-synonymous coding SNPs

- Most polymorphisms may have no functional relevance
- Exonic
  - Non-synonymous coding SNPs
  - Lymphoid tyrosine kinase (PTPN22) R620W SNP
- Regulatory regions and introns
  - Transcription levels and alternative splicing
  - Serotonin transporter protein (SLC6A4) Ins/del in promoter region and an intronic VNTR
- Intragenic
  - Gene regulation

Functional relevance of genetic variation

http://genetics.bwh.harvard.edu/pph/data/
http://blocks.fhcrc.org/sift/SIFT.html/

Prediction the functional consequences of non synonymous coding SNPs

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Allele specific differential gene expression

Conserved non-coding sequences (CNC)

Implications for future treatments

- To date there are few examples of genetic predictors being useful in the clinic
- However gene expression and variation is beginning to make an impact
- Over the next decade it is likely that our understanding of human genetic variation will impact both on the diagnosis and treatment of disease

Lin W et al., Genomics, 2005, 86, 518-527

Drake et al., Nature Genet, 2006, 38, 223-227

47% SNPs showed differential allele specific expression
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Redefining disease...

...will drive future treatment approaches

...and bring increased benefits for patients

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Marketed targeted treatments

<table>
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<th>Product</th>
<th>Primary Indication</th>
<th>Diagnostic</th>
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<tbody>
<tr>
<td>Herceptin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gleevec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kineret</td>
<td></td>
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</tbody>
</table>

http://www.herceptin.com/professional/index.jsp
http://www.gleevec.com/index.jsp
http://www.kineret.com/professional/index.jsp

Targeted treatments - overview

Summary

- The study of human genetic variation is a key consideration and component in the discovery and development of new medicines.
- It will drive a greater understanding of the causes and treatment of disease.
- Future medicines will incorporate predictive diagnostics to target patients more effectively.